Environment and Seasons in an Aging Population: an Epidemiological Approach

Milieu en Seizoenen in een vergrijzende bevolking: Een epidemiologische aanpak

Thesis

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Manuscripts that form the basis of this thesis

1. **Cepeda M***, Koolhaas CM*, van Rooij FJA, Tiemeier H, Guxens M, Franco OH, Schoufour JD. Seasonality of physical activity, sedentary behavior, and sleep in a middle-aged and elderly population: The Rotterdam Study. Maturitas 2018; 110:41-50.
2. **Cepeda M*,** van der Toorn J*, Franco OH, Schoufour JD. Seasonality of dietary intake among participants of the Rotterdam Study. Under review in European Journal of Nutrition.
3. **Cepeda M**, Schoufour J, Erler N, Marques-Vidal P, Franco OH. Effect of meteorological factors and physical activity on the seasonality of cardiovascular risk factors: The Rotterdam Study. Under review in PLOS Medicine.
4. **Cepeda M**, Muka T, Ikram MA, Franco OH, Schoufour JD. Seasonality of insulin resistance, glucose and insulin levels among participants of the Rotterdam Study. J Clin Endocrinol Metab 2018; 103(3):946-55.
5. **Cepeda M***, Licher S*, Schoufour JD, Franco OH, Ikram A. Seasonal variation of cognitive function in The Rotterdam Study population. Draft in preparation.
6. Martínez P*, **Cepeda M***, Schoufour JD, Franco OH. Seasonality of antibiotic resistance. Systematic review and meta-analysis. Under review in BMJ Open.
7. **Cepeda M**, Schoufour JD, Freak-Poli R, Koolhaas CM, Dhana K, Bramer W, Franco OH. Levels of ambient air pollution according to mode of transport: a systematic review. The Lancet Public Health 2016; 2 (1), e23-e34.
8. **Cepeda M**, Schoufour JD, Freak-Poli R, Koolhaas CM, Dhana K, Guxens M, Franco OH. Exposure to ultrafine particles according to mode of transport: a systematic review. Submitted in European Journal of Epidemiology.
9. **Cepeda M**, Schoufour JD, Franco OH, Guxens M. Exposure to air pollution among participants of the Rotterdam Study. Draft in preparation.

Chapter 1 Introduction

BACKGROUND

Current trends of urbanization have had several benefits. These have contributed to adapt the environment where the human society thrives and to reduce the burden of multiple diseases with high and acute mortality, thus contributing largely to the extension of human life expectancy.[1] Nevertheless, urbanization has also increased the exposure to several risks that menace both the environment and global health,[1,2] such as the man-made acceleration of climate change and the widespread of antibiotic resistance due to misuse of antibiotics. This burden is projected to increase progressively, as with the extension of life expectancy and average age, so does the pool of population with a high susceptibility to environmental challenges and chronic diseases. Specifically in the elderly, the higher susceptibility occurs, among others, due to the age-related decline of physiological reserve capacity, the deterioration in the responding immune system, and reductions in cognitive capacity.

In spite there is consensus that the ongoing trend of climate change is mostly caused by man-made actions,[3] the current course of action seems unable to stop, let alone reverse, its progression. Therefore, it is necessary to anticipate the challenges of environmental deterioration and climate change, in order to enhance the adaptive capacity of the society, understood as the reduction of disease, mortality and poor quality of life.[4] A first step implies understanding the health burden that is attributable to environmental factors. For example, whereas the seasonality of the cardiovascular risk is a well described phenomenon,[5,6] it remains unclear the contribution of meteorological factors[7-15] and the seasonality of lifestyle factors, such as physical activity [16,17] and diet.[18-21] Additionally, it is necessary to understand the susceptibility of population subgroups, such as the elderly. For example, elderly populations are more susceptible to meteorological challenges given the age-related impairment of thermoregulation and the occurrence of comorbidities. The coping capabilities are further impaired by a higher vulnerability, given the frequently observed isolation, disability, and poverty of this subgroup.[22] During the scientific work presented in this thesis, susceptibility is understood as the biological responses to environmental stressors, such as the age-related impairment of thermoregulation mechanisms and comorbidities. Vulnerability is understood as the capacity to cope with environmental stressors.[22] This vulnerability contributes to reduce the adaptive capacity of the population.

In this thesis, I examined some of the most urgent health issues that potentially will affect the susceptibility of elderly population under the upcoming challenges of climate change, including the seasonal variation of lifestyle factors, cardiovascular risk factors, cognition, and antibiotic resistance; and exposure to air pollution.

OUTLINE OF THE THESIS

This thesis is composed of two parts. In part 1 I examined the seasonal variation and the influence of meteorological factors in the seasonal variation of lifestyle factors, cardiovascular risk factors, and cognition in the Rotterdam Study; furthermore, I evaluated the seasonality of

antibiotic resistance using a systematic review of the literature. In part 2, I examined the exposure to air pollution according to mode of transport in two systematic reviews and describe the exposure to air pollution in the population of the Rotterdam Study.

All but the systematic reviews studies were based in the Rotterdam Study, a prospective Dutch cohort comprised in 1987 with middle-aged and elderly population living in the district of Ommoord, in Rotterdam.[23] The cohort is comprised by three cohorts, the first one which started in 1987, the second one in 1996 and the third one in 2006. The age for inclusion in the cohort has moved across the cohorts, with participants being recruited at age of 55 in the first cohort and currently, being recruited at age of 45.

The relevant visits varied across the studies included in this thesis, depending on the aim of the specific study. In the

Figure 1 (page 8) we show the overview of the cohorts as well as the relevant visits for each study that composes the chapters regarding seasonal variation and health effects of air pollution.

Figure 1. Overview of the Rotterdam Study

Sub-cohort	Visits						
RS-I	1 n=7,983 ▲	2 n=6,315	3 n=4,785 ★■	4 n=3,558 ★■		5 n=2,147 ●▲★+■¶	6 n=1,153 ●■
RS-II			1 n=3,011 ▲★+■	2 n=2,506 ★■		3 n=1,893 ●▲★+■¶	4 n=1,408 ●■
RS-III					1 n=3,932 ▲★+■	2 n=3,122 ●★+■¶	
Timeline ->	1989-1993	1993-1995	1996-2001	2002-2005	2006-2008	2009-2013	

● Chapter 2.1.1 ▲ Chapter 2.1.2 ★ Chapter 2.2.1 + Chapter 2.2.2 ■ Chapter 2.2.3 ¶ Chapter 3.3

Seasonal variation of lifestyle factors in aging population

Previous studies have examined the seasonality of lifestyle factors, namely physical activity and diet, in several populations. First, in winter months, physical activity levels are usually lower,[16,17] while sedentary behavior increases.[24,25] However, it has not been examined if nighttime sleep duration is also related to this variation. Additionally, both age and meteorological factors have been suggested to be important determinants of the seasonality of activity levels,[26,27] but an age-specific assessment of the impact of meteorological factors in the seasonal variation of activity levels has not been performed. Therefore, in chapter 2.1.1 I examined the seasonality of the complete 24-hour spectrum, including physical activity, sedentary behavior, and sleep, measured objectively with accelerometers, according to an age-specific approach, while accounting for the influence of meteorological factors in the seasonal variation. Additionally, although several studies showed a seasonal variation of food groups and nutrients intake,[18-21] these do not take into account the high inter-correlation of food groups and nutrients within the diet. Such inter-correlation could also lead to a seasonality of the overall diet quality, hence potentially explaining the seasonality of morbidity and mortality of diet-related health outcomes, such as cardiovascular.

Yet, the seasonality of overall diet quality has not been examined. Therefore, in chapter 2.1.2 I examined the seasonality of diet quality among the participants of the Rotterdam Study.

Seasonal variation of morbidity in aging population

Although the seasonality of cardiovascular risk has been widely described,[5,28-33] the mechanisms underlying this phenomenon are not well understood. It is suggested that the variation can be attributed to the seasonality of lifestyle markers,[17,34] such as body mass index or physical activity, but meteorological factors may also contribute.[7-15] However, the influence of both lifestyle markers and meteorological factors on the seasonality of cardiovascular risk factors have not been investigated. Moreover, it was unclear if such influence differs according to age. Understanding the mechanisms underlying the seasonality of cardiovascular risk is relevant to identify interventions that are more likely to mitigate the potential influence of this phenomenon on the well-described seasonality of cardiovascular risk and the upcoming challenges of climate change. Therefore, in chapter 2.2.2 I examined the role of lifestyle markers and meteorological factors on the seasonality of seven hemodynamic, metabolic, and anthropometric risk factors, stratified by age (middle-aged (<65 years) vs elderly (≥65 years)).

The aging trend of the population has also increased the burden of cognitive decline, thus the need to understand its causes and determinants. Previous studies have shown that besides individual factors, such as educational attainment, vascular and lifestyle factors, cognitive decline can also relate with environmental factors,[35] some of which could have a seasonal influence. Nevertheless, few studies have addressed the seasonal variation of cognitive function in the general population [36,37] and the findings are contradictory. We examine in chapter 2.2.3 the seasonality of cognitive functioning among community-dwelling individuals.

The potential burden of infectious diseases within the current trends of climate change cannot be overstated, given the expected increase of environmental conditions that will favor the widespread of infections.[4] Elderly populations are among the most threatened population, as they are more sensitive to become infected and experience complications due to reduced responsiveness of immune system and presence of comorbidities; and are extra vulnerable, due to isolation and economic insecurity.[22] In this scenario, the widespread of antibiotic resistance may worsen the consequences of climatic change, by reducing the therapeutic alternatives to treat infections and by increasing the fatal outcomes of previous underlying health conditions. Especially, given the rapid worldwide increase of the intake of last-resort antibiotics.[38] Much of these infections, such as respiratory infections, have a well described seasonal variation that may lead to a seasonal variation of antibiotic resistance as well,[39-45] either because the circulating resistant strains increase, or induced by the increase of antibiotic use, or most likely a combination of both. Nevertheless, since these conditions underlying the development of antibiotic resistance change across settings, there is great heterogeneity in the evidence regarding the seasonality of antibiotic resistance. Therefore, in chapter 2.2.4 I systematically review the evidence about the seasonal variation of antibiotic resistance in bacteria that pose a serious threat to public health and examine the sources of heterogeneity of such variation.

Air pollution exposure and health effects in aging population

Air pollution is one of the other great threats of climate change. Air pollution is composed, among others, by heat-trapping gases, which are an important cause of climate change, and under and scenario of global warming, air pollution levels will worsen due to temperature inversion. Traffic emissions are an important source of air pollution,[46] and air pollution exposure while commuting often reaches levels above air quality standards.[47] Active commuters are usually considered as less exposed to air pollutants compared to motorized commuters.[48,49] However, active commuters are more likely to inhale more air pollutants due to larger trip times and breathing parameters. Although active and public transport are encouraged as environmentally-friendly and healthier (e.g. increased physical activity), it is necessary to better understand what determines the exposure to air pollution while commuting and the health effects of exposure to air pollution. I undertook a systematic review of available studies that compared the exposure to five air pollutants (CO, BC, NO_x, fine particles (particulate matter ≤ 2.5μm) and coarse particles (PM<10μm)) in chapter 3.1 and ultrafine particles in chapter 3.2 between active and motorized commuters. Previous systematic reviews have addressed the comparison of air pollution exposure between modes of transport,[48-50] but do not address the positive effect of physical activity and the difference of inhaled pollutants dose, taking into account length of the trip and increased breathing parameters.

By 2012, outdoor air pollution exposure caused about 3 million deaths worldwide, about 94% occurring in adults and up to 72% due to stroke, and ischemic heart disease, followed by chronic obstructive pulmonary disease and lung cancer.[51] Although traffic is the major source of pollutants in urban areas, it is less clear which are the components of traffic explaining its health effects. Indeed, in spite air pollution exposure is a strong candidate, traffic also increases the exposure to other risk factors, such as noise,[52] less green space, and heat islands, which are strongly spatially correlated, what makes difficult to disentangle their individual effects. Few previous studies have approached the environmental risk factors affecting elderly populations, air pollution exposure among the most important. Addressing the health effects of traffic related air pollution exposure in the population of the Rotterdam Study, which comes from the relatively small geographic area (about 4.5km^2), has the value of contributing to control for traffic-related covariates that may confound such associations, for example those related to the urban built environment. Moreover, the detailed longitudinal nature of the Rotterdam Study data permits a better characterization of the health outcomes related to air pollution exposure. In this context, we estimated the exposure to traffic-related air pollutants (NO_2, NO_x, PM_{10}, $PM_{2.5}$, and $PM_{2.5}$ absorbance) using a model previously validated for the Netherlands, which allow to estimate geographical gradients of air pollution in this small area. In chapter 3.3, I describe the methods for the estimation of pollutants in one specific visit of the cohort, where the exposure was calculated up to the writing of this thesis. Additionally, I describe the distribution of the pollutants according to selected characteristics of the participants at the visit date.

Discussion

In the last section, we discuss the methodological implications of our findings and discuss the major findings of our study in relation with the seasonality of mortality and morbidity, climate change, and air pollution exposure among elderly population.

Chapter 2 Seasonality and meteorological factors

2.1 Seasonality of lifestyle factors

2.1.1 Seasonality of physical activity, sedentary behavior, and sleep in a middle-aged and elderly population: the Rotterdam Study

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ABSTRACT

Introduction: Physical activity (PA) and sedentary behavior (SB) have seasonal patterns. It remains unclear how these patterns are associated with sleep, meteorological factors, and health.

Methods: Activity levels were continuously measured with an accelerometer for seven days between July- 2011 and May- 2016, among middle-aged (50-64 years), young-elderly (65-74 years) and old-elderly (≥75 years) participants of a population-based Dutch cohort study (n=1,116). Meteorological factors (ambient temperature, wind speed, sunlight hours, precipitation, and minimum visibility) were locally recorded. We first examined the seasonality of PA, SB, and nighttime sleep, stratified by age group. Second, we examined the influence of meteorological factors. Third, we modeled the potential seasonality of the all-cause mortality risk due to the seasonality of PA and SB, by using previously published relative risks.

Results: Levels of light and moderate-to-vigorous PA were higher in summer than in winter among middle-aged (seasonal variation=18.1 and 14.8 minutes/day) and young-elderly adults (12.8 and 8.6 minutes/day). The pattern was explained by ambient temperature and sunlight hours. Nighttime sleep was 31.8 minutes/day longer in winter among middle-aged adults. SB did not show a seasonal pattern. No seasonality in activity levels was observed among old-elderly adults. The all-cause mortality risk may be higher in winter than in summer due to the accumulation of low levels of moderate to vigorous PA and high levels of SB.

Conclusion: PA has a larger degree of seasonality than SB and nighttime sleep among middle-aged and young-elderly adults. SB appears strongly ingrained in daily routine. Recommending the interruption of SB with light PA might be a good starting point for public health institutions.

INTRODUCTION

Population ageing, urbanization, and automatization of daily activities have contributed to a predominantly sedentary lifestyle, with low levels of physical activity (PA) and high levels of sedentary behavior (SB), but also to suboptimal nighttime sleep duration (i.e. not sleeping 7-8 hours).[53,54] However, although low levels of PA cluster with high SB and suboptimal nighttime sleep duration [53,55], these are partly independent phenomena. Moreover, the proportions of the various types of daily (in) activity (i.e. PA, SB, sleep) may influence cardio-metabolic health beyond their independent effects. [56-58] Therefore, there is increasing interest in the factors determining the composition of daily activity levels.

Objective measurements with accelerometers have demonstrated that levels of PA and SB are not constant throughout the year. Studies performed in young and middle-aged population report that time spent in PA decreases in winter,[16,17] whereas sedentary time increases.[24,25] However, it is unclear whether sleep duration is related to this variation because previous studies have used sleep diaries [25] rather than objective measures or because sleep was omitted within daily routine.[24]

Several factors determine the seasonality of activity levels. For example, with increasing age, time spent in PA and nighttime sleep tends to decrease, while sedentary behavior increases.[59,60] Retirement may also explain this pattern, as leisure PA is more sensitive to seasonal changes than occupational PA.[17] Additionally, age interacts with meteorological factors to influence PA levels [26,27] and PA seasonality is more marked in geographical regions with more climatic variation.[61,62] However, an age-specific assessment of the impact of meteorological factors on the seasonality of activity levels has not been performed.

The seasonality of activity levels is of relevance to public health, as PA is often prescribed as a first means to improve health, e.g. to reduce dyslipidemia and high blood pressure. [63] Indeed, it is hypothesized that the seasonal pattern of cardio-metabolic risk and mortality can be partly explained by the seasonality of PA.[16,32] Nevertheless, it is not clear whether this seasonality is large enough to influence all-cause mortality on a seasonal basis.

We therefore examined the seasonality of objectively measured daily levels of PA, SB, and nighttime sleep duration according to age, using around-the-clock measurements. Furthermore, we examined to what extent meteorological factors explained the seasonality of activity levels. Finally, we modeled the seasonality of the all-cause mortality risk produced by the seasonal variation in levels of moderate to vigorous PA and SB.

METHODS

Study design

We performed a cross-sectional study to analyze the annual seasonal variation in PA, SB and nighttime sleep duration. This study was embedded in The Rotterdam Study (RS), a prospective population-based cohort established in 1989, which has invited the participation of all middle-aged and elderly people living in the Ommoord district of Rotterdam, the Netherlands. Baseline invitations were sent to all the home addresses within the district, including senior housing facilities, retirement homes and assisted living facilities. The aim of the Rotterdam Study was to examine the incidence of risk factors for neurological, cardiovascular, psychiatric, and other

chronic diseases.[23] The study is composed by three cohorts (RS-I, RS-II and RS-III) and follow-up visits are performed every five years.[23] The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (MEC 02.1015) and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to their involvement in the study and to obtain information from their treating physicians.

Between June-2011 and June-2014 (wave 1) and between July-2014 and May-2016 (wave 2), 3,507 participants were invited to wear an accelerometer for seven days, to measure their activity levels. 482 participants were invited in both waves. Along with wearing the accelerometer, participants reported overnight sleep periods in a sleep diary. For the current study, we selected 1,166 sets of observations (48 from participants who participated in both wave 1 and wave 2) obtained from non-disabled participants. Disability was defined as having a disability index > 0.5.[64] The participation flowchart is provided in **Appendix 1** (page 29).

Physical activity, sedentary behavior and nighttime sleep duration

To measure activity, we used a GENEActiv device (GENEActiv; Activinsights Ltd, Kimbolton, Cambridgeshire, UK, http://www.GENEActiv.org/), a tri-axial accelerometer that can be worn like a watch. Participants were instructed to wear the accelerometer on the non-dominant wrist for 7 consecutive days and nights. Accelerometer data were extracted and used to designate SB, as well as light, moderate, or vigorous PA. Detailed information on the assessment of accelerometer-derived PA can be found in the Appendix 2 (page 30) and has been described in detail elsewhere.[65] Nighttime sleep duration was detected using a validated algorithm,[66] which combines the accelerometer data and the time when participants reported they went to bed and the reported time of waking from the sleep diary. Time-in-bed was also extracted from sleep diaries. Sleep efficiency was calculated as (nighttime sleep duration/time in bed)*100.

Meteorological factors

Daily information on meteorological factors in Rotterdam was obtained from the Koninklijk Nederlands Meteorologisch Instituut (KNMI).[67] The monitor is located approximately 8km from the Ommoord district (coordinates: 51° 58' N 04° 27' E). The daily meteorological data were linked to the dates on which the accelerometer was worn. In the current study, we included daily average temperature (°C), average relative humidity (percentage), total number of sunlight hours, accumulated precipitation (mm), average wind speed (m/s), and minimum visibility (km), classified as <1.8 km, 1.8-3.9 km, 3.9-7 km, 7-12 km and ≥12 km.

Covariates

Data on covariates were collected through home interviews or measured at the Rotterdam Study research center by trained research assistants,[68] and included sex, age (years), body mass index (BMI) (kg/m^2), history of comorbidities (cardiovascular disease, diabetes, cancer or chronic obstructive pulmonary disease), smoking behavior, housing status, disability score, occupation, and alcohol intake. Data collection procedures are described in detail in Appendix 2 (page 30).

Statistical methods

All analyses were stratified by age group: 50-64 years (middle-aged), 65-74 years (young-elderly) and aged 75 years or older (old-elderly). General characteristics of the population are presented as absolute frequencies and percentage for categorical variables and as median and interquartile range (25^{th} and 75^{th} percentile) for continuous variables. Differences in distributions between the age- groups were evaluated using the Kruskal-Wallis test for continuous variables and the χ^2 test for categorical variables.

In our analysis, we first examined the seasonality of light and moderate-to-vigorous PA, SB, and nighttime sleep duration (in minutes/day) using a linear mixed effects model to account for the correlation within days contributed per participant. We used the participant id as clustering variable. Because 48 participants wore the accelerometer in both waves, we accounted for the correlation between these repeated measurements by adding a second random intercept, using the wave as clustering variable. The seasonality was evaluated using a cosinor model assuming a sinusoidal pattern with a period of one year,[17] by adding sine and cosine terms of the accelerometer wear-date in the fixed part of the model.[69] All models were adjusted for the covariates listed above, plus the day of the week (weekday or weekend day).

The seasonality is reported as the seasonal variation, corresponding to the peak-to-nadir difference in activity levels throughout the year. Procedures to estimate the seasonal variation are provided elsewhere.[69] A subgroup analysis stratified by sex was performed, including the seasonal variation in time in bed (minutes/day) and sleep efficiency (%).

Second, to examine to what extent the meteorological factors explained the seasonality of activity levels, we included one meteorological factor at a time in the fully adjusted model. Then, we calculated the difference of the seasonal variation before and after the inclusion of the meteorological factor. The influence of a meteorological factor on the seasonality of activity levels was considered significant if the seasonal variation became non-significant or was reduced by more than 5%. Average temperature was categorized in quintiles to account for the non-linear association. Wind speed, sunlight hours, precipitation, and humidity were converted to z-scores and added as continuous variables.

Finally, we examined the potential seasonality of the all-cause mortality risk as a function of moderate to vigorous PA and SB, as described in detail in Appendix 3 (page 31). Briefly, we first multiplied the time/day spent in moderate-to-vigorous PA and in SB with the log transformed relative risk (RR) for the association of moderate to vigorous PA (RR=0.72) and SB (RR=1.24) with all-cause mortality, as obtained from published meta-analyses.[70,71] The sum of these products was considered the hypothetical all-cause mortality risk due to moderate to vigorous PA and SB combined. Next, we modeled the seasonality of this hypothetical all-cause mortality risk using cosinor analysis, adjusted for the covariates listed above, and stratified by age-group. Using the seasonal variation obtained in the previous step, we calculated the hypothetical all-cause mortality risk at the peak and the nadir of the seasonal variation and, using standard life tables, we calculated the corresponding life expectancy at each extreme. The difference between the life expectancy at the peak and at the nadir is expressed in months. The analyses were repeated for PA and SB separately and using the lower and upper boundaries of the 95%

confidence interval of the RRs. All analyses were performed in Stata version 14.1 SE (StataCorp LP, College Station, Texas).[72]

RESULTS

General characteristics

A total of 1,166 sets of measurements were included, 34% (n=394) among middle-aged adults, 39% among young-elderly (n=449), and 28% among old-elderly participants (n=323). Old-elderly adults more often participated in the study during winter than during summer (43.6% vs. 6.2% of the days contributed). The other age-groups did not show this difference in participation across the seasons. Additionally, old-elderly adults were more often living in assisted living facilities and were less often in paid employment than middle-aged and young-elderly adults (Table 1, page 21).

Seasonal variation in activity levels

Among middle-aged participants, levels of light PA were highest in early August (seasonal variation=18.1 minutes/day (standard error (SE)=4.0)), and levels of moderate-to-vigorous PA were highest in late-July (seasonal variation=14.8 minutes/day (SE=3.8)), whereas nighttime sleep duration was highest mid-January (seasonal variation=31.8 minutes/day (SE=6.6)). No significant seasonal variation in SB was observed (Figure 2, page 21). Among young-elderly adults, levels of light PA and moderate-to-vigorous PA were highest in late-July (seasonal variation=12.8 minutes/day (SE=3.9) and 9.9 minutes/day (SE=3.3), respectively), but no significant seasonal variation was observed for nighttime sleep duration. Among old-elderly participants, no significant seasonal variation was observed for any activity level. No large sex differences in the seasonality of activity levels were observed (Appendix 4, page 33).

Impact of meteorological factors on seasonality of activity levels

Among middle-aged adults, the seasonality of levels of light PA was explained by ambient temperature (seasonal variation change=-16.3%) and sunlight hours (-16.0%). The seasonality of levels of moderate-to-vigorous PA was explained by sunlight hours (-21.5%) and the seasonality of nighttime sleep duration was explained by ambient temperature (-49.4%). Among young-elderly participants, the seasonality of levels of light PA was explained by ambient temperature (-46.7%) and relative humidity (-17.7%), and the seasonality of levels of moderate to vigorous PA was explained by ambient temperature (-14.0%), minimum visibility (-12.7%), and relative humidity (-11.0%). The meteorological factors had a large significant association with PA levels among the old-elderly, but none explained the seasonality (Table 2 and Table 3, pages 23 and 25).

Seasonal variation in all-cause mortality risk and life expectancy as a function of the seasonality of activity levels

If the all-cause mortality risk depended entirely on the levels of moderate to vigorous PA and SB, it would increase by 1.09 (95%CI 0.99, 1.21) times in winter compared with summer among middle-aged participants, 1.11 (95%CI 1.01, 1.21) times in winter compared with summer among young-elderly participants, and 1.04 (95%CI 0.95, 1.15) times in autumn compared with

spring among old-elderly participants (Table 4, page 26). The estimates were similar when using the 95% CI of the RR (Appendix 5, page 34).

DISCUSSION

In this population-based cohort, middle-aged and young-elderly participants spent more time in light and moderate to vigorous PA in summer than in winter, but no seasonality of PA was observed among old-elderly adults. Also, no seasonality was observed for SB in any age group. Nighttime sleep duration was higher in winter than in summer among middle-aged participants. The seasonality of PA and nighttime sleep duration was mostly explained by ambient temperature and sunlight hours. The modeled all-cause mortality risk might increase in winter because of the accumulation of low levels of moderate to vigorous PA and high levels of SB.

The heterogeneous seasonal patterns according to activity levels and age group can be explained by several factors. First, the magnitude of the seasonal variation in PA decreased with age, which can be explained by the age-specific domain composition of PA (i.e. occupational, transportation, leisure, and household). Indeed, while up to 30% of daily PA among middle-aged adults corresponds to occupational PA,[59] this domain nearly disappears after retirement, around age 65 (i.e. the young-elderly).[59] Therefore, the summer increase in PA among middle-aged participants, and to a lesser extent among young-elderly participants, likely reflects an increase in leisure, household, and transportation PA, while levels of occupational PA remain constant. In contrast, because old-elderly adults have less structured daily PA (due to absence of occupational PA), they are less sensitive to the variation led by holidays and vacations. Second, the summer reduction in nighttime sleep duration among middle-aged adults suggests its seasonality is led by PA, which appears to replace sleep time in summer. Third, a small and non-statistically significant seasonality of SB was observed in our population (about 10 minutes/day), which is in contrast with O'Connell et al, who reported a winter increment of SB of about 30 minutes/day.[25] This difference could be explained by the large proportion of the waking time our population spent in SB (around 77%), and because we classified the non-sleep time in bed as SB time. Taken together, our findings suggest that middle-aged and young-elderly participants replaced their nighttime sleep with more light and moderate to vigorous PA, and that SB is much more ingrained in the daily routine of the population.

We found a discrete influence of meteorological factors on the seasonality of activity levels. Klenk et al [73] similarly found a strong association of objectively measured daily walking time with several meteorological factors, but not with season, among elderly German participants. The domain composition of activity levels could contribute to this finding. For example, while active transportation might be sensitive to meteorological factors, it represents a small proportion of the daily PA. In contrast, indoors occupational PA would be less sensitive to meteorological factors, but corresponds to a larger proportion of daily PA. Leisure PA could also be sensitive to meteorological conditions, [62] but people will remain sedentary if it is ingrained in their daily occupational routine,[74,75] irrespective of favorable weather. Therefore, although meteorological factors have a strong influence on daily activity levels, they may be less relevant than the domain composition of PA and SB to explain the seasonality in activity levels.

Previous evidence suggested that the seasonality of PA plays a role in the well-described seasonality of cardiovascular disease and mortality.[5,16,32] Our results suggest that the all-cause mortality risk will increase in winter among middle-aged and young-elderly adults due the accumulation of low levels of moderate to vigorous PA and high levels of SB. Our results need to be interpreted cautiously, because this approach does not take into account physical fitness, as a measure of functional reserve.[76] Also, we assumed a linear association of PA with all-cause mortality risk, although it is suggested that the association is steeper at lower than at higher PA levels.[71] Nevertheless, this analysis illustrates the potential seasonality of all-cause mortality risk as a function of the seasonal variation in activity levels. Consequently, these findings suggest that season and age should be taken into consideration when interventions are designed to improve activity levels both in clinical practice and in public health. For example, interventions can be designed to avoid people replacing active time with SB. Strategies may include promoting active transportation, by offering facilities to change wet clothes, fans and showers, encouraging people to wear lighter clothes during warm and humid days, and ensuring safe transportation during adverse climate conditions (e.g. snow and high wind speed). People can also be encouraged to exercise (e.g. yoga and strength training) and to undertake regular activities of daily living of light and moderate intensity, such as housework. These interventions could also contribute to interrupting long bouts of SB, since SB is also associated with several adverse health outcomes.[56-58,77]

There is an ongoing discussion regarding the potential of accelerometers and other wearable devices that measure activity levels within interventions aimed to promote PA and reduce SB. Based on our findings, wearable devices could provide feedback regarding the declining levels of PA in winter and could prompt people to interrupt long SB periods, even in real time. The high compliance with accelerometer use in our study showed the relative ease of evaluating activity in a middle-aged and elderly population, using a device with a minimal burden for the participant, as it is worn as a watch and participants did not have to remove it during the measurement week. This improves the precision of the measurements [78] and avoids the need for assumptions to be made about activity levels when the device is not being worn.[78,79] Nevertheless, there is controversy regarding the effectiveness of interventions based on wearable devices to change behavior,[80,81] partly because these changes appear not to be sustained in the longer term.[82] Additionally, it is yet to be examined whether there are differences in the effectiveness of interventions using standard feedback based on average individual data [80] and that of personalized feedback and targets. These issues are sensitive to an elderly population, for whom standard targets for moderate to high intensity PA may be less feasible than improving light PA [81] and reducing long SB periods; but also because barriers, either individual (e.g. lack of self-efficacy, frailty, or fear of falling) or environmental (e.g. meteorological conditions and built environment),[83] may hamper the effectiveness of such interventions. Therefore, improving PA and reducing SB through the use of wearable devices may be a promising strategy in clinical practice. Nevertheless, long-term clinical trials are required of interventions with user-friendly, precise, and low-cost devices,[80] with relevant, age-appropriate targets for PA and SB.

Strengths and limitations

The main strength of our study is the objective round-the-clock measurement of activity levels in middle-aged and elderly adults, allowing us to evaluate the seasonality of 24-hour age-specific activity levels. Moreover, we improved the accuracy of SB and nighttime sleep measurements, because our participants were instructed not to remove the accelerometer during the measurement week and because we calculated non-sleep time in bed, which seemed to contribute to overall sedentary time. Second, to our knowledge, we are the first to examine the seasonality of the all-cause mortality risk and life expectancy, under the assumption that it will depend solely on levels of moderate-to-vigorous PA and SB. In these analyses, we used RR estimates obtained from comprehensive systematic reviews with meta-analysis,[70,71] thereby enhancing the representativeness of our modeling. Third, all our participants were resident in a single area, the Ommoord district, which reduces the variation in activity levels that can be attributed to other determinants, such as the built environment.

Nevertheless, we acknowledge several limitations. First, we could not test which domains of PA and SB might explain seasonality. Furthermore, we had repeated sets of measurements of activity for only 48 participants and each participant contributed only one week of data in each wave. Given their uneven participation throughout the year, this might lead to under- or over-representation of certain traits at specific periods of the year. Therefore, some seasonal variation could be explained by residual confounding or selection bias. The lack of detailed information on the type of jobs participants engaged in and the lack of information on community-based seasonal activities (e.g. walking or cycling events) might also have contributed to residual confounding. Second, our all-cause mortality risk estimations are based on a modeled distribution of the all-cause mortality risk and were assumed to be determined only by the seasonality of activity levels. Moreover, although we adjusted all our estimates by several covariates, the generalizability of our findings is limited to middle-aged and elderly adults with rather high BMIs and a high prevalence of comorbidities. Third, we used the same cut-offs to define activity intensity in middle-aged, young-elderly and old-elderly adults, whereas it might be argued that a particular activity would be experienced as vigorous by old-elderly adults but as moderate activity by middle-aged adults. Consequently, we might have some misclassification of activity. Finally, not all physical activities are captured by the device, as it depends on acceleration of the wrist to detect movement. Therefore, we may have not captured activities performed mostly by the legs, such as cycling, which is a common mode of transport in the Netherlands.

In conclusion, middle-aged and young-elderly adults spent more time in light and moderate to vigorous PA in the summer than in the winter. In the summer, PA appears to replace nighttime sleep, especially among middle-aged adults. The small seasonal variation observed in SB may be explained by the large proportion of the day dedicated to SB, as this is a behavior strongly ingrained in the daily routine. Meteorological factors had a discrete impact on the seasonality of activity levels. However, on a daily basis, the meteorological factors had a strong association with PA and SB, especially among old-elderly individuals. The all-cause mortality risk would increase in winter due to the accumulation of low levels of PA and high levels of SB.

The use of wearable devices may contribute to the design of interventions to improve PA and reduce SB, which are relevant targets within clinical practice to improve health. These interventions should be designed to attend to specific needs according to season and age. Since

we observed the largest seasonality in levels of light PA, recommending the interruption of SB with light PA might be a good starting point for public health interventions.

Table 1. Characteristics of the Population at Visit-Date, The Rotterdam Study, the Netherlands, 2011-2016

Covariate	Middle-aged (50-64 years, n=394)			Young-elderly (65-74 years, n=449)			Old-elderly (≥75 years, n=323)			p-value
	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	
Age (years)	59.1	56.1	62.4	71.3	67.3	72.8	78.9	77.0	81.4	
Waist circumference (cm)	92.2	84.5	101.8	94.9	87.3	102.3	92.5	85.8	101.1	0.014
Body mass index (kg/m2)	26.7	24.2	29.4	26.7	24.7	28.9	26.1	24.0	28.5	0.026
Disability index[a]	0.1	0.0	0.3	0.1	0.0	0.3	0.3	0.1	0.4	<0.001
Depression	3.0	0.0	6.0	2.0	0.0	4.0	2.0	0.0	6.0	0.004
Activity levels (minutes/day)[b, c]										
Light PA	154.1	154.8	144.5	150.7	151.9	142.1	147.4	147.5	139.5	<0.001
Moderate-to-vigorous PA	99.6	100.0	90.8	87.7	88.0	80.0	76.4	76.3	69.3	<0.001
Sedentary while awake	806.3	808.7	783.7	818.6	815.4	798.2	825.4	826.1	809.0	<0.001
Nighttime sleep duration	370.4	369.1	354.4	374.0	373.6	364.7	381.3	381.0	372.2	<0.001
	n	Percentage		n	Percentage		n	Percentage		
Sex										
Men	203	51.5		256	57.0		189	58.5		0.127
Women	191	48.5		193	43.0		134	41.5		
Comorbidities										
Cancer	23	5.8		62	13.8		109	33.8		<0.001
Cardiovascular disease	13	3.3		59	13.1		53	16.4		<0.001
Diabetes	34	8.6		60	13.4		48	14.9		0.022
Chronic obstructive pulmonary disease	47	11.9		71	15.8		46	14.2		0.266
Medication intake [d]										
Antihypertensive	107	27.2		206	46.2		154	48.0		<0.001
Diabetic medication	24	6.1		29	6.5		27	8.4		0.442
Statin	72	18.3		138	30.9		96	29.9		<0.001
Smoking behavior										
Never	118	29.9		219	48.9		249	77.1		<0.001
Currently	68	17.2		48	10.7		16	5.0		
Previously	209	52.9		181	40.4		58	18.0		
Housing conditions										

Covariate	Middle-aged (50-64 years, n=394)			Young-elderly (65-74 years, n=449)			Old-elderly (≥75 years, n=323)			p-value
	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	
	n	Percentage		n	Percentage		n	Percentage		
Living independent	392	99.5		440	98.0		297	92.0		<0.001
Assisted living facilities	2	0.5		9	2.0		26	8.1		
Occupation										
Working	340	86.1		136	30.4		17	5.3		<0.001
Not working	55	13.9		312	69.6		306	94.7		
Alcohol intake										
<2.5 glass/day	285	72.3		337	75.1		272	84.2		
2.44.4 glass/day	90	22.8		92	20.5		44	13.6		<0.001
≥4.5 glass/day	19	4.8		20	4.5		7	2.2		
Contribution of days										
1-3 days	128	32.4		156	34.8		108	33.4		0.776
4-6 days	197	49.9		222	49.6		168	52.0		
7 days	70	17.7		70	15.6		47	14.6		
Seasons[c]										
Winter	480	22.1		651	25.5		805	43.6		<0.001
Spring	638	29.3		661	25.9		398	21.5		
Summer	594	27.3		459	18.0		114	6.2		
Autumn	465	21.4		781	30.6		531	28.7		
Day of the week[c]										
Weekend	755	34.7		865	33.9		626	33.9		0.817
Weekday	1,422	65.3		1687	66.1		1222	66.1		

PA: Physical activity [a]Measured with Stanford Health Assessment Questionnaire[64]. [b]Adjusted for cosinor terms, age, gender, body mass index, comorbidities (cancer, cardiovascular disease, diabetes, chronic obstructive pulmonary disease), depression, medication intake (antihypertensive, statins, antidiabetic), smoking behavior, housing status, disability index, occupation status, alcohol intake and day of the week (weekend day or weekday). [c]Sample sizes/total are days contributed per participant: 2,171 days (middle-aged), 2,541 days (young-elderly) and 1,836 days (old-elderly). [d]Medication intake information was not available for 1 middle-aged participant, 2 young-elderly and 2 old-elderly.

Table 2. Seasonality of Light and Moderate-to-Vigorous Physical Activity After Accounting for Meteorological Factors, The Rotterdam Study, the Netherlands, 2011-2016[a]

Meteorological factors	Middle–aged (50–64 years)				Young–elderly (65–74 years)				Old–elderly (≥75 years)			
	SV	% SV change	Co.	95% CI	SV	% SV change	Co.	95%CI	SV	% SV change	Co.	95% CI
(a) Light PA, minutes/day												
Model												
+ temperature (°C)	15.2[d]	−16.3			6.81	−46.7			4.2	121.5		
−9.8 – 6.6 (Ref)												
6.7 – 10.3			−2.5	−6.6, 1.7			−1.0	−4.8, 2.8			−4.5	−8.0, −1.0
10.4 – 14.0			0.6	−5.4, 6.6			3.7	−1.1, 8.6			−3.1	−7.7, 1.6
14.1 – 27			2.2	−5.2, 9.5			4.5	−1.3, 10.3			2.3	−4.2, 8.8
+ wind speed (SD m/s)[b]	16.8[c]	−7.1	−0.3	−2.0, 1.3	11.4[c]	−10.9	−1.0	−2.5, 0.5	8.0	287.2	−3.3	−5.3, −1.4
+ sunlight (SD h)[b]	15.2[c]	−16.0	1.8	0.0, 3.6	11.2[d]	−12.1	1.0	−0.5, 2.6	10.0	385.5	4.2	2.2, 6.3
+ precipitation (SD mm)[b]	17.3[c]	−4.7	−0.9	−2.6, 0.8	12.1[c]	−5.4	−1.7	−3.2, −0.2	5.3	157.1	−3.4	−5.1, −1.6
+ relative humidity (SD%)[b]	16.5[c]	−9.0	−1.1	−2.9, 0.8	10.5	−17.7	−2.5	−4.1, −0.8	6.3	208.4	−2.4	−4.5, −0.2
+ minimum visibility (km)	18.8[c]	3.9			10.9[d]	−15.1			2.2	5.2		
<1.8 (Ref)												
1.8–3.9			0.3	−3.8, 4.3			−3.7	−7.1, −0.2			−1.1	−4.3, 2.2
3.9–7.0			−1.2	−5.3, 2.8			1.4	−2.1, 4.8			−0.7	−4.2, 2.9
7.0–12.0			−1.2	−5.2, 2.8			3.2	−0.2, 6.6			0.0	−3.4, 3.4
≥12.0			−1.4	−5.7, 2.8			4.4	0.6, 8.2			0.8	−3.3, 4.8
(b) Moderate–to–vigorous PA, minutes/day												
Model												
+ temperature (°C)	14.2[c]	−4.1			8.5[d]	−14.0			4.4	13.2		
−9.8 – 6.6 (Ref)												
6.7 – 10.3			−2.7	−6.4, 0.9			−1.9	−5.0, 1.1			−3.6	−6.1, −1.1
10.4 – 14.0			0.8	−4.5, 6.1			−0.3	−4.2, 3.6			−2.5	−5.8, 0.9
14.1 – 27			−0.3	−6.8, 6.2			0.2	−4.4, 4.9			0.4	−4.3, 5.1
+ wind speed (SD m/s)[b]	13.5[c]	−8.9	−1.3	−2.7, 0.1	9.8[c]	−0.9	−0.9	−2.1, 0.3	8.3	113.8	−2.8	−4.3, −1.4
+ sunlight (SD h)[b]	11.6[c]	−21.5	2.2	0.6, 3.8	9.3[d]	−6.1	1.3	0.0, 2.5	9.1	132.2	3.3	1.8, 4.8
+ precipitation (SD mm)[b]	14.6[c]	−1.4	−1.9	−3.4, −0.4	10.c[d]	4.6	−2.3	−3.4, −1.1	6.3	60.6	−3.2	−4.5, −1.9
+ relative humidity (SD%)[b]	12.8[c]	−13.1	−18.5	−34.5, −2.4	8.8	−11.0	−22.7	−36.0, −9.4	6.2	59.4	−15.2	−31.3, 0.9
+ minimum visibility (km)	14.8[c]	0.5			8.6[d]	−12.7			4.3	9.1		
<1.8 (Ref)												
1.8–3.9			−1.7	−5.3, 1.8			−1.9	−4.7, 0.9			−1.9	−4.3, 0.4
3.9–7.0			−1.6	−5.1, 2.0			2.2	−0.5, 5.0			−1.7	−4.2, 0.9
7.0–12.0			−0.2	−3.7, 3.3			2.2	−0.5, 4.9			0.2	−2.2, 2.7
≥12.0			−0.4	−4.1, 3.3			4.2	1.2, 7.3			1.4	−1.5, 4.3

CI = Confidence interval. SD = Standard deviation. SV = Seasonal variation. ** At least one significant cosinor term at 0.025 confidence level. * At least one significant cosinor term at 0.05 confidence level. [a]All estimates are adjusted for cosinor terms, sex, age, body mass index, prevalent comorbidities (cancer, cardiovascular disease, diabetes, and chronic obstructive pulmonary disease), smoking behavior, housing status, occupation, alcohol intake, disability index and day of the week. [b] Increment in activity levels (minutes/day) per increment of one standard deviation of meteorological factor: wind speed 2.17 m/s, wind chill: 2.06 m/s, sunlight hours: 3.97, precipitation: 4.6mm, relative humidity: 8.5%. Bold coefficients are statistically significant at 95% confidence level.

Table 3. Seasonality of Sedentary Behavior and Nighttime Sleep Duration After Accounting for Meteorological Factors, The Rotterdam Study, the Netherlands, 2011-2016[a]

Meteorological factors	Middle–aged (50–64 years)				Young–elderly (65–74 years)				Old–elderly (≥75 years)			
	SV	% SV change	Co.	95% CI	SV	% SV change	Co.	95%CI	SV	% SV change	Co.	95% CI
(a) Sedentary behavior, minutes/day												
Model												
+ temperature (°C)	15.9	33.9			17.3	24.8			7.0	9.2		
−9.8 – 6.6 (Ref)												
6.7 – 10.3			10.4	−0.5, 21.4			6.2	−4.2, 16.6			8.2	−2.5, 19.0
10.4 – 14.0			10.4	−5.4, 26.2			2.7	−10.5, 15.8			6.2	−8.1, 20.6
14.1 – 27			13.3	−5.9, 32.6			8.2	−7.7, 24.1			0.3	−19.5, 20.1
+ wind speed (SD m/s)[b]	13.3	12.0	0.5	−3.6, 4.7	15.9	14.8	3.3	−0.8, 7.4	23.8	269.6	10.2	4.3, 16.2
+ sunlight (SD h)[b]	17.4	46.2	−3.4	−8.0, 1.3	18.0	29.9	−0.7	−4.9, 3.5	18.7	190.2	−7.1	−13.6, −0.6
+ precipitation (SD mm)[b]	13.6	14.2	1.4	−3.0, 5.8	18.3	32.0	3.9	0.0, 7.9	20.3	216.4	5.4	−0.1, 11.0
+ relative humidity (SD%)[b]	14.1	18.8	6.6	−40.3, 53.6	17.1	23.2	17.0	−28.6, 62.5	20.9	225.5	1.0	−67.4, 69.5
+ minimum visibility (km)	11.3	−4.9			11.4	−17.6			7.9	23.1		
<1.8 (Ref)												
1.8–3.9			3.6	−7.3, 14.4			6.2	−3.5, 15.8			7.5	−2.7, 17.6
3.9–7.0			10.4	−0.4, 21.2			−3.5	−13.2, 6.1			8.3	−2.8, 19.3
7.0–12.0			9.1	−1.6, 19.7			−3.1	−12.6, 6.3			1.6	−9.0, 12.2
≥12.0			2.2	−9.1, 13.5			−4.7	−15.2, 5.9			5.9	−6.7, 18.4
(b) Nighttime sleep duration, minutes/day												
Model												
+ temperature (°C)	16.1	−49.4			2.2	−60.8			6.7	−27.7		
−9.8 – 6.6 (Ref)												
6.7 – 10.3			−4.8	−13.7, 4.2			−1.3	−9.7, 7.2			−0.4	−9.5, 8.7
10.4 – 14.0			−12.5	−25.4, 0.4			−3.4	−14.1, 7.3			2.1	−10.1, 14.3
14.1 – 27			−16.7	−32.3, −1.0			−8.6	−21.5, 4.4			−5.4	−22.1, 11.4
+ wind speed (SD m/s)[b]	33.5[c]	5.6	1.1	−2.4, 4.5	2.5	−56.2	−0.7	−4.1, 2.7	17.7	90.1	−3.9	−8.9, 1.1
+ sunlight (SD h)[b]	33.3[c]	5.0	−0.6	−4.4, 3.2	4.4	−23.5	−1.9	−5.3, 1.6	18.2	95.4	0.0	−5.4, 5.5
+ precipitation (SD mm)[b]	34.2[c]	7.6	0.2	−3.4, 3.8	2.5	−56.0	0.2	−3.0, 3.5	18.6	99.6	1.6	−3.0, 6.3
+ relative humidity (SD%)[b]	32.4[c]	2.1	1.8	−2.0, 5.7	5.5	−4.3	2.2	−1.5, 6.0	21.0	125.5	2.0	−3.7, 7.7
+ minimum visibility (km)	32.3[c]	1.8			5.3	−7.9			10.0	7.1		
<1.8 (Ref)												

Meteorological factors	Middle–aged (50–64 years)				Young–elderly (65–74 years)				Old–elderly (≥75 years)			
	SV	% SV change	Co.	95% CI	SV	% SV change	Co.	95%CI	SV	% SV change	Co.	95% CI
1.8–3.9			−1.2	−10.2, 7.8			−1.8	−9.6, 6.1			−3.8	−12.5, 4.8
3.9–7.0			−5.4	−14.3, 3.6			0.0	−7.8, 7.8			−3.9	−13.3, 5.5
7.0–12.0			−4.4	−13.2, 4.5			−1.4	−9.1, 6.3			−1.6	−10.6, 7.4
≥12.0			0.5	−8.8, 9.8			−3.6	−12.1, 5.0			−6.9	−17.6, 3.8

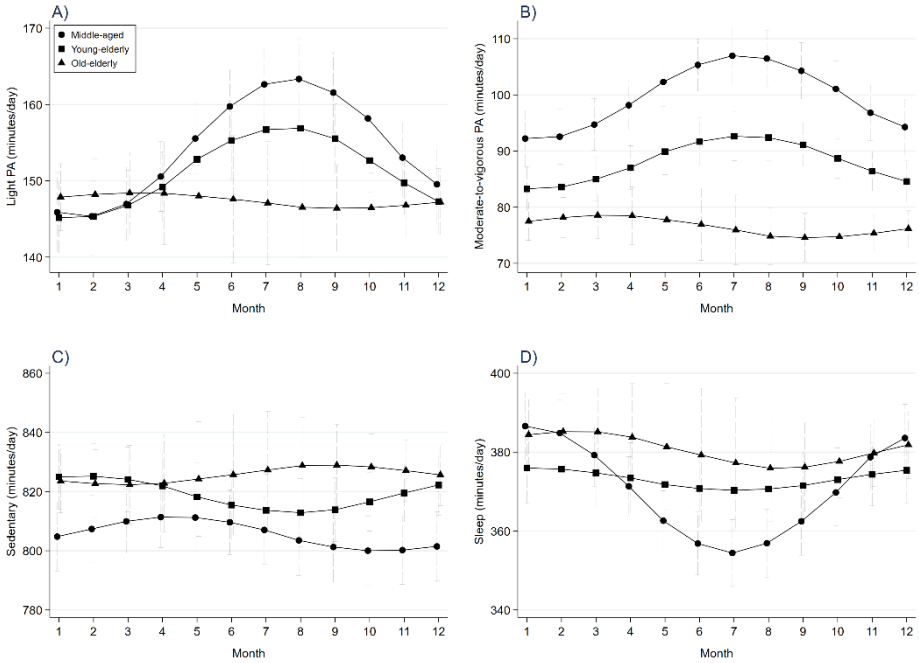
CI = Confidence interval. SD = Standard deviation. SV = Seasonal variation. ** At least one significant cosinor term at 0.025 confidence level. * At least one significant cosinor term at 0.05 confidence level. [a] All estimates are adjusted for cosinor terms, sex, age, body mass index, prevalent comorbidities (cancer, cardiovascular disease, diabetes, and chronic obstructive pulmonary disease), smoking behavior, housing status, occupation, alcohol intake, disability index and day of the week. [b] Increment in activity levels (minutes/day) per increment of one standard deviation of meteorological factor: wind speed 2.17 m/s, wind chill: 2.06 m/s, sunlight hours: 3.97, precipitation: 4.6mm, relative humidity: 8.5%. Bold coefficients are statistically significant at 95% confidence level.

Table 4. Variation of Life Expectancy Along With Seasonal Variation of Moderate-to-Vigorous PA and SB, The Rotterdam Study, the Netherlands, 2011-2016

Age group [a,b]	Peak-to-nadir all-cause mortality risk ratio [c]			Peak	Life expectancy at the peak of the variation (years)	Life expectancy at the nadir of the variation (years)	Peak-to-nadir difference of life expectancy [d] (months)
	Seasonal variation	95%	CI				
(a) Variation due to both moderate-to-vigorous PA and sedentary behavior combined							
Middle-aged	1.09	0.99,	1.21	23-Feb	16.6	17.2	-8.2
Young-elderly	1.11	1.01,	1.21	4-Feb	8.1	8.5	-4.5
Old-elderly	1.04	0.95,	1.15	13-Sep	2.6	2.7	-1.4
(b) Variation due to moderate-to-vigorous PA alone							
Middle-aged	1.08	1.04,	1.13	25-Jan	27.5	28.0	-6.3
Young-elderly	1.06	1.02,	1.09	27-Jan	16.1	16.4	-3.7
Old-elderly	1.02	0.99,	1.06	18-Sep	9.1	9.3	-2.4
(c) Variation due to sedentary behavior alone							
Middle-aged	1.04	0.97,	1.12	27-Apr	19.2	20.0	-9.0
Young-elderly	1.05	0.98,	1.12	13-Feb	18.7	19.3	-7.8
Old-elderly	1.02	0.95,	1.10	8-Sep	18.6	19.0	-5.3

CI: Confidence interval. PA: Physical activity. Bold estimates are statistically significant at 95% confidence level. [a] Age groups are: middle-aged (40-64 years), young-elderly (65-75 years) and old-elderly (≥76 years). [b] For middle-aged and young-elderly, estimates are calculated at the middle of the range: 57 years and 69.5 years, respectively. For old-elderly, estimates are obtained at 79 years for analysis with moderate-to-vigorous PA component, and at 80 years for analysis with light-to-moderate PA component. [c] Represents the risk ratio of all-cause mortality at the peak of the seasonal variation, compared with its nadir. [d] Life expectancy for Dutch population at each age categories was 24.4, 13.8 and 7.1 years, respectively, using mortality rates from .[84]

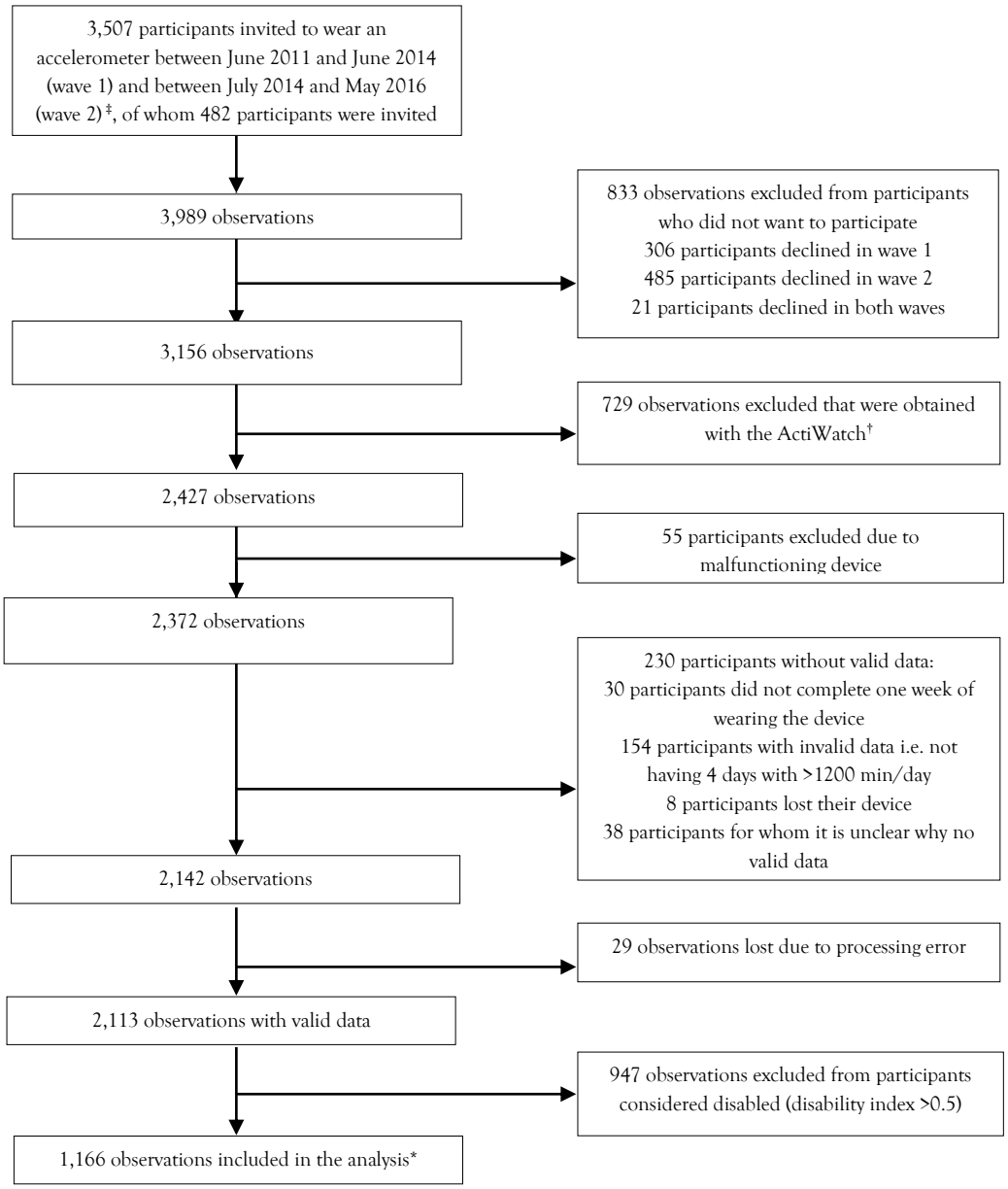
Figure 2. Seasonal pattern of activity levels according to age group



Monthly averages of A) light physical activity; B) moderate-to-vigorous physical activity; C) Sedentary behavior; D) Nighttime sleep behavior. PA: Physical activity. All estimates are adjusted for cosinor terms, sex, age, body mass index, prevalent comorbidities (cancer, cardiovascular disease, diabetes, and chronic obstructive pulmonary disease), smoking behavior, housing status, occupation, alcohol intake, disability index and day of the week. Middle-aged participants included those aged 50-64 years, young-elderly participants included those aged 65-74 years and old-elderly included adults aged ≥75 years.

SUPPLEMENTARY MATERIAL

Appendix 1. Flow chart of participant inclusion for the Rotterdam Study



† The ActiWatch could not be used to measure physical activity. *The 1,166 observations included 48 participants with two sets of observations. ‡In wave 1, participants from the fifth follow-up visit of RS-I (RS-I-5), the third visit of RS-II (RS-II-3), and the second visit of RS-III (RS-III-2) were invited. In wave 2, participants from the sixth follow-up visit of RS-I (RS-I-6) and the fourth visit of RS-II (RS-II-4) were invited.

Appendix 2. Covariates data collection and analysis procedures

Physical activity, sedentary behavior and nighttime sleep duration calculation based on GENEActiv devices

The GENEActiv was sampled at 50 Hz and acceleration was expressed relative to gravity (g units; 1 g =9.81 m/s^2).[85-87] To quantify acceleration related to the movement registered, we computed the high-pass filtered vector magnitude (HPFVM). Accelerometer data were processed in Python (2.6.6) using the open access PAMPRO software.[88] Data were extracted from the first wearing day up to seven days later. Participants were included in the analysis if they wore the watch for >1,200 min/day, for at least 4 days. Activity was categorized into sedentary (<48 mg), light (48 < 154 mg), moderate (154 <389 mg) and vigorous activity (>389 mg), based on a recent validation study.[89]

Covariates

Data on covariates were collected through home interviews or measured in the Rotterdam Study research center by trained research assistants.[68] Height and weight were measured and were used to calculate body mass index (BMI; calculated as weight divided by height squared). History of cardiovascular disease, diabetes mellitus, chronic obstructive pulmonary disease (COPD) and cancer at the visit date was obtained from medical records. Smoking behavior was categorized as never, current or former and requested via questionnaires. Housing condition was classified as living independent vs. living in assisted living facilities (i.e., service flat, nursing home). A disability index was calculated using the Stanford Health Assessment Questionnaire.[64] Depression was screened during the home interview using the Center for Epidemiology Studies Depression Scale (CES-D Scale).[90] Alcohol intake was obtained using the AUDIT tool.[91]

Appendix 3. Procedures for the modeling of the all-cause mortality risk and LE as function of moderate to vigorous PA and SB

To examine the potential health impact of the seasonality of moderate to vigorous PA and SB, we modeled the all-cause mortality risk using risk ratios (RR) and the estimates for the 95% confidence interval (95%CI) obtained from literature. For moderate to vigorous PA, we extracted the RR (95%CI) of 0.72 (0.65, 0.80) from a recently published systematic review with meta-analysis.[71] The RR expresses the all-cause mortality risk of those achieving an equivalent of 150 minutes of moderate-to-vigorous PA per week, compared to those not achieving this equivalent. We assumed the RR was equivalent to achieving 30 minute/day of moderate-to-vigorous PA,[92] to be able to use our daily data.

For SB, we used the RR (95%CI) of 1.24 (1.09, 1.41) reported in a recent review and meta-analysis.[70] It expresses the all-cause mortality risk of people with high SB levels compared to those with low SB. Because no specific cut-off was provided to define high SB, we used the median of the cut-offs indicated in the primary studies included in the review to define high versus low SB (8 hours/day).

Hypothetical all-cause mortality risk due to moderate-to-vigorous PA

For each participant, we modeled the all-cause mortality risk due to moderate-to-vigorous PA. To do so, we multiplied the time/day spent in moderate to vigorous PA with the natural log transformed risk ratio of the association of moderate-to-vigorous PA with all-cause mortality, estimated by Hupin et al [71] (RR=0.72; log transformed = -0.33). Because the RR represents the association between accumulating 150 minutes/week of moderate-to-vigorous PA vs. less than 150 minutes/week, we did two assumptions: 1) that the RR would be equivalent if the person accumulated 30minutes/day of moderate to vigorous PA, 2) that the association is linear, and the RR represents the reduction of the risk when accumulating 150 minutes per day, and could increase or reduce linearly and proportionally to the levels of moderate-to-vigorous PA of the participants. To fulfill these assumptions, we first centered the moderate-to-vigorous variable at 30 minutes/day, by subtracting 30 and then, dividing the difference by 60, to express the difference in hours. To model the lower and upper boundary of the 95% confidence interval (95%CI), we used the lower and upper boundary of the 95%CI of the risk ratio (0.65 and 0.80)

Hypothetical all-cause mortality risk due to SB

For each participant, we modeled the all-cause mortality risk due to SB. To do so, we repeated the previous process but using the SB variable and the natural log transformed risk ratio of the association between SB and all-cause mortality, as estimated by Biswas et al[70] (RR=1.24; log transformed = 0.22). We modeled the 95%CI by using the lower and upper boundary of the 95%CI of the risk obtained risk ratio (1.09 and 1.41). The RR calculated by Biswas et al represents the increment of the all-cause mortality risk at high levels of SB, compared to low. They meta-analyzed the RR at the levels of high SB as defined by every paper included in the meta-analysis, so the definition of high SB corresponds to a range of categories between ≥4hours/day to ≥11 hours/day. We defined high SB at the median of the cut-offs of the studies included in the meta-analysis, at 8 hours/day. Therefore, for the calculation of the daily individual mortality risk due to SB, the time was centered at 8 hours/day.

Hypothetical all-cause mortality risk due to PA and SB

The newly generated variables of the modeled all-cause mortality risk per participant and day recorded are summed up between them.

Example: The average middle-aged participant of the study had 99minutes/day of moderate-to-vigorous PA and 805minutes/day of SB. The centered time of moderate-to-vigorous PA would be calculated as (99-30)/60=1.15, and for SB would be (805-480)/60=5.4. Therefore, the all-cause mortality risk due to moderate-to-vigorous PA would be ln(0.72)*1.15=-0.38, and for SB would be ln(1.24)*5.4=1.16. Therefore, the overall all-cause mortality risk would be (-0.38)+1.16=0.78. This procedure was performed for each participant for each day contributed per participant. Also, the procedure was calculated using the lower and upper boundaries of the 95% confidence intervals of the RR for both moderate-to-vigorous PA and SB, for sensitivity analyses.

Seasonality of the hypothetical all-cause mortality risk

The seasonality of the modeled overall (PA and SB) all-cause mortality risk was calculated using the standard cosinor analysis. The seasonal variation (peak-to-nadir difference of the risk) was estimated in this analysis. The difference between

the modeled risk at the peak and at the nadir represents the seasonal variation of the modeled all-cause mortality risk. Because the difference is expressed in log units, the exponential of this difference represents the quotient between the risk at the peak and at the nadir of the variation, it means, the risk ratio for the modeled mortality risk. Therefore, a RR higher than one can be interpreted as the number of times the modeled all-cause mortality risk ratio increases at the peak compared to the nadir of the variation. Because the PA seasonal patterns were different for each age group, the seasonality of the modeled all-cause mortality risk was calculated stratified according to age group, the same way the seasonality of the activity levels was calculated.

Seasonality of the life expectancy as a consequence of the hypothetical all-cause mortality risk

To calculate how the variation of the modeled all-cause mortality risk would impact the life expectancy (LE), we created a hypothetical baseline scenario, using the age-specific mortality rates in the Dutch population in 2015, obtained from the Global Burden of Disease (GBD) data. Using these mortality rates, a standard life table was constructed.

Then, to calculate how would be the LE at the peak of the seasonality of the hypothetical mortality risk, we multiplied the mortality rate of each group by the exponential of the sum of the predicted all-cause mortality risk of each age group plus the amplitude of the seasonal variation [mortality rate (expressed in deaths/100,000) * exp(average adjusted all-cause mortality risk+amplitude of the seasonality of all-cause mortality risk)]. The average adjusted all-cause mortality risk was obtained with the Stata postestimation command margins after fitting the cosinor model of the all-cause mortality risk. The amplitude corresponds to the seasonal variation divided by two. The updated mortality rates at the peak of the seasonality of the all-cause mortality risk provided the LE at the peak of the seasonal pattern.

To calculate how would be the LE at the nadir of the seasonality of the hypothetical mortality risk, we multiplied the mortality rate of each group by the exponential of the difference between the predicted all-cause mortality risk of each age group (same as defined above) and the amplitude of the seasonal variation (same as defined above) [mortality rate (expressed in deaths/100,000)*exp(average adjusted all-cause mortality risk - amplitude of the seasonality of all-cause mortality risk)]. The updated mortality rates were used to calculate the LE at the nadir of the seasonal pattern.

Finally, the difference in months of the LE was calculated as the difference between the peak and the nadir of the seasonal variation of the all-cause mortality risk.

Appendix 4. Seasonal variation of activity levels (minutes/day) according to age group and sex

Activity level/ Strata	Middle-aged (50-64 years, n=394)						Young-elderly (65-74 years, n=447)						Old-elderly (≥75 years, n=321)					
	Seasonal variation	SE		Peak	Participants	Number of days	Seasonal variation	SE		Peak	Participants	Number of days	Seasonal variation	SE		Peak	Participants	Number of days
Light, minutes/day																		
All	18.1	4.0	**	9-Aug	394	2,171	12.8	3.9	**	4-Aug	447	2,541	1.9	5.6		17-Mar	318	1,818
Men	19.6	5.3	**	1-Aug	203	1,131	19.9	5.2	**	21-Aug	254	1,454	7.6	7.5		17-Dec	188	1,076
Women	18.6	6.0	**	16-Aug	191	1,040	9.5	6.0		5-Jul	193	1,087	11.4	8.3		17-Jun	130	742
Moderate-to-vigorous, minutes/day																		
All	14.8	3.8	**	26-Jul	394	2,171	9.9	3.3	**	27-Jul	447	2,541	3.9	4.3		19-Mar	318	1,818
Men	15.0	5.2	**	20-Jul	203	1,131	12.0	4.4	*	10-Aug	254	1,454	8.0	5.7		9-Jan	188	1,076
Women	16.3	5.7	*	7-Aug	191	1,040	9.0	5.0		10-Jul	193	1,087	12.5	6.6		8-Jun	130	742
Sedentary awake, minutes/day																		
All	11.9	9.1		27-Apr	394	2,171	13.9	9.3		13-Feb	447	2,539	6.4	13.7		8-Sep	318	1,818
Men	23.9	12.4		30-Jun	203	1,131	31.2	12.3	*	21-Feb	254	1,454	29.2	18.0		10-Aug	188	1,076
Women	21.7	13.3		5-Mar	191	1,040	10.6	14.4		6-Oct	193	1,085	38.8	20.8		17-Jan	130	742
Nighttime sleep duration, minutes/day																		
All	31.8	6.6	**	17-Jan	394	2,171	5.7	7.6		17-Jan	447	2,539	9.3	11.4		26-Feb	318	1,818
Men	51.7	8.3	**	17-Jan	203	1,131	2.7	10.4		13-May	254	1,454	22.4	14.7		11-Mar	188	1,076
Women	17.7	10.3		25-Jan	191	1,040	15.2	11.5		31-Jan	193	1,085	6.8	17.9		9-Sep	130	742
Time-in-bed, minutes/day																		
All	27.5	6.7	**	13-Jan	394	2,171	4.4	7.3		30-Nov	447	2,539	12.3	11.0		21-Apr	318	1,818
Men	45.2	8.4	**	18-Jan	203	1,131	2.6	9.2		19-Nov	254	1,454	24.0	14.0	*	16-Apr	188	1,076
Women	17.3	10.4		8-Jan	191	1,040	7.1	12.4		10-Feb	193	1,085	5.2	18.1		19-Jun	130	742
Sleep efficiency (%)																		
All	1.9	0.8	*	17-Jan	394	2,171	0.8	0.9		28-Feb	447	2,537	2.5	1.4		19-Dec	318	1,814
Men	3.4	1.1	**	11-Jan	203	1,131	1.2	1.4		31-May	254	1,454	3.5	2.0		7-Jan	188	1,072
Women	0.8	1.1		24-Mar	191	1,040	2.3	1.1		19-Jan	193	1,083	2.1	1.6		17-Oct	130	742

SE=Standard error. ** At least one significant cosinor term at 0.025 confidence level. * At least one significant cosinor term at 0.05 confidence level. Seasonal variation adjusted for cosinor terms, sex, age, body mass index, prevalent comorbidities (cancer, cardiovascular disease, diabetes, chronic obstructive pulmonary disease), smoking behavior, housing status, occupation, alcohol intake, disability index and day of the week.

Appendix 5. Potential seasonality of all-cause mortality risk as a function of moderate-to-vigorous PA and SB, sensitivity analyses

Age group[1,2]	Peak-to-nadir all-cause mortality risk ratio[3]			Peak	LE at the peak of the variation (years)	LE at the nadir of the variation (years)	Peak-to-nadir difference of LE[4] (months)
	Seasonal variation	95%	CI				
Sensitivity analysis 1: Lower 95%CI boundary of all-cause mortality risk estimates							
(a) Variation due to both moderate-to-vigorous PA and sedentary behavior combined							
Middle-aged	1.11	1.03	1.20	4-Feb	23.3	24.1	-8.6
Young-elderly	1.09	1.03	1.17	31-Jan	12.6	13.0	-4.9
Old-elderly	1.04	0.97	1.11	15-Sep	6.0	6.2	-2.6
(b) Variation due to moderate-to-vigorous PA alone							
Middle-aged	1.11	1.05	1.18	25-Jan	28.5	29.2	-8.2
Young-elderly	1.07	1.02	1.12	27-Jan	16.9	17.3	-5.0
Old-elderly	1.03	0.98	1.08	18-Sep	9.8	10.1	-3.4
(c) Variation due to sedentary behavior alone							
Middle-aged	1.02	0.99	1.05	27-Apr	39.6	40.2	-6.4
Young-elderly	1.02	0.99	1.05	13-Feb	39.4	39.8	-5.6
Old-elderly	1.01	0.98	1.04	8-Sep	39.3	39.7	-4.4
Sensitivity analysis 2: Upper 95%CI boundary of all-cause mortality risk estimates							
(a) Variation due to both moderate-to-vigorous PA and sedentary behavior combined							
Middle-aged	1.09	0.96	1.24	18-Mar	10.6	11.2	-7.3
Young-elderly	1.12	1.00	1.26	8-Feb	5.1	5.5	-4.3
Old-elderly	1.05	0.92	1.20	11-Sep	1.1	1.2	-0.7
(b) Variation due to moderate-to-vigorous PA alone							
Middle-aged	1.06	1.03	1.09	25-Jan	26.5	26.8	-4.3
Young-elderly	1.04	1.01	1.06	27-Jan	15.3	15.5	-2.4
Old-elderly	1.01	0.99	1.04	18-Sep	8.4	8.5	-1.5
(c) Variation due to sedentary behavior alone							
Middle-aged	1.07	0.96	1.19	27-Apr	9.6	10.2	-7.8
Young-elderly	1.08	0.98	1.20	13-Feb	8.9	9.5	-7.3
Old-elderly	1.04	0.92	1.17	8-Sep	8.8	9.1	-4.0

Bold estimates are statistically significant. [1] Age groups are: middle-aged (40-64 years), young-elderly (65-75 years) and old-elderly (>=76 years). [2]For middle-aged and young-elderly, estimates are calculated at the middle of the range: 57 years and 69.5 years, respectively. For old-elderly, estimates are obtained at 79 years for analysis with moderate-to-vigorous PA component, and at 80 years for analysis with light-to-moderate PA component. [3] Represents the risk ratio of all-cause mortality at the peak of the seasonal variation, compared with its nadir. [4] Life expectancy for Dutch population at each age categories was 24.4, 13.8 and 7.1 years, respectively, using mortality rates from. [84] CI: Confidence interval. LE: Life expectancy.

2.1.2 Seasonal variation of diet quality in a large middle-aged and elderly Dutch population-based cohort

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ABSTRACT

Introduction: Several studies have reported seasonal variation in intake of food groups and certain nutrients. However, whether this could lead to a seasonal pattern of diet quality has not been addressed. We aimed to describe the seasonality of diet quality, and to examine the contribution of the food groups included in the dietary guidelines to this seasonality.

Methods: Among 9,701 middle aged and elderly participants of the Rotterdam Study, a prospective population-based cohort, diet was assessed using food-frequency questionnaires (FFQ). Diet quality was measured as adherence to the Dutch dietary guidelines and expressed in a diet quality score ranging from 0-14 points. The seasonality of diet quality and of the food group intake was examined using cosinor linear mixed models. Models were adjusted for sex, age, cohort, energy intake, physical activity, body mass index, comorbidities, and education.

Results: Diet quality had a seasonal pattern with a winter-peak (seasonal variation=0.10 points, December-peak) especially among participants who were men, obese and of high socio-economic level. This pattern was mostly explained by the seasonal variation in the intake of legumes (seasonal variation=3.52 grams/day, December-peak), nuts (seasonal variation=0.78 grams/day, January-peak), sugar-containing beverages (seasonal variation=12.96 milliliters/day, June-peak), and dairy (seasonal variation=17.52 grams/day, June-peak).

Conclusion: Diet quality varies seasonally with a heterogeneous seasonality of food groups counteractively contributing to the seasonal pattern in diet quality. This seasonality should be considered in future research on dietary behavior. Also, season specific recommendations and policies are required to improve diet quality throughout the year.

INTRODUCTION

There are several approaches to study diet behavior, including the 'nutrient approach', 'foods or food group approach' and 'dietary pattern approach'.[93,94] The food group and nutrient approaches have contributed to identify specific diet components and nutrients that are relevant for health.[95,96] However, these approaches often do not account for the high correlation between nutrients and food groups.[94] Therefore, there is increasing interest in dietary pattern approaches to study dietary behavior,[93,94,97] for example, using diet quality scores.

Several factors determine the diet quality of individuals. Diet quality varies across age groups, sex, ethnicity,[98] and socio-economic status (SES).[99,100] Emerging evidence shows that diet is not constant throughout the year, as nutrient and food groups intake follow a seasonal pattern.[18-21] Nevertheless, less is known about how diet quality varies throughout the year and how food groups interact to convey such variation.

Understanding the seasonality of diet quality can contribute to unveil determinants underlying the variation between seasons of diet behavior as a lifestyle factor and the seasonality of diet-related morbidity and mortality [6,101]. It also contributes to the ongoing debate regarding the factors that could be efficiently targeted to improve diet quality and to identify the role of specific food groups on diet quality. Therefore, we aimed to describe the seasonality of diet quality defined as adherence to dietary guidelines, and to examine which food groups included in these guidelines explain the seasonal pattern of diet quality in the population of the Rotterdam Study.

METHODS

Study design and participants

This is a cross-sectional analysis based on the Rotterdam Study, a large prospective population-based cohort initiated in 1989 including adults living in the Ommoord district in Rotterdam, the Netherlands. The Rotterdam Study was initially designed to examine risk factors of cardiovascular, neurological, respiratory, psychiatric, locomotor, ophthalmological, endocrine, and dermatological diseases.[23] The study is composed by three sub-cohorts (RS-I: 7,893 participants aged 55 years or above; RS-II: 3,011 participants aged over 55 years of age or who moved into the district; and RS-III: 3,932 participants aged 45 years and over). Study visits are scheduled throughout the year at participant convenience. Follow-up visits are performed every four to five years.[23]

We selected cohort visits with available data of dietary intake using a semi-quantitative food frequency questionnaire (FFQ), i.e. first and fifth visits of first cohort (RS-I-1, RS-I-5), first and third visits of the second cohort (RS-II-1, RS-II-3), and first visit of the third cohort (RS-III-1). Each participant contributed with up to two visits (observations). Out of 13,008 observations with diet data available, we excluded those that reported unreliable data or reported daily energy intakes <500 kcal or ≥5,000 kcal (n=419). Consequently, our sample was 12,589 observations obtained from 9,701 participants (full flowchart provided in Appendix 6, page 49).

Diet quality assessment

For visits RS-I-1 and RS-II-1, a self-administrated FFQ with 170 food items was used; a trained dietician identified the amounts of food intake over the past year and estimated the daily average intakes. This FFQ was previously validated against four 24h urinary excretion samples and fifteen 24h dietary records, which showed adequate ability to rank participants' food group and nutrient intake.[102] For visits RS-I-5, RS-II-3 and RS-III-1, an extended self-administrated FFQ based on 389 food items about the frequency and amount of consumed food items in days, weeks, and months according to the previous month, and was filled out at home. This FFQ was previously validated against a 9-day dietary record and a 4-week dietary history among two Dutch populations.[103,104] To estimate portion sizes in grams, standardized household measures were applied.[105] For calculation of the nutritional data, the Dutch Food Composition Table (NEVO) was used.[106]

Based on the FFQ, adherence to the Dutch dietary guidelines was calculated and expressed in a score.[107,108] This a priori dietary index is based on the Dutch dietary guidelines 2015 for an optimal healthy diet,[108,109] consisting of fifteen components: vegetables and fruit, whole grain products, legumes, nuts, dairy, fish, tea, coffee, unsaturated fats and oils ratio, whole grain ratio, red and processed meat, sugar-containing beverages, alcohol, salt, and supplement use.[109] For the purpose of this study, we omitted coffee and supplements because no complete information was available.[108] Adherence for each food group was predefined at specific cut-off values (Table 5, page 34); adherence per food group was scored as 1 and non-adherence as 0. Thus, total diet quality ranged from zero to fourteen points, with a higher score representing a higher adherence, i.e. a better diet quality.

Covariates assessment

Data collection included a standardized home interview and two visits to the research center for clinical examination and blood sampling. Energy intake was estimated from FFQ responses. Weight and height were measured with participants standing straight without wearing shoes or heavy clothes. Weight was measured in kilograms using an electronic floor scale and height was measured in centimeters with a wall-mounted stadiometer. BMI was calculated dividing weight by height squared (kg/m^2), and was stratified into normal weight (18.5 – 25 kg/m^2) and overweight/obesity (>25 kg/m^2). Participants' level of education, monthly household income, living status and smoking behavior was obtained by trained interviewers. Level of education was expressed in primary (primary education), low-intermediate (lower/intermediate general education or lower vocational education), intermediate-high (intermediate vocational education or higher general education) or high (higher vocational education or university). Monthly household income was classified as <€1,500 or ≥ €1,500. Education and income information were used to calculate SES; low SES was defined as low primary/low education level or income below <€1500, high SES was defined as intermediate/high education and income ≥€1,500[100,110,111] Living status was expressed as 'living alone' or 'living with partner, relatives, or others'. Smoking status was expressed as 'never smoked', 'ever smoked', or 'current smoker'. Prevalent comorbidities was determined by a combination of blood examinations, continuous digital linkage of medical records and by information of medical specialists,[112-114] it was operationalized as "yes" if at least one of the following was present: myocardial infarction (MI), stroke, type 2 diabetes mellitus (T2DM), and cancer, and "no" otherwise. Physical activity at RS-I-

3 (as a proxy for RS-I-1) and RS-II-1 was assessed using a validated version of the Zutphen Physical Activity Questionnaire,[115] and was expressed in MET-hours/week.[116] At RS-I-5 and RS-II-3, physical activity was assessed using the LASA Physical Activity Questionnaire (LAPAQ) and expressed in MET-hours/week.[117] We accounted for heterogeneity between the questionnaires by estimating a cohort and follow-up visit specific *z*-score of the MET-hours/week.

Statistical analyses

Characteristics of the participants at study visit are described per season using descriptive statistics. Absolute values and percentages were used for categorical variables and medians and interquartile ranges (IQRs) for continuous variables; differences per season were tested with Chi-Square test and Kruskal-Wallis Test, respectively. Seasons were defined according to the light season definition, centered at the equinoxes (winter: November 6 to February 4; spring: February 5 to May 6; summer: May 7 to August 5; and fall: August 6 to November 5).[32]

To account for potential bias associated with missing data, we imputed missing values of covariates using multiple imputation (n=5 imputations) by chained equations.[118] Further details of imputation procedures are provided in Appendix 6 (page 49).

We examined the seasonality of diet quality and daily intake (grams, milliliters or ratio per day) of each food group using cosinor linear mixed models.[18] Date of study visit was included in the model transformed into its cosinor terms (i.e. sine and cosine) [101,119] with an assumed annual seasonality.[19] The model was further adjusted for age, sex, cohort, and kilocalories/day (Model 1). The coefficients of the cosinor terms were used to calculate the amplitude, seasonal variation, and the date with highest (peak) or lowest (nadir) diet quality score.[119] The amplitude is the distance from the annual average of diet quality to the peak or the nadir. The seasonality was presented as the seasonal variation, which is the maximal difference between the peak and nadir, i.e. 2*amplitude. Detailed descriptions to estimate the amplitude, seasonal variation, peak, and nadir are provided elsewhere.[101,119] The variance of the seasonal variation was estimated using the delta method.[120]

Model 2 was fitted to examine the seasonality of the diet quality after taking into account the non-random attendance of the participants to the study center throughout the year. The potential covariates were selected on the basis of literature,[99,121-123] of the differences of the population at specific periods of the year, and the percentage of change in the amplitude. The final set of covariates included physical activity, BMI, smoking, prevalent comorbidities, living status, income and education (Model 2).

Subsequently, we examined the seasonality of each food group included in the Dutch dietary guidelines. Model 1 and Model 2 were fitted for each of the fourteen food groups, using as outcome the continuous daily intake of each food group. The seasonality of total energy intake was also examined. To provide consistency and comparability, Models 1 and 2 included the same covariates as for the diet quality score. To examine what food groups contributed the most to the seasonality of diet quality, we re-calculated the seasonal variation of the diet quality score after excluding one food group at a time from the total score.

Finally, we performed several subgroup analyses to test effect modification. We performed stratified analyses for age,[124,125] sex,[126] BMI,[127] SES,[100,110] and living status.[128,129] As two

different types of FFQs were used to measure dietary intake, we also performed a stratified analysis to assess differences in seasonality of diet quality according to FFQ. Finally, to better characterize the population according to diet quality score, we compared participants with low diet quality (below one standard deviation of adjusted average diet quality score), high diet quality (above one standard deviation), and intermediate diet quality (in between low and high diet quality). For all analysis, we used Stata version 14.1 SE (StataCorp LP, College Station, Texas).[72]

RESULTS

Characteristics of the study population

Overall, the study population comprised more women than men (58% vs 42%) and the median age was approximately 66 years (IQR: 59 – 74), most of the participants had a lower/intermediate education (68.9%) and median BMI was 26.5 kg/m^2 (IQR: 24.3 – 29.1). Participants attending in autumn were about three years older than those who attended in summer, and a larger consumption of energy intake was observed in autumn than in summer. Participants with comorbidities were more likely to attend in winter than in summer (Table 6, page 44).

Seasonality of diet quality and daily intake of food groups

Diet quality had a significant seasonality with peak in December (seasonal variation=0.10, 95%-CI: 0.01 to 0.18), indicating a higher adherence to guidelines in winter than in summer. Seasonal variation was observed for intake of legumes, nuts, tea, red and processed meat, salt and kilocalories, with a winter peak; and for sugar-containing beverages, dairy, and fish intake, with a summer peak (Figure 3, page 47). The largest seasonal variation was observed for legumes, with an intake of up to 3.5 grams/day higher in winter than in summer, which represented 39% relative to the average legumes intake (9.1 grams/day) (Table 7, page 45). No large seasonality was observed for intake of vegetables, fruits, whole grain products, whole grains ratio, unsaturated fats and oils ratio, or alcohol. Results were similar when using the non-imputed dataset (Appendix 7, page 50).

Diet quality seasonality was reduced by 80% after excluding legumes from score, by 40% after excluding fruit, and by 30% after excluding nuts. In contrast, diet quality seasonality increased by 30% and 20% after excluding dairy and vegetables from the score, respectively (Table 8, page 46).

Subgroup analyses

Diet quality and more food groups had a larger seasonal variation among men, participants with BMI >25 kg/m^2, those living with relatives/others, and participants with high SES, than among their respective counterparts. No large differences in seasonal pattern were observed according to age group or FFQ used (Appendix 8 to Appendix 13, pages 51 to 56).

Participants with a lower overall diet quality were more likely to be men, lower educated, current or ever smokers, were more often having comorbidities, and living with relatives. In addition, participants with a lower diet quality had a lower energy intake (Appendix 14, page 57).

DISCUSSION

In this Dutch population, diet quality had a seasonal pattern with a peak, i.e. better diet quality, in winter. This pattern was mostly explained by the seasonality of legumes, nuts, sugar-containing beverages, dairy, fruits, and vegetables intake. A larger seasonality in more food groups and a lower diet quality was observed among men, subjects with a higher BMI, higher SES, and those living with a partner or relatives, than among their respective counterparts.

Diet quality increased in winter, mostly due to the winter peak of legumes and nuts intake and to the summer peak (and winter nadir) of dairy and sugar-containing beverages intake. The winter peak of legumes intake has been previously reported,[20,130] and is likely explained by the preference among Dutch population to consume legume-based dishes during the winter, such as lentil- and split pea soup. We are not aware of comparable studies addressing the seasonality of nuts intake, although people could prefer them in colder months for its fat content. The summer peak of sugar-containing beverages intake has also been reported before [131,132], and is attributed to the preference for sweet refreshing beverages in summer. Probably, these are replaced in winter by warmer beverages, such as tea and coffee, as we and others [132] found. Finally, the summer peak of dairy intake is consistent with one study performed among Spanish men, but not among Finnish women.[20,132] In our population, the pattern could be attributed to the increment of ice creams intake in summer.

Interestingly, the seasonal pattern was also modified by vegetables and fruits intake, which did not show a significant seasonality. We hypothesize that the exclusion of vegetables from the score reveals the pattern of a lower diet quality, which is less stable throughout the year. Indeed, diet quality and vegetables intake among people who regularly eat vegetables may be less influenced by season because of diet consciousness. As for fruits intake, we hypothesize that those who do not eat fruits regularly are more likely to eat it along with other food groups with a strong seasonal pattern, e.g. legumes and nuts. The stable intake of vegetables and fruits throughout the year in our study opposes the seasonality observed in previous studies,[19,20,130,131] and could be attributed to the constant availability of affordable vegetables and fruits in the Netherlands.[133] However, because only 50% of our population met the guidelines for vegetables and fruits intake,[108] aiming to increase the intake of vegetables and fruits may contribute to enhance overall diet quality.

Overall, a larger seasonality was observed in those food groups for which less people followed the intake guideline recommendations (i.e. fish, tea, nuts and legumes). For these food groups, intake was below the recommendations in more than 60% of the participants.[108] This suggests that addressing the mechanisms underlying the large seasonal variation of these food groups could contribute to improve the adherence to guideline recommendations.

Seasonality of alcohol intake appears also influenced by age. In contrast with previous studies showing a summer peak of alcohol intake among younger population,[20] we did not find such variation in our study. Arguably, our middle-aged and elderly population would be less inclined to increase their alcohol intake during summer activities.

A larger seasonality in diet quality and in more food groups was observed among men and among participants with high BMI than in their counterparts. The sex-differences in the seasonality of food groups are in agreement with previous studies[18,20,131] and can be explained by a better diet consciousness among women.[108,134] A better diet consciousness could also explain the

more stable diet quality of participants with lower BMI. Interestingly, participants with higher SES and subjects living with a partner or relatives exhibited a larger seasonality of food groups' intake than their corresponding counterparts. However, this pattern appears to reflect that of men, as the proportion of men was higher among participants with higher SES and those living with a partner or relatives. The larger seasonality of food groups' consumption among participants with high SES also contradicted our working hypothesis about the role of the price of food products on the seasonality of diet,[135] which would lead to a larger seasonality in the lower SES-group. However, it is possible that those in the lower-SES group replace food items with other less expensive within the same food group, or that they purchase food items without prices varying seasonally. These hypotheses need to be tested in other populations with different distribution of SES.

Taken together, our findings suggest that policies aimed to improve diet quality need to address the seasonal factors leading to a lower intake of legumes, nuts, and tea in summer and of fish in winter. Because the seasonality of the food groups appears to have cultural and behavioral mechanisms underlying, stakeholders can collaborate with markets and food producers to make certain food groups more attractive when the intake is anticipated to decline. For example, legumes intake could be promoted during summer with legume-based salads or other palatable recipes containing legumes. Also, fish intake could be promoted to replace red and processed meat, which appeared strongly ingrained in our population diet. Indeed, less than 20% of the participants reported an intake of red and processed meat below 300g/w, and the intake had a small seasonality. In contrast, fish intake had a summer-peak that coincided with the period of lowest intake of red and processed meat, but also with the traditionally Dutch herring season. Therefore, the factors underlying the summer preference for fish could be accounted for to increase the intake in other seasons. Finally, the summer-peak of sugar-containing beverages intake can be reduced by aiming to replace it by other non-sugar-containing beverages during summer activities.

Several strengths of this study are worth mentioning. To our knowledge, we are the first to address the seasonality of diet quality and to examine the food groups that influence this pattern. In addition, we used validated FFQs to determine dietary intake [102,103]. Furthermore, our study uses data from a large population-based study and is representative for the general adult and elderly population; and we accounted for the non-randomness of the participation over the season by adjusting for several covariates. However, some limitations need to be acknowledged. First, we used two different FFQs to assess diet quality; one asks about dietary intake in the past year and the other requests for the intake of the last month. However, this had a small impact in our findings, as these remained similar in the stratified analysis according to FFQ. Nevertheless, the fact that the seasonality estimates remained similar in the stratified analysis suggests that people are more likely to report their current diet behavior than the actual average during the last year.[136] Therefore, researchers addressing the long-term diet behavior need to account for this limitation, especially in geographic areas with seasonal variation. Second, the use of the FFQ to measure dietary intake, instead of 24h dietary recalls or dietary records to avoid recalling bias could have led to an underestimation of the actual seasonality. Third, we were able to include up to two repeated measurements per participant, what reduced the within-subject variation of our

seasonality estimates. It would be valuable to conduct a similar study using dietary record methods with more measurements per person during different seasons to improve the understanding of the seasonal patterns.

In conclusion, diet quality has a significant seasonality, with specific food groups counteractively contributing to this pattern. The pattern was mostly explained by the seasonality in intake of legumes, sugar-containing beverages, tea, dairy and nuts. Men and those with highest BMI had the largest seasonality of diet quality and food groups' intake throughout the year. Season should be accounted for when measuring diet quality. Reducing the seasonality in the intake of the food groups with largest seasonality could contribute to improve the adherence to intake guidelines recommendations, and arguably, to improve the overall diet quality.

Table 5. Components of the Dutch dietary guidelines 2015 and corresponding cut-off scores

Ítem	Food groups	Guideline
1	Vegetables	≥200 g/d
2	Fruit	≥200 g/d
3	Whole grain products	≥90 g/d
4	Legumes	≥135 g/wk
5	Nuts	≥15 g/d
6	Dairy	≥350 g/d
7	Fish	≥100 g/wk
8	Tea	≥450 ml/d
9	Unsaturated fats and oils ratio	Replace fats ≥50% of total fats as healthy fats[1]
10	Whole grains ratio	Replace refined grains ≥50% of total grains as whole grains
11	Red and processed meat	<300 g/wk
12	Sugar-containing beverages	<150 ml/d
13	Alcohol	≤10 g/d
14	Salt	≤6 g/d

[1]Total fats: margarine, oils and butter. Healthy fats: soft margarine, oils

Table 6. Characteristics of the study population, stratified by season

Characteristics	Overall		Winter		Spring		Summer		Autumn		p-value*
	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	
Age (years)	66.3 (59.4 - 73.7)	12,589	66.3 (59.8 - 73.5)	3,239	66.5 (58.8 - 74.1)	3,951	67.3 (59.7 - 74.5)	2,868	64.7 (59.3 - 72.5)	2,531	<0.01
Physical activity[1] (z-score MET-hrs/week)	-0.22 (-0.77 – 0.58)	10,412	-0.19 (-0.75 – 0.55)	2,643	-0.26 (-0.78 – 0.48)	3,301	-0.23 (-0.78 – 0.56)	2,388	-0.11 (-0.77 – 0.78)	2,080	<0.01
BMI	26.5 (24.3 - 29.1)	12,397	26.3 (24.2 – 29.0)	3,204	26.6 (24.3 - 29.3)	3,882	26.4 (24.2 – 29.0)	2,820	26.4 (24.3 - 29.1)	2,491	0.04
Energy intake (kilocalories/day)	1996 (1653 - 2392)	12,589	2001 (1671 - 2396)	3,239	1984 (1654 - 2391)	3,951	1982 (1625 - 2371)	2,868	2021 (1657 - 2416)	2,531	0.06
Diet quality score	7 (5 - 8)	12,589	7 (6 - 8)	3,239	7 (5 - 8)	3,951	7 (5 - 8)	2,868	7 (6 - 8)	2,531	0.63
	n	%	n	%	n	%	n	%	n	%	
Sex											
Men	5,306	42,1	1,391	43.0	1,679	42.5	1,181	41.18	1,055	41.7	0.50
Women	7,283	57.9	1,848	57.0	2,272	57.5	1,687	58.82	1,476	58.3	
Education											
Primary	1,695	13.6	410	12.8	550	14.0	409	14.35	326	13.0	<0.01
Lower	5,189	41.6	1,370	42.6	1,553	39.7	1,196	41.96	1,070	42.8	
Intermediate	3,626	29.0	911	28.4	1,128	28.8	828	29.05	759	30.4	
Higher	1,968	15.8	521	16.2	685	17.5	417	14.63	345	13.8	
Smoking status											
Never	4,031	32.1	1,046	32.5	1,261	32.0	908	31.76	816	32.5	0.42
Ever	5,879	46.9	1,537	47.8	1,855	47.0	1,345	47.04	1,142	45.4	
Current	2,626	21.0	635	19.7	829	21.0	606	21.20	556	22.1	
Prevalent diseases[2]											
Yes	2,155	17.1	590	18.2	692	17.5	476	16.60	397	15.7	0.06
No	10,434	82.9	2,649	81.8	3,259	82.5	2,392	83.40	2,134	84.3	

BMI = Body Mass Index; MET = Metabolic Equivalent of Task. ** Some characteristics do not sum up, because the table is based on non-imputed data. * P-value of population differences between seasons estimated using Kruskal-Wallis test for continuous variables, and chi-square test for categorical variables. [1]Physical activity is based on non-imputed data, because (imputed) standardized values of physical activity were used for the analyses. [2] Prevalent diseases include stroke, myocardial infarction (MI), diabetes mellitus type 2 (T2DM), and cancer.

Table 7. Seasonality of the diet quality score and of each contributing food group

Outcome	Model	Seasonal variation **	95%-confidence interval	Mean daily score/ intake	Seasonal variation in percentages[1]	Peak	Nadir
Diet quality score (0-14)	Model 1	0.12	0.03 - 0.21*				
	Model 2	0.10	0.01 - 0.18*	6.72	1.49	19-Dec	19-Jun
Kilocalories/day	Model 1	45.93	17.92 - 73.95*				
	Model 2	46.03	18.27 - 73.80*	2067.43	2.23	29-Nov	30-May
Food groups							
Vegetables (g/d)	Model 1	4.67	-2.19 - 11.54				
	Model 2	4.81	-1.96 - 11.58	210.09	2.29	1-Sep	2-Mar
Fruits (g/d)	Model 1	6.57	-5.360 - 18.50				
	Model 2	3.18	-8.63 - 14.99	284.84	1.12	3-Dec	4-Jun
Wholegrain (g/d)	Model 1	2.95	-0.37 - 6.27				
	Model 2	2.95	-0.37 - 6.27	125.36	2.35	12-Feb	12-Aug
Legumes (g/d)	Model 1	3.52	2.62 - 4.42*				
	Model 2	3.51	2.61 - 4.41*	9.09	38.61	30-Dec	30-Jun
Nuts (g/d)	Model 1	0.82	0.20 - 1.45*				
	Model 2	0.78	0.16 - 1.41*	8.25	9.45	25-Jan	26-Jul
Dairy (g/d)	Model 1	16.95	5.03 - 28.87*				
	Model 2	17.52	5.60 - 29.44*	365.65	4.79	17-Jun	16-Dec
Fish (g/d)	Model 1	1.45	0.57 - 2.33*				
	Model 2	1.52	0.64 - 2.40*	14.82	10.26	1-Jun	30-Nov
Tea (mL/d)	Model 1	21.48	9.21 - 33.76*				
	Model 2	19.82	7.65 - 32.00*	288.42	6.87	9-Feb	9-Aug
Whole grains ratio	Model 1	0.59	-0.66 - 1.83				
	Model 2	0.50	-0.75 - 1.75	68.54	0.73	10-Oct	10-Apr
Unsaturated fats and oils ratio	Model 1	0.45	-1.00 - 1.91				
	Model 2	0.32	-1.16 - 1.79	52.41	0.61	19-Feb	19-Aug
Red and processed meat (g/d)	Model 1	2.11	-0.26 - 4.47				
	Model 2	2.43	0.11 - 4.75*	89.70	2.71	4-Nov	6-May
Sugar-containing beverages (mL/d)	Model 1	13.01	7.22 - 18.80*				
	Model 2	12.96	7.16 - 18.77*	75.52	17.16	1-Jun	1-Dec
Alcohol (g/d)	Model 1	0.41	-0.25 - 1.07				
	Model 2	0.34	-0.31 - 0.99	11.47	2.96	16-Jun	16-Dec
Salt (mg/d)	Model 1	84.87	18.37 - 151.38*				
	Model 2	80.70	14.50 - 146.90*	5658.87	1.43	5-Feb	5-Aug

Model1 includes cosinor terms, sex, age, cohort and energy intake

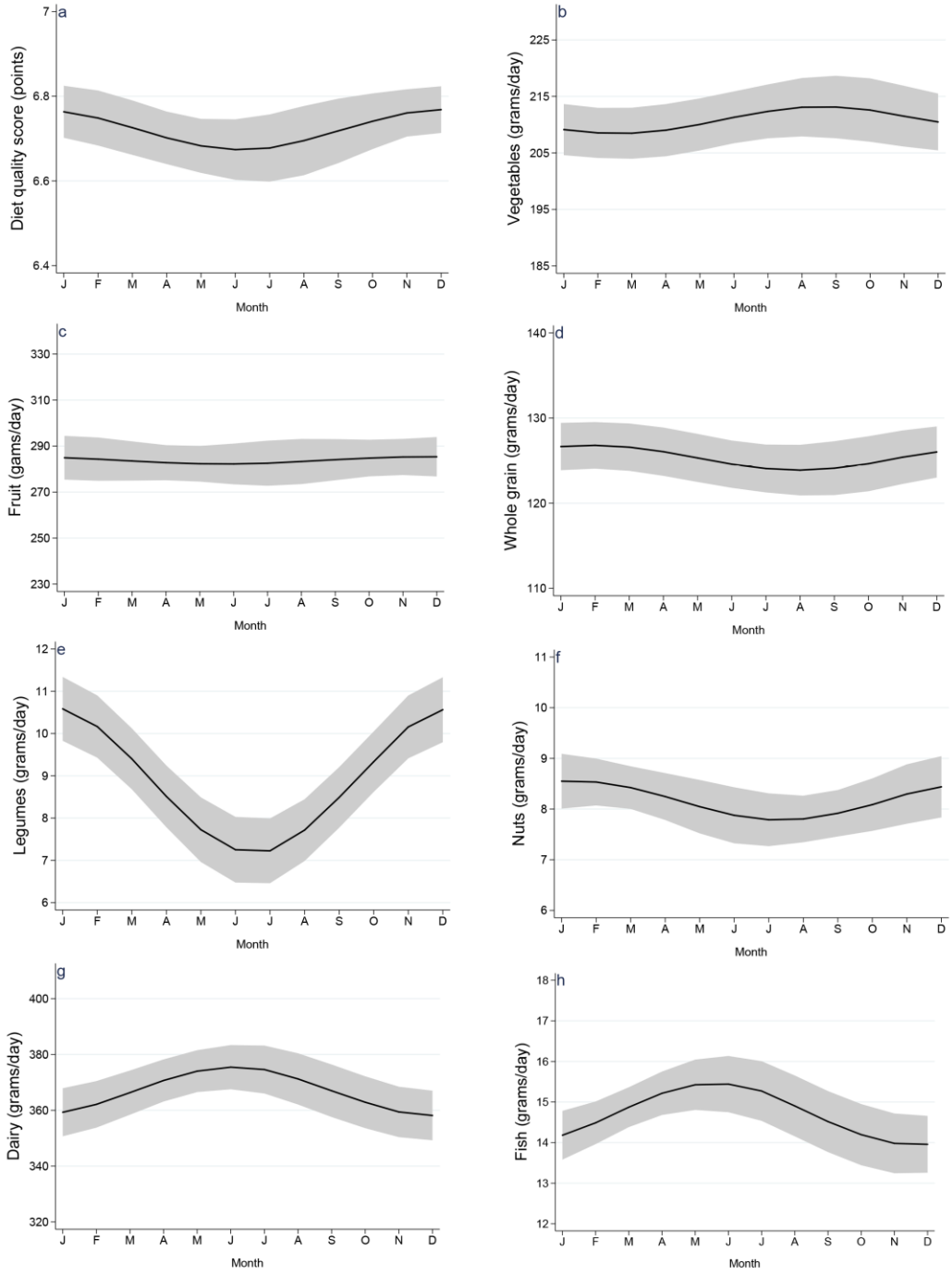
Model 2 additionally adjusted for physical activity, smoking behaviour, body mass index, prevalent diseases (stroke, myocardial infarction, diabetes mellitus type 2, and cancer), and education. ** Seasonal variation = maximum difference between the highest annual average (peak) and lowest annual average (nadir). * Statistically significant [1] Seasonal variation in percentages (seasonal variation/mean daily score or intake*100%)

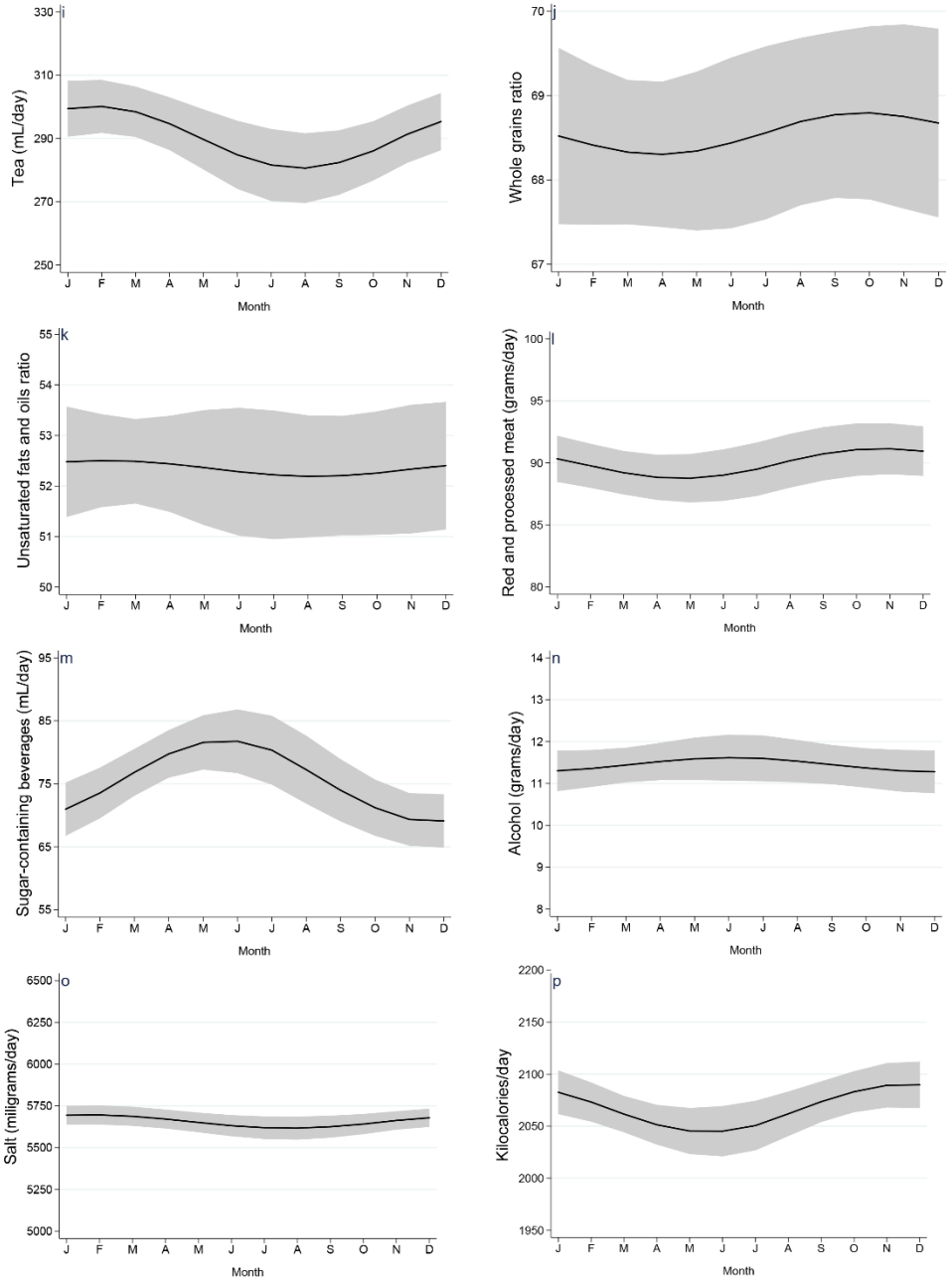
Table 8. Seasonal variation of diet quality score excluding one food group at a time

Outcome	Seasonal variation **	95%-confidence interval	%[1]
Diet quality score	0.10	0.01 - 0.18*	
Diet quality score excluding			
vegetables	0.12	0.04 – 0.20*	+20
fruit	0.06	-0.02 – 0.13	-40
wholegrain products	0.10	0.02 – 0.18*	0
legumes	0.02	-0.06 – 0.10	-80
nuts	0.07	-0.01 – 0.16	-30
dairy	0.13	0.05 – 0.21*	+30
fish	0.11	0.03 – 0.19*	+10
tea	0.08	-0.00 – 0.17	-20
wholegrains ratio	0.09	0.01 – 0.17*	-10
unsaturated fats and oils ratio	0.10	0.02 – 0.18*	0
red and processed meat	0.11	0.03 – 0.20*	+10
sugar-containing beverages	0.07	-0.01 – 0.15	-30
alcohol	0.09	0.01 – 0.17*	-10
salt	0.11	0.03 – 1.19*	+10

Estimates are adjusted for cosinor terms, sex, age, cohort, energy intake, physical activity, smoking behaviour, body mass index, prevalent diseases (stroke, myocardial infarction, diabetes mellitus type 2, and cancer), and education. ** Seasonal variation = maximum difference between the highest annual average (peak) and lowest annual average (nadir)
* Statistically significant. [1] Percentage reduction or increment of the seasonal variation by excluding food groups, compared to the total diet score (SV - 0.10/(0.10*100%)).

Figure 3 (a – p). Seasonal variation of diet quality and food groups

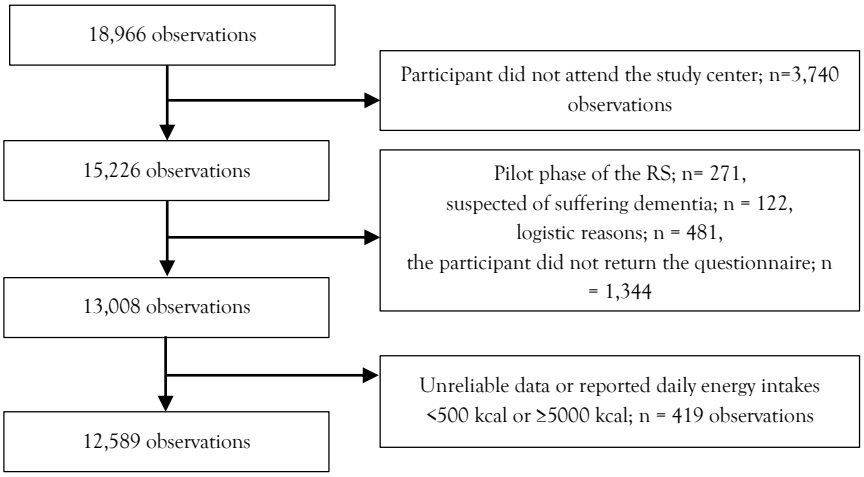




Graphical representation of the seasonal variation of the diet quality score and food groups intake. The gray area represents the 95% confidence interval around the pattern. Estimates are adjusted for cosinor terms, age, sex, cohort, (kilocalories), physical activity, smoking behavior, BMI, diseases and education.

SUPPLEMENTARY MATERIAL

Appendix 6. Flowchart of sample size selection



Imputation procedures: Sequential multiple imputation using chained equations was performed to impute missing values only of covariates. The predictors used to impute the covariates were age, sex, cohort, standardized MET hours/week, smoking behavior, BMI, education, diet quality score. We performed the imputation stratified per number of visits, in order to use the data from other visits for those participants with more than one visit. For ordered categorical variables (education) we used an ordered logit function. For categorical non-ordered variables (smoking behavior) we used multinomial logit function. For BMI and physical activity, we used linear functions. To ensure reproducibility, we used a random seed (2005). We created five imputed datasets. Covariates with missing values were: education: 111missing, smoking behavior: 53missing, BMI: 192missing, physical activity: 2177missing. Imputations were performed using the *mi impute* command of Stata software.

Appendix 7. Seasonal variation of diet quality score and of contributing food group, based on non-imputed dataset.

Outcome	Model	Seasonal variation **	95%-confidence interval	Peak	Nadir	Observations
Diet quality score (0-14)	Model 1	0.12	0.03 – 0.21*	14-Dec	14-Jun	12,589
	Model 2	0.15	0.06 – 0.25*	14-Dec	14-Jun	10,173
Kilocalories/day	Model 1	45.93	17.92 – 73.95*	20-Nov	22-May	12,589
	Model 2	43.58	13.08 – 74.07*	12-Dec	12-Jun	10,173
Food groups						
Vegetables (g/d)	Model 1	4.67	-2.19 – 11.54	13-Sep	14-Mar	12,589
	Model 2	6.46	-1.23 – 14.15	30-Sep	31-Mar	10,173
Fruits (g/d)	Model 1	6.57	-5.36 – 18.50	2-Dec	3-Jun	12,589
	Model 2	1.54	-12.08 – 15.16	27-Nov	28-May	10,173
Wholegrain (g/d)	Model 1	2.95	-0.37 – 6.27	7-Feb	8-Aug	12,589
	Model 2	3.51	-0.17 – 7.19	2-Mar	31-Aug	10,173
Legumes (g/d)	Model 1	3.52	2.62 – 4.42*	29-Dec	29-Jun	12,589
	Model 2	4.01	2.95 – 5.07*	26-Dec	26-Jun	10,173
Nuts (g/d)	Model 1	0.82	0.20 – 1.45*	25-Jan	25-Jul	12,589
	Model 2	1.11	0.40 – 1.81*	11-Jan	12-Jul	10,173
Dairy (g/d)	Model 1	16.95	5.03 – 28.87*	15-Jun	15-Dec	12,589
	Model 2	16.67	3.58 – 29.75*	12-Jun	11-Dec	10,173
Fish (g/d)	Model 1	1.45	0.57 – 2.33*	2-Jun	2-Dec	12,589
	Model 2	1.23	0.25 – 2.22*	10-Jun	9-Dec	10,173
Tea (mL/d)	Model 1	21.48	9.21 – 33.76*	6-Feb	6-Aug	12,589
	Model 2	19.65	6.05 – 33.25*	7-Feb	7-Aug	10,173
Whole grains ratio	Model 1	0.59	-0.66 – 1.83	13-Oct	13-Apr	12,589
	Model 2	0.40	-0.90 – 1.69	25-Jan	25-Jul	10,173
Unsaturated fats and oils ratio	Model 1	0.45	-1.00 – 1.91	19-Jan	20-Jul	12,589
	Model 2	0.81	-0.82 – 2.45	28-Nov	29-May	10,173
Red and processed meat (g/d)	Model 1	2.11	-0.26 – 4.47	31-Oct	1-May	12,589
	Model 2	1.93	-0.65 – 4.50	28-Oct	29-Apr	10,173
Sugar-containing beverages (mL/d)	Model 1	13.01	7.22 – 18.80	2-Jun	2-Dec	12,589
	Model 2	12.14	5.56 – 18.73	1-Jun	1-Dec	10,173
Alcohol (g/d)	Model 1	0.41	-0.25 – 1.07	4-Jun	4-Dec	12,589
	Model 2	0.71	-0.02 – 1.44	1-Jun	1-Dec	10,173
Salt (mg/d)	Model 1	84.87	18.37 – 151.28*	4-Feb	5-Aug	12,589
	Model 2	93.17	19.20 – 167.15*	15-Jan	15-Jul	10,173

Model1 includes cosinor terms, sex, age, cohort and energy intake

Model 2 additionally adjusted for physical activity, smoking behaviour, body mass index, prevalent diseases (stroke, myocardial infarction, diabetes mellitus type 2, and cancer), and education. ** Seasonal variation = maximum difference between the highest annual average (peak) and lowest annual average (nadir). * Statistically significant

Appendix 8. Stratified analyses: Seasonal variation of diet quality score and of each contributing food group according to sex

Outcome	Men n=5,306 observations					Women n=7,283 observations				
	Seasonal variation**	%[1]	95%-confidence interval	Peak	Nadir	Seasonal variation**	%[1]	95%-confidence interval	Peak	Nadir
Diet quality score (0-14)	0.11	1.73	-0.02 – 0.24	6-Jan	6-Jul	0.09	1.29	-0.02 – 0.21	4-Dec	4-Jun
Kilocalories/day	53.84	2.34	7.34 – 100.33*	13-Nov	15-May	41.82	2.20	8.17 – 75.47*	14-Dec	14-Jun
Food groups										
Vegetables (g/d)	11.39	5.55	1.68 – 21.09*	17-Sep	18-Mar	2.33	1.09	-6.90 – 11.57	16-Jun	16-Dec
Fruit (g/d)	6.95	2.76	-11.30 – 25.21	1-May	31-Oct	10.49	3.40	-5.46 – 26.45	11-Nov	12-May
Whole grain products (g/d)	4.54	3.22	-1.25 – 10.32	27-Dec	27-Jun	3.73	3.28	-0.23 – 7.68	24-Mar	23-Sep
Legumes (g/d)	4.12	39.47	2.59 – 5.65*	23-Dec	24-Jun	3.13	38.60	2.05 – 4.22*	4-Jan	5-Jul
Nuts (g/d)	0.62	6.11	-0.44 – 1.68	29-Jan	30-Jul	0.96	13.99	0.21 – 1.72*	27-Jan	28-Jul
Dairy (g/d)	28.96	8.00	9.72 – 48.20*	11-Jun	11-Dec	10.46	2.84	-4.59 – 25.50	27-Jun	27-Dec
Fish (g/d)	2.47	15.50	1.02 – 3.92*	22-May	20-Nov	0.90	6.43	-0.18 – 1.98	22-Jun	22-Dec
Tea (mL/d)	26.54	10.70	8.99 – 44.09*	18-Feb	19-Aug	15.98	5.03	-0.64 – 32.61	28-Jan	28-Jul
Unsaturated fats and oils ratio	0.18	0.26	-1.76 – 2.13	5-May	4-Nov	1.01	1.47	-0.59 – 2.61	6-Oct	6-Apr
Whole grains ratio	0.47	0.89	-1.73 – 2.67	17-Feb	17-Aug	0.25	0.48	-1.74 – 2.24	26-Feb	27-Aug
Red and processed meat (g/d)	0.82	0.80	-3.05 – 4.69	21-Nov	22-May	3.55	4.43	0.73 – 6.38*	28-Oct	28-Apr
Sugar-containing beverages (mL/d)	17.66	20.31	7.91 – 27.41*	14-Jun	14-Dec	9.97	14.84	2.89 – 17.06*	16-May	15-Nov
Alcohol (g/d)	0.59	3.51	-0.69 – 1.87	28-May	27-Nov	0.30	3.96	-0.34 – 0.94	28-Jul	27-Jan
Salt (mg/d)	112.21	1.79	-0.78 – 225.19	19-Jan	20-Jul	68.83	1.32	-11.25 – 148.92	28-Feb	29-Aug

Seasonal variation adjusted for cosinor terms, age, sex, cohort, physical activity, smoking behaviour, body mass index, comorbidities, education, and kilocalories/day (except when used as outcome). ** Seasonal variation expresses the maximum difference between the average highest intake/score (peak) and average lowest intake/score (nadir). * Seasonal variation is statistically significant. [1] (Seasonal variation/average intake or score)*100%

Appendix 9. Stratified analyses: Seasonal variation of diet quality score and of each contributing food group according to age group

Outcome	<65 years-old n=5,544 observations					≥65 years-old n=7,045 observations				
	Seasonal variation**	%[1]	95%-confidence interval	Peak	Nadir	Seasonal variation**	%[1]	95%-confidence interval	Peak	Nadir
Diet quality score (0-14)	0.13	1.94	-0.01 – 0.26	9-Dec	10-Jun	0.10	1.48	-0.01 – 0.22	2-Feb	3-Aug
Kilocalories/day	76.06	3.49	31.70 – 120.43*	18-Nov	19-May	33.01	1.67	-3.54 – 69.57	6-Dec	7-Jun
Food groups										
Vegetables (g/d)	7.93	3.42	-3.10 – 18.96	12-Sep	13-Mar	2.38	1.23	-6.47 – 11.23	7-Jun	6-Dec
Fruit (g/d)	8.57	3.18	-7.70 – 24.84	21-Jan	22-Jul	5.24	1.76	-11.09 – 21.56	5-Mar	4-Sep
Whole grain products (g/d)	5.19	3.80	-0.16 – 10.54	9-Dec	9-Jun	3.88	3.33	-0.50 – 8.26	18-Mar	17-Sep
Legumes (g/d)	3.63	35.62	2.24 – 5.02*	26-Nov	27-May	4.15	50.46	2.94 – 5.36*	23-Jan	23-Jul
Nuts (g/d)	0.65	6.59	-0.38 – 1.67	1-Feb	2-Aug	0.94	13.48	0.14 – 1.75*	10-Feb	10-Aug
Dairy (g/d)	28.26	7.59	8.74 – 47.79*	10-Jun	10-Dec	13.70	3.80	-1.66 – 29.06	31-Jul	30-Jan
Fish (g/d)	0.96	6.27	-0.37 – 2.28	25-May	24-Nov	1.94	13.45	0.70 – 3.18*	31-May	30-Nov
Tea (mL/d)	22.07	8.48	2.66 – 41.47*	9-Feb	10-Aug	12.78	4.12	-3.74 – 29.31	3-Jan	4-Jul
Unsaturated fats and oils ratio	1.65	2.41	-0.16 – 3.45	2-Dec	2-Jun	0.60	0.87	-1.08 – 2.27	31-Jul	31-Jan
Whole grains ratio	1.12	2.06	-0.97 – 3.21	21-Jan	22-Jul	2.05	4.04	0.00 – 4.10	31-Mar	30-Sep
Red and processed meat (g/d)	3.74	3.94	0.10 – 7.38*	21-Sep	23-Mar	3.52	4.11	0.44 – 6.60*	17-Nov	18-May
Sugar-containing beverages (mL/d)	13.39	15.23	4.16 – 22.63*	1-Jul	0-Jan	12.27	18.66	4.99 – 19.56*	24-May	23-Nov
Alcohol (g/d)	0.40	3.01	-0.84 – 1.65	17-Apr	17-Oct	0.40	3.99	-0.44 – 1.24	6-Jul	5-Jan
Salt (mg/d)	104.50	1.72	-1.72 – 210.73	24-Nov	26-May	99.97	1.88	12.87 – 187.06*	7-Mar	6-Sep

Seasonal variation adjusted for cosinor terms, age, sex, cohort, physical activity, smoking behaviour, body mass index, comorbidities, education, and kilocalories/day (except when used as outcome). ** Seasonal variation expresses the maximum difference between the average highest intake/score (peak) and average lowest intake/score (nadir). * Seasonal variation is statistically significant. [1] (Seasonal variation/average intake or score)*100%

Appendix 10. Stratified analyses: Seasonal variation of diet quality score and of each contributing food group according to age group

Outcome	BMI 18.5 – 25 kg/m² n=2,714 observations					BMI >25 kg/m² n=9,799 observations				
	Seasonal variation**	%[1]	95%-confidence interval	Peak	Nadir	Seasonal variation**	%[1]	95%-confidence interval	Peak	Nadir
Diet quality score (0-14)	0.02	0.29	-0.17 – 0.21	4-Jun	3-Dec	0.13	1.95	0.04 – 0.23*	22-Dec	22-Jun
Kilocalories/day	72.57	3.45	15.67 – 129.46*	7-Jan	7-Jul	44.87	2.18	12.76 – 76.98*	15-Nov	16-May
Food groups										
Vegetables (g/d)	10.17	4.90	-3.41 – 23.76	19-Jul	19-Jan	5.19	2.46	-2.71 – 13.09	27-Sep	28-Mar
Fruit (g/d)	3.05	1.09	-20.92 – 27.02	0-Jan	1-Jul	3.05	1.06	-10.41 – 16.51	10-Dec	10-Jun
Whole grain products (g/d)	1.45	1.06	-6.85 – 9.74	21-Apr	20-Oct	3.77	3.09	0.14 – 7.39*	10-Feb	10-Aug
Legumes (g/d)	2.76	32.66	0.75 – 4.77*	7-Jan	7-Jul	3.79	40.80	2.78 – 4.80*	29-Dec	29-Jun
Nuts (g/d)	0.95	10.90	-0.47 – 2.37	14-Apr	14-Oct	1.01	12.43	0.30 – 1.71*	7-Jan	8-Jul
Dairy (g/d)	21.79	5.84	-5.04 – 48.63	16-Jul	16-Jan	17.99	4.95	4.63 – 31.35*	10-Jun	10-Dec
Fish (g/d)	0.24	1.82	-1.34 – 1.83	1-Dec	1-Jun	2.04	13.35	1.00 – 3.07*	30-May	29-Nov
Tea (mL/d)	17.03	5.30	-11.59 – 45.64	1-Apr	30-Sep	25.02	8.97	11.45 – 38.59*	2-Feb	3-Aug
Unsaturated fats and oils ratio	1.07	1.54	-1.63 – 3.76	4-Nov	5-May	0.30	0.45	-1.11 – 1.71	20-Sep	21-Mar
Whole grains ratio	2.20	4.21	-1.08 – 5.48	25-May	23-Nov	0.77	1.47	-0.87 – 2.41	4-Jan	4-Jul
Red and processed meat (g/d)	2.93	3.58	-1.64 – 7.51	29-Sep	31-Mar	2.45	2.66	-0.23 – 5.12	10-Nov	11-May
Sugar-containing beverages (mL/d)	11.80	16.19	-1.15 – 24.74	1-Jun	1-Dec	13.29	17.44	6.79 – 19.80*	31-May	30-Nov
Alcohol (g/d)	0.45	4.33	-0.82 – 1.72	8-Jan	9-Jul	0.69	5.85	-0.07 – 1.45	23-Jun	22-Dec
Salt (mg/d)	53.71	0.96	-79.75 – 187.18	21-Jan	21-Jul	88.51	1.56	11.80 – 165.22*	11-Feb	11-Aug

Seasonal variation adjusted for cosinor terms, age, sex, cohort, physical activity, smoking behaviour, body mass index, comorbidities, education, and kilocalories/day (except when used as outcome). ** Seasonal variation expresses the maximum difference between the average highest intake/score (peak) and average lowest intake/score (nadir). * Seasonal variation is statistically significant. [1](Seasonal variation/average intake or score)*100%

Appendix 11. Stratified analyses: Seasonal variation of diet quality score and of each contributing food group according to socioeconomic status

	Lower SES n=8,937 observations					Higher SES n=3,652 observations				
Outcome	**Seasonal variation****	**%[1]**	**95%-confidence interval**	**Peak**	**Nadir**	**Seasonal variation****	**%[1]**	**95%-confidence interval**	**Peak**	**Nadir**
Diet quality score (0-14)	0.09	1.34	-0.01 – 0.20	3-Jan	4-Jul	0.13	1.94	-0.03 – 0.29	5-Dec	5-Jun
Kilocalories/day	42.62	2.12	10.51 – 74.73*	4-Dec	5-Jun	57.81	2.62	3.31 – 112.30*	20-Nov	22-May
Food groups										
Vegetables (g/d)	2.26	1.11	-5.29 – 9.81	13-Jul	12-Jan	12.69	5.62	-1.08 – 26.46	24-Sep	26-Mar
Fruit (g/d)	10.85	3.91	-2.24 – 23.95	16-Dec	16-Jun	15.47	5.11	-8.60 – 39.55	26-Jun	25-Dec
Whole grain products (g/d)	3.10	2.54	-0.89 – 7.09	5-Mar	4-Sep	3.90	2.92	-2.19 – 9.98	29-Dec	30-Jun
Legumes (g/d)	2.66	33.09	1.64 – 3.69*	5-Jan	6-Jul	5.77	49.46	3.95 – 7.59*	24-Dec	24-Jun
Nuts (g/d)	0.66	9.15	-0.05 – 1.37	21-Jan	22-Jul	1.05	9.74	-0.21 – 2.31	2-Feb	2-Aug
Dairy (g/d)	13.70	3.73	-0.81 – 28.21	2-Jun	2-Dec	30.23	8.36	9.05 – 51.41*	29-Jun	29-Dec
Fish (g/d)	1.17	8.65	0.17 – 2.17*	9-May	8-Nov	2.79	15.52	1.00 – 4.57*	26-Jun	26-Dec
Tea (mL/d)	20.15	6.66	5.31 – 34.98*	7-Mar	5-Sep	32.68	12.89	10.91 – 54.45*	1-Jan	2-Jul
Unsaturated fats and oils ratio	0.63	0.92	-0.84 – 2.10	28-Aug	27-Feb	1.13	1.63	-1.00 – 3.26	2-Dec	2-Jun
Whole grains ratio	0.76	1.47	-1.02 – 2.53	6-Jan	7-Jul	1.14	2.10	-1.45 – 3.73	23-May	22-Nov
Red and processed meat (g/d)	2.48	2.77	-0.26 – 5.22	25-Oct	26-Apr	2.21	2.46	-2.14 – 6.56	17-Nov	19-May
Sugar-containing beverages (mL/d)	12.69	18.24	5.82 – 19.56*	16-May	14-Nov	18.73	20.80	7.81 – 29.66*	7-Jul	7-Jan
Alcohol (g/d)	0.46	4.64	-0.27 – 1.20	18-Jun	17-Dec	0.24	1.57	-1.09 – 1.57	15-Jul	15-Jan
Salt (mg/d)	78.70	1.43	1.74 – 155.66*	13-Mar	12-Sep	171.33	2.86	36.45 – 306.20*	27-Dec	28-Jun

SES = Socioeconomic status. Seasonal variation adjusted for cosinor terms, age, sex, cohort, physical activity, smoking behaviour, body mass index, comorbidities, education, and kilocalories/day (except when used as outcome). ** Seasonal variation expresses the maximum difference between the average highest intake/score (peak) and average lowest intake/score (nadir). * Seasonal variation is statistically significant. [1] (Seasonal variation/average intake or score)*100%

Appendix 12. Stratified analyses: Seasonal variation of diet quality score and of each contributing food group according to living status

	Living alone n=3,361 observations					Living with partner/relatives/others n=9,228 observations				
Outcome	**Seasonal variation****	**%[1]**	**95%-confidence interval**	**Peak**	**Nadir**	**Seasonal variation****	**%[1]**	**95%-confidence interval**	**Peak**	**Nadir**
Diet quality score (0-14)	0.06	0.88	-0.11 – 0.23	27-Mar	26-Sep	0.16	2.40	0.06 – 0.26*	13-Dec	14-Jun
Kilocalories/day	15.54	0.80	-39.92 – 71.00	2-Apr	2-Oct	64.42	3.05	32.01 – 96.83*	24-Nov	26-May
Food groups										
Vegtables (g/d)	4.59	3.33	-9.49 – 18.68	15-Feb	16-Aug	8.15	3.79	0.51 – 15.80*	1-Sep	3-Mar
Fruit (g/d)	21.31	7.18	-2.54 – 45.16	8-Dec	8-Jun	3.35	1.19	-10.04 – 16.75	16-Jun	16-Dec
Whole grain products (g/d)	3.94	3.31	-2.88 – 10.76	3-May	2-Nov	4.26	3.34	0.45 – 8.06*	19-Jan	20-Jul
Legumes (g/d)	2.47	25.94	0.49 – 4.46*	27-Dec	27-Jun	4.04	45.22	3.06 – 5.02*	28-Dec	29-Jun
Nuts (g/d)	0.84	11.81	-0.42 – 2.11	1-Mar	31-Aug	0.88	10.16	0.16 – 1.61*	16-Jan	17-Jul
Dairy (g/d)	21.43	5.49	-4.51 – 47.37	10-Jul	9-Jan	14.47	4.06	1.17 – 27.77*	12-Jun	12-Dec
Fish (g/d)	0.47	3.08	-1.50 – 2.44	2-May	1-Nov	1.79	12.22	0.81 – 2.76*	1-Jun	1-Dec
Tea (mL/d)	3.03	1.02	-22.84 – 28.89	20-Sep	22-Mar	27.47	6.63	13.56 – 41.38*	10-Feb	10-Aug
Unsaturated fats and oils ratio	0.26	0.38	-2.23 – 2.75	17-Dec	18-Jun	0.62	0.91	-0.81 – 2.05	20-Oct	20-Apr
Whole grains ratio	3.08	6.31	-0.04 – 6.19	1-May	31-Oct	1.10	2.05	-0.60 – 2.79	3-Dec	3-Jun
Red and processed meat (g/d)	4.04	5.03	-0.24 – 8.31	30-Nov	31-May	1.98	2.13	-0.78 – 4.74	7-Oct	7-Apr
Sugar-containing beverages (mL/d)	12.63	17.93	1.43 – 23.83*	20-Apr	20-Oct	14.80	19.13	8.04 – 21.57*	12-Jun	11-Dec
Alcohol (g/d)	0.68	7.77	-0.45 – 1.82	14-Sep	15-Mar	0.54	4.33	-0.27 – 1.35	2-Jun	2-Dec
Salt (mg/d)	120.89	2.26	-8.21 – 249.99	24-Apr	23-Oct	111.36	1.93	33.74 – 188.97*	9-Jan	10-Jul

Seasonal variation adjusted for cosinor terms, age, sex, cohort, physical activity, smoking behaviour, body mass index, comorbidities, education, and kilocalories/day (except when used as outcome). ** Seasonal variation expresses the maximum difference between the average highest intake/score (peak) and average lowest intake/score (nadir). * Seasonal variation is statistically significant. [1] (Seasonal variation/average intake or score)*100%

Appendix 13. Stratified analyses: Seasonal variation of diet quality according to FFQ

Outcome	Seasonal variation*	95%-confidence interval	Peak	Nadir
FFQ 170 items, n= 8,572.observations				
Diet quality score (0-14)	0.10	-0.01 - 0.20	2-Jan	3-Jul
FFQ 389 items, n= 4,017 observations				
Diet quality score (0-14)	0.13	-0.02 - 0.29	17-Dec	18-Jun

FFQ = Food Frequency Questionnaire. * Seasonal variation expresses the maximum difference between the average highest intake/score (peak) and average lowest intake/score (nadir). Seasonal variation adjusted for cosinor terms, age, sex, cohort, physical activity, smoking behaviour, body mass index, comorbidities, education, and kilocalories/day.

Appendix 14. Characteristics of the study population stratified by participants with a lower, intermediate, and higher diet quality score

Covariate	Diet score below 1SD of average* (<6.145) n=2,071		Diet score between -1 SD and + 1 SD of average* n=8,549		Diet score above 1SD of average* (>7.302) n=1,969	
	Median (IQR)		Median (IQR)		Median (IQR)	
Age (years)	65.9 (59.7 - 71.9)		66.8 (59.8 - 73.9)		63.4 (57.3 - 75.12)	
Physical activity (MET-hours per week)	43.0 (21 - 70.5)		62.4 (30.3 - 95.1)		85.3 (47.4 – 128.0)	
BMI, kg/m^2	27.0 (24.7 - 29.6)		26.6 (24.4 - 29.3)		25.2 (23.2 - 27.6)	
Kilocalories/day	1942 (1589 – 2312)		1946 (1612 – 2336)		2264 (1934 – 2721)	
	n	%	n	%	n	%
Sex						
men	1,767	85.3	3,414	39.9	125	6.4
women	304	14.7	5,135	60.1	1,844	93.6
Education						
primary	276	13.4	1,227	14.5	192	9.8
lower	854	41.5	3,640	43.0	695	35.5
intermediate	748	36.4	2,363	27.9	515	26.4
higher	178	8.7	1,236	14.6	554	28.3
Smoking status						
never	84	4.0	2,671	31.4	1,276	65.2
ever	626	30.3	4,594	54.0	659	98.7
current	1,359	65.7	1,246	14.6	21	1.1
Comorbidities						
no	1,566	75.6	7,089	82.9	1,779	90.4
yes	505	24.4	1,460	17.1	190	9.6
Socioeconomic status						
lower	1,438	69.4	6,213	72.7	1,286	65.3
higher	633	30.6	2,336	27.3	683	34.7
Living status						
alone	389	18.8	2,285	26.7	687	34.9
with others	1,682	81.2	6,264	73.3	1,282	65.1

BMI = Body mass index. IQR = Interquartile range. MET = Metabolic Equivalent of Task. SD = Standard deviation.
*Predicted average of diet score after adjusted for covariates of Model 2.

2.2 Seasonality of health outcomes

2.2.1 Influence of lifestyle markers and meteorological factors on the seasonality of cardiovascular risk factors: The Rotterdam Study

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ABSTRACT

Introduction: The seasonality of multiple cardiovascular risk factors (CVRF), with peak in winter, has been widely described. Nevertheless, it remains unclear the mechanisms underlying this variation and if it differs according to age. We examined if lifestyle markers (body mass index (BMI) and physical activity) or meteorological factors (daily average of ambient and apparent temperature and total sunlight hours and precipitation) explained the seasonality of seven CVRF, according to age group [middle-aged (<65 years) vs elderly (≥65 years)]. CVRF were hemodynamic (systolic and diastolic blood pressure), metabolic (glucose, total-, high-density, and low-density lipoprotein cholesterol), and anthropometric (waist-to-hip ratio).

Methods: Seasonality estimates were obtained using cosinor analysis based on data from a prospective population-based Dutch cohort. We analyzed 20,723 observations from 10,405 participants (57% woman, mean age 68 years) obtained between 1997 and 2014. Meteorological factors were obtained from local records, BMI was measured, and physical activity was questionnaire-based. Additionally, we estimated the population attributable fraction (PAF) to lifestyle markers and meteorological factors for the prevalence of selected CVRF (i.e. high blood pressure, high glucose levels, high LDL-cholesterol and high/very high cardiovascular mortality risk according to SCORE charts).

Results: CVRF had peak values in winter and autumn in both age groups. Among middle-aged, meteorological factors explained the seasonality of systolic and diastolic blood pressure, total-, and HDL-cholesterol. Among elderly, meteorological factors explained the seasonality of all CVRF. Lifestyle markers explained a modest part of the seasonality of CVRF in both age groups. The PAF to ambient temperature for the prevalence of selected CVRF was higher in winter than in summer (up to 27.7 percentage points among middle-aged and up to 9.1 percentage points among elderly). In contrast, the PAF to lifestyle markers was constant across seasons. The main limitation of our analysis is that meteorological factors are local measures which may not reflect the exposure of participants.

Conclusions: Our findings suggest that ambient temperature explains most of the seasonality of the CVRF, especially among elderly. This provides a plausible mechanism for the winter increase of cardiovascular mortality and health risks associated to meteorological factors within climate change.

INTRODUCTION

Seasonality of cardiovascular risk factors (CVRF) has been widely described.[5,28-33] Compared to summer, a higher systolic (SBP), diastolic (DBP) blood pressure, and glucose levels and a more unfavorable lipid profile have been reported in winter.[5,28-33] Several factors can explain the seasonality of CVRF. For example, most CVRF are associated with lifestyle markers, such as physical activity and body mass index (BMI), which have been reported to have a seasonal pattern.[17,34] Also, some CVRF have been associated with meteorological factors, like ambient temperature, precipitation and sunlight hours;[7-15] thus, the prevailing adverse meteorological conditions in winter could explain the increase of cardiovascular risk in this season.

However, it remains unclear if meteorological factors, lifestyle markers, or both, explain the seasonality of the CVRF. Moreover, it has not been addressed whether the impact of lifestyle markers and meteorological factors in the seasonality of CVRF differs according to age. Age-related differences are expected as physical activity and dietary patterns change with age,[59,137] as well as thermoregulation response.[138] Understanding the mechanisms underlying the seasonality of CVRF is of relevance to identify interventions that are most likely to impact this phenomenon, also in preparation for the projected effects of climate change in global health.

In this study, we examined the role of lifestyle markers and meteorological factors on the seasonality of seven CVRF: (hemodynamic (SBP and DBP), metabolic (serum levels of total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and glucose), and anthropometric (waist-to-hip ratio (WHR)), stratified by age (middle-aged (<65 years) vs elderly (≥65 years)). We also calculated the population attributable fraction (PAF) to lifestyle markers and meteorological factors for the seasonal prevalence of cardiovascular risk.

METHODS

Study population and selection criteria

The Rotterdam Study is a population-based prospective cohort initiated in 1989 by inviting all elderly people living in the Ommoord district in Rotterdam, the Netherlands.[23] The study is composed by three cohorts (RS-I: 7,893 participants aged 55 years or above; RS-II: 3,011 participants aged over 55 years of age or who moved into the district; and RS-III: 3,932 participants aged 45-54 years). Follow-up visits are performed approximately every 3-4 years. For this study, we included the visits third to fifth of RS-I, first to third of RS-II and, first to second of RS-III; each visit corresponds to an observation. We excluded the first and second visit of RS-I for consistency in measurement procedures. From 24,954 observations (11,939 participants) we excluded 4,231 (1,534 participants) with missing data on CVRF of interest (Appendix 15, page 73).

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of cardiovascular risk factors

Data collection included a standardized home interview and two visits to the research center for clinical examination and blood sampling. Hemodynamic CVRF were SBP and DBP (mm Hg), corresponding to the average of two measurements obtained after a resting period of 5 minutes. Metabolic CVRF were total-, HDL-, LDL-cholesterol, and glucose (mmol/L), obtained from fasting blood drawn during the examination at the research center. Anthropometric CVRF was WHR, which was calculated as waist circumference divided by hip circumference. The SCORE risk was calculated according to the SCORE (Systematic COronary Risk Evaluation) charts for low-risk countries were used to define high or very high 10-year risk of fatal cardiovascular events.[139] Details on measurement procedures are provided in Appendix 16 (page 74).

Assessment of lifestyle markers

BMI (kg/m^2) was calculated as weight divided by height squared. Height and weight were measured with the participants standing without shoes and heavy outer garments, and with emptied out pockets, breathing out gently. Physical activity was assessed using a validated adapted version of the Zutphen Physical Activity Questionnaire [140] on RS-I-3 and RS-II-1, and the LASA Physical Activity Questionnaire (LAPAQ) on RS-I-5, RS-II-3 and RS-II-2.[117] Physical activity is expressed in MET-hours/week.[116] Details on questionnaires are provided on Appendix 16, page 74. To account for the heterogeneity between the questionnaires, we estimated a z-score per follow-up visit and cohort. Because physical activity was not measured for RS-I-4 and RS-II-2, we estimated for each participant the best-linear unbiased prediction using a mixed model with the observed zMET-hours/week as outcome, adjusted for age, sex, date of visit, date squared, cohort, systolic and diastolic blood pressure, glucose, BMI and WHR, and using the individual id as clustering variable.

Assessment of meteorological factors

Mean ambient temperature (°C), relative humidity (%), accumulated precipitation (mm) and total sunlight hours in Rotterdam, at participants' blood sampling date and the six days before, were obtained from the Koninklijk Nederlands Meteorologisch Instituut (KNMI, Royal Dutch Meteorological Institute).[67] The monitor is located approximately at 8km from Ommoord district (coordinates: 51° 58' N. 04° 27' E). Apparent temperature (°C) was calculated using the formula: $-2.653 + 0.994Temperature + 0.00153DewPoint^2$,[141] where $DewPoint = \left(\frac{Relative\ humidity(\%)}{100}\right)^{1/8} * (112 + 0.9Temperature) + 0.1Temperature - 112$. Visit and blood sampling dates were classified in seasons according to the light definition, centered at equinoxes (winter: November 6 to February 4, spring: February 5 to May 6, summer: May 7 to August 5, autumn: August 6 to November 5).[32]

Statistical analysis

All analyses were stratified by age at visit date: middle-aged (<65 years) vs. elderly (≥65 years). This corresponds to the age of retirement in The Netherlands; retirement explains part of the age-related variation of physical activity.[142-144] General characteristics at the visit date are presented using descriptive statistics. Categorical variables are described in absolute frequencies and

percentages. Continuous variables are described as median and interquartile range (25th and 75th percentile). Differences in the distribution of general characteristics according to age group were tested with Chi^2-test for categorical and Mann Whitney U-test for continuous variables.

To account for potential bias associated with missing data, we used multiple imputation procedures (n=5 imputations) to impute missing values of covariates. We applied sequential multiple imputation using chained equations with outcomes and covariates as predictors (a full description of the imputation procedure is provided in Appendix 16 (page 74)). General characteristics of the population according to age-group and season on the imputed dataset are presented using descriptive statistics. Categorical variables were described with absolute frequency and percentage; the distribution was compared using Chi-squared test. Continuous variables were described with median and interquartile range (25th and 75th percentile); the distribution was compared using the Kruskal-Wallis test. For all analyses, we used Stata version 14.1 SE (StataCorp, College Station, Texas, USA).[72]

First, CVRF seasonality was examined using linear mixed models, to account for repeated measurements per participant. Glucose seasonality was modelled using generalized linear mixed model with a log link, due to its skewed distribution. CVRF were assumed to have a sinusoidal seasonality with a period of one-year [5] and, accordingly, a crude model was fitted with the cosinor terms of visit or blood sampling date.[69] A model 1 was fitted with covariates: sex; age (years); self-reported use of antihypertensive, statin or anti-diabetic medication; self-reported smoking behavior (never, current, former); living situation [community-dweller vs. other (i.e., service flat, nursing home)]; monthly household income at baseline visit, categorized as <1,700 (<1,890), ≥1,700 to <3,000 (≥1,890 to <3,336), ≥3,000 to <4200 (≥3,336 to <4,670) and ≥4200 € (≥4,670 U.S. dollars); cohort; and date and date squared to account for long-term patterns. All covariates, except income, were obtained at visit date. Confounders were chosen on the basis of literature and model fit, according to the lowest Akaike's information criterion (AIC) value. CVRF seasonality was reported as the seasonal variation, which corresponds to the distance between peak and nadir levels throughout the annual period. Procedures to estimate the seasonal variation and standard error are described elsewhere.[17,69]

To examine the influence of lifestyle markers and meteorological factors on the seasonality of CVRF, we first fitted a Lifestyle markers model, by adding BMI (kg/m^2) and physical activity (z-score of MET-hours/week) in the model 1 (these were included as cubic splines with 2 degrees-of-freedom), and then we fitted a set of Meteorological factor models, by adding to model 1 the meteorological factors at the visit date and six previous days. To account for changes in the strength of the association at increasing lagged days, we applied one constraint between day 0 and day lag 1 and another between day lag 2 to 6. The influence of either lifestyle markers and meteorological factors on the seasonality of the CVRF was operationalized as the relative difference of the seasonal variation, which was calculated as

$$(Seasonal\ variation_{Model\ 2\ or\ 3} - Seasonal\ variation_{Model\ 1})/Seasonal\ variation_{Model\ 1}$$

Finally, we examined the proportion of the seasonal prevalence of selected CVRF that could be attributed to the lifestyle markers and the meteorological factors. First, selected CVRF (blood pressure, LDL cholesterol, glucose) and the SCORE risk calculated for each participant were dichotomized, i.e. high-very high SCORE (≥5%), high blood pressure (SBP ≥140 and/or

DBP ≥90mm Hg), high LDL cholesterol (≥1.8, ≥2.6 and ≥3.0 mmol/L for participants with very high (≥10%), high (≥5%), and low to moderate (<5%) SCORE risk), and high glucose levels(fasting glucose ≥7.0 mmol/L).[145] Then, we fitted fully adjusted Poisson mixed models to calculate the relative risk of the association of lifestyle markers (BMI and physical activity) and each meteorological factor (first and second constraint) with these dichotomized outcomes. Each exposure was included in the model stratified into its deciles. Then, we calculated the PAF per season of each exposure using the formula $PAF_{season} = 1 - \sum_{i=0}^{k} \frac{pd_i}{RR_i}$ [146], where pd_i corresponds to the proportion of cases that fall within each exposure category per season and RR_i corresponds to the relative risks obtained from the Poisson model. Finally, to calculate the combined PAF of the lifestyle markers, according to season, we used the formula $PAF_{lifestyle_{season}} = 1 - \left(1 - PAF_{BMI_{season=i}}\right) * \left(1 - PAF_{physical\ activity_{season}}\right)$. The same formula was used to combine the PAF of the first and second constraint of each meteorological factor. 95% confidence intervals were calculated with the delta method, using the command *nlcom* from Stata.

RESULTS

General characteristics

We included 20,723 observations belonging to 10,405 participants. Overall, elderly participants (aged 65 years and over) were more frequently former smokers, reported more often the use of antihypertensive, antidiabetic and statins medication, and were less frequently community-dweller (Table 9, page 68). No large differences were observed in the distribution of population characteristics according to season (Appendix 17, page 75). The seasonal patterns of the CVRF are shown in Figure 4, page 71. Seasonal patterns of meteorological factors and lifestyle markers are shown in Appendix 18 and Appendix 19 (pages 76 and 77).

Effect of lifestyle markers and meteorological factors on cardiovascular risk factors

SBP and DBP showed a clear seasonal variation with a peak in early-January (SBP=4.61mmHg (95%CI 3.69, 5.52)/4.17 mmHg (3.24, 5.11) and DBP=2.91 mmHg (2.38, 3.45) /1.69 mmHg (1.18, 2.20) among middle-aged and elderly participants, respectively; Table 10 (page 69)). The amplitude was mainly explained by ambient and apparent temperature.

Total cholesterol had a larger seasonal variation among middle-aged (0.11mmol/L (95%CI=0.06, 0.16) than among elderly adults (0.04mmol/L (0.00, 0.07)). Precipitation explained part of the pattern in both groups. Sunlight hours also explained the pattern among the middle-aged. LDL-cholesterol had a larger seasonality among the middle-aged (0.10mmol/L (0.05, 0.15)) than among the elderly (0.05mmol/L (0.00, 0.10)). The pattern was partly explained by ambient and apparent temperature among the elderly. The seasonal variation of HDL-cholesterol was not significant in any age group.

Glucose levels were higher in mid-Spring among the middle-aged (0.01mmol/L (0.00-0.02)). The variation was partly explained by lifestyle markers. Among elderly participants, peak levels of glucose were observed in early-December (0.01mmol/L (0.00-0.02)), and the pattern was mostly explained by ambient temperature, followed by lifestyle markers and apparent temperature.

WHR seasonality was similar in both age groups, with values around 0.01 higher in summer than in winter. Ambient and apparent temperature and sunlight hours explained the pattern among elderly participants. All estimates were similar in the non-imputed analysis (Appendix 20, page 78).

Analyses according to sex were similar for most CVRF. Nevertheless, there were differences in LDL-cholesterol, which seasonality among elderly was not explained by ambient or apparent temperature; HDL-cholesterol, which seasonality was explained by lifestyle markers among elderly men and by ambient temperature among middle-aged men; and glucose, which seasonality was not explained by ambient temperature among elderly men neither lifestyle markers among middle-aged women and elderly men (Appendix 22, page 80).

Population attributable fraction to lifestyle markers and meteorological factors of the seasonal prevalence of selected CVRF

The PAF to ambient and apparent temperature was higher in winter than in summer between 2.4 and 27.7 percentage points (pp) among middle-aged and up to 9.1pp among elderly, for the seasonal prevalence of the selected CVRF. In contrast, the PAF to lifestyle markers was consistent across seasons. The PAF to ambient temperature was higher than that of lifestyle markers for the winter prevalence of high blood pressure (middle-aged=6.1pp, elderly=11.7pp), high HDL-cholesterol (middle-aged=1.6pp, elderly=7.8pp), and high/very high SCORE risk (middle-aged = 13.8pp, elderly = 14pp). No lifestyle or meteorological factor had a significant PAF to the seasonal prevalence of high LDL-cholesterol (Figure 5, page 72). Procedures and PAF estimations are provided in the Appendix 23 (page 82).

DISCUSSION

In this cohort of middle-aged and elderly participants, CVRF had a seasonal variation and most CVRF had peak values towards winter/autumn. Lifestyle markers explained a modest proportion of the seasonality of glucose in both age groups and of DBP among the elderly. Among middle-aged participants, ambient and apparent temperature explained the seasonality of SBP and DBP. Among the elderly, ambient and apparent temperature explained the seasonality of SBP, DBP, glucose, LDL-cholesterol, and WHR. The PAF to meteorological factors, especially ambient and apparent temperature, for the seasonal prevalence of the selected CVRF and of high/very high SCORE risk was higher in winter. Additionally, PAF to lifestyle markers was constant throughout the year. Our findings suggest that exposure to low ambient and apparent temperature explains most of the seasonality of the CVRF, especially among elderly, and provides a plausible mechanism for the winter increase of cardiovascular mortality.

The seasonality of SBP and DBP was explained by ambient and apparent temperature in both age groups. Ambient and apparent temperature have been associated with blood pressure through endothelium-dependent mechanisms and sympathetic activation.[7,9,147,148] In contrast, the seasonality of glucose, LDL-cholesterol, and WHR was explained by ambient and apparent temperature only among the elderly. Previous studies have reported a negative association between exposure to low ambien temperature and glucose [149] and LDL-cholesterol levels.[13] These findings can be attributed to the seasonal variation of insulin resistance [150] and the increase of

insulin resistance and energy expenditure under exposure to low ambient temperature.[151] It has been also suggested that glucose, WHR, and lipid profile can be modified through the activation of brown adipose tissue under exposure to low ambient temperature,[152,153] although our findings do not support this hypothesis, probably because our population is older and have more comorbidities than in previous evidence. The specific influence of ambient and apparent temperature among elderly but not among middle-aged participants suggests that elderly are more sensitive to the exposure to low ambient and apparent temperature, probably due to the age-related impairment of thermoregulation mechanisms.[138]

HDL- and total-cholesterol seasonality was partly explained by sunlight hours and precipitation. Precipitation may explain the seasonality of total-cholesterol by modifying the air moisture,[12,154-156] which may induce changes in plasma volume and hemodilution, with a consequent apparent reduction of cholesterol levels.[32] Sunlight exposure may influence total-cholesterol seasonality through vitamin D levels.[157]

We found a discrete influence of lifestyle markers in the seasonality of CVRF, as BMI and physical activity only explained part of the seasonality of DBP and total cholesterol. It is possible that the seasonality of lifestyle markers is not large enough to have a major influence on the other CVRF. For example, it has been shown that the seasonality of physical activity is not large enough to induce changes in physical fitness [76] that would link the seasonality of physical activity with the seasonality of blood pressure.[158] Additionally, our study population would be less likely to change their lifestyle behavior on a seasonal basis, because we included overall population taking statins. Indeed, we observed in our sensitivity analysis according to sex that the seasonality of lifestyle markers was larger among men than among women, thus lifestyle markers were more likely to explain the seasonality of SBP, DBP, HDL-cholesterol, and glucose among men than among women. The influence of lifestyle markers in the seasonality of DBP among elderly and of glucose in both age groups suggests that these CVRF may be more senstive to the even small seasonal variation of the lifestyle markers.

We additionally examined the proportion of CVRF prevalence that can be attributed to high BMI and low physical activity or to adverse climatic conditions, per season. In agreement with our previous findings, the proportion of CVRF prevalence that can be attributed to exposure to low ambient temperature was larger in winter, suggesting that the increase of CVRF prevalence in winter can be attributed to low ambient and apparent temperature. In contrast, whereas a substantial proportion of the CVRF prevalence was attibutable to high BMI and low physical activity, this proportion was constant across seasons. A counterintuitive finding was the higher PAF to both lifestyle markers and meteorological factors among middle-aged than among elderly. However, this finding can be explained by the higher prevalence of participants taking medication (antihypertensive, antidiabetic and statins) among the elderly population, what may have contributed to counter the influence of lifestyle markers and meteorological factors. Nevertheless, these findings suggest that the achievement of therapeutic targets may be lower and the prevalence of CVRF may increase in winter time, mostly due to the adverse climatic conditions of the season.

Our findings have several relevant implications, both for clinical and public health perspectives. From the clinical perspective, it is likely that the accumulation of peak levels of most

CVRF in winter time has effects on the well-stablished seasonality of cardiovascular mortality,[6] and people would benefit of accounting for exposure to adverse climatic conditions as a cardiovascular risk factor. From a public health perspective, our findings provide evidence of the substantial contribution of meteorological factors in the seasonality and prevalence of CVRF, in preparation for the challenges ahead due to climate change. Although it has been projected an increase in the health risks associated to climate change due to heat waves, it is also expected that without adaption, irrespective of the increasing average temperature the burden of low ambient temperature will remain high, partly due to the worldwide ageing trend.[159] Elderly people are among the most vulnerable population under the projected scenarios of climatic change, partly due to the age-related impairment of thermoregulation, what may alter the perception of ambient temperature and increase the risk of sustained exposure to adverse climatic conditions.[160,161] Such impairment of thermoregulation has also been associated with increased insulin resistance and endothelial dysfunction.[162] Therefore, the public health adaption aimed to mitigate the influence of meteorological factors on global health should include sustainable and affordable policies for building design and urban planning standards to reduce heat islands and efficiently maintain indoors temperature, and strong surveillance and warning systems to increase awareness under adverse climatic conditions.[163,164] Furthermore, a better understanding of the mechanisms of exposure to such climatic conditions is required, to better identify vulnerable population. For example, despite the substantial proportion of CVRF prevalence that was attributable to ambient temperature in our study, we do not have information about the actual exposure of our participants. Although high blood pressure has been previously correlated with improper clothing at outdoor excursions in winter [7] and poor housing isolation,[11,165,166] the Netherlands has fared relatively well, compared to other European countries, in housing standards and measures of fuel poverty related with relative excess of winter mortality.[167] Additionally, although our elderly population was more likely to report baseline household income below 1,700eur than the middle-aged, all our estimates were adjusted per income and no differences were observed in the distribution of income across seasons. Therefore, future studies are urgently required to have a better understanding of the susceptibility and mechanisms of exposure to adverse climatic conditions, in order to efficiently address their influence in the seasonality of cardiovascular risk and to identify strategies to mitigate the upcoming effects of climate change on global health.

Strengths and limitations

Our study has several strengths. First, using repeated measurements reduced the variability of CVRF levels between participants. Second, we adjusted for a broad range of confounders to avoid spurious seasonal patterns due to participants attending the research center not at random throughout the year (crude vs. model 1).

However, some limitations deserve attention. First, the seasonality of the metabolic CVRF was smaller than in previous studies,[5,29,32,33,168] what can be explained because a sizeable proportion was taking antihypertensive, antidiabetic, and statins medication. Additionally, about two third of our participants was overweight or obese and predominantly of European descent. Therefore, our findings might not be generalizable and should be confirmed in other settings. Second, we were unable to evaluate the effect of diet quality, which might have provided a more

comprehensive understanding of the effect of lifestyle markers on CVRF seasonality.[169] Third, physical activity was measured with two different questionnaires, and the physical activity not measured in one of the visits of two cohorts was assumed to be reflected by the data of other visits, which led to a heterogeneous seasonality of physical activity. Also, our elderly population exhibited peak values of physical activity in winter. Although more physical activity in winter has been observed among elderly population,[17,170-173] objective measurements are necessary to confirm this pattern. Fourth, we calculated our PAF estimates using stratified levels of the exposures, what may reduce the power of the calculations. Finally, the meteorological factors used in the analyses are derived from locally obtained measurements, which may not necessarily represent the individual exposure to adverse climatic conditions, nor account for other environmental factors that might be involved, such as air pollution.

Conclusion

The CVRF examined had a seasonal variation, leading to a worsened cardiovascular risk profile worsened in winter/autumn, when most CVRF had peak values. The worsening of the cardiovascular risk in winter is largely attributable to the exposure to low ambient and apparent temperature and is likely to have a role in the well-described seasonality of cardiovascular mortality. Therefore, population may benefit of a close monitoring when elevated levels of CVRF are observed, both to confirm the diagnosis during summer and to address the excess of burden during winter. In preparation for the projected health effects of climate change, future research is required to improve our understanding about the mechanisms of exposure to adverse meteorological factors, to efficiently address its effect as risk factors for cardiovascular risk.

Table 9. Distribution of general characteristics of participants at visit date

Covariates	Middle-aged (<65 years) (n=8,263 observations)			Elderly (≥65 years) (n=12,460 observations)			p-value
	Median	IQR		Median	IQR		
Age (years)	59.7	56.0	62.2	73.3	68.8	78.5	
Physical activity (zMETs-hours/week)	-0.1	-0.7	0.5	-0.1	-0.6	0.4	0.09
Body mass index, kg/m²	26.9	24.5	29.8	26.9	24.6	29.6	0.56
Hemodynamic risk factors (mmHg)							
Systolic blood pressure	133.0	121.0	146.0	147.0	134.0	162.0	<0.01
Diastolic blood pressure	81.0	73.5	88.0	79.0	72.0	87.0	<0.01
Metabolic risk factors (mmol/L)							
Total-cholesterol	5.7	5.0	6.3	5.6	4.9	6.3	<0.01
Low-density lipoprotein cholesterol‡	3.6	2.9	4.2	3.5	2.8	4.1	<0.01
High-density lipoprotein cholesterol	1.4	1.1	1.7	1.4	1.1	1.7	0.15
Glucose	5.4	5.1	5.9	5.6	5.2	6.1	<0.01
Anthropometric risk factor							
Waist-to-hip ratio	0.9	0.8	1.0	0.9	0.8	1.0	0.84
	n	%		n	%		
Sex*							
Men	43.7	3,613		42.8	5,334		0.19
Women	56.3	4,650		57.2	7,126		
Medication intake							
Antihypertensive							
No	72.8	6,015		51.4	6,402		<0.01
Yes	27.2	2,248		48.6	6,058		
Antidiabetic							
No	95.3	7,871		91.5	11,405		<0.01
Yes	4.7	3,92		8.5	1,055		
Statin							
No	81.8	6,758		76.6	9,547		<0.01
Yes	18.2	1,505		23.4	2,913		
Living situation							
Community-dweller	99.9	8,256		98.9	12,323		<0.01
Non-community dweller	0.1	7		1.1	137		
Smoking status							
Never	30.3	2,507		31.4	3,916		<0.01
Current	25.0	2,066		14.2	1,771		
Former	44.7	3,690		54.4	6,772		
Monthly household income at baseline*,†							
<1,700 euros	7.7	634		24.0	2,988		<0.01
1,700-3000 euros	12.3	1,015		24.9	3,100		
3,000-4,200 euros	21.6	1,786		28.1	3,496		
>4,200 euros	58.4	4,829		23.1	2,875		
Body mass index categories							
Normal/underweight (<25kg/m²)	30.5	2,519		29.2	3,638		0.57
Pre-obesity (25-29.9kg/m²)	46.4	3,833		48.5	6,041		
Obesity (≥30kg/m²)	23.1	1,911		22.3	2,781		

zMET: score of Metabolic Equivalent of Task. IQR = Interquartile risk (25th to 75th percentile) *Sex and income are constant during follow-up visit and are summarized at baseline visit. †Categories are <1,890; ≥1,890 to <3,336; ≥3,336 to <4,670 and ≥4,670 U.S. dollars. ‡ Sample sizes for LDL-cholesterol estimates: 7,178 observations among 5,387 middle-aged participants and 8,174 observations among 6,769 elderly participants

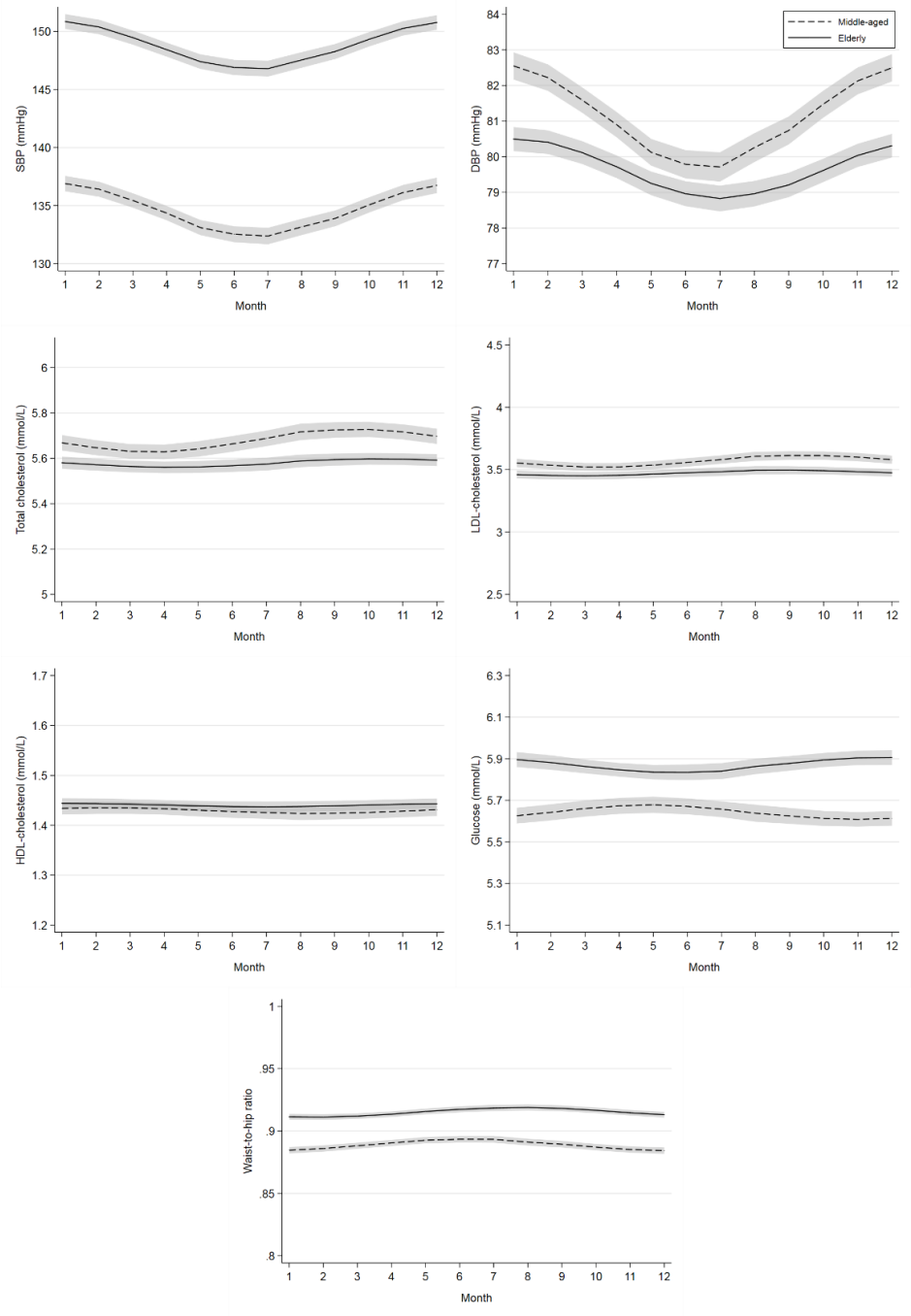
Table 10. Seasonality and effect of lifestyle markers and meteorological factors in the seasonality of cardiovascular risk factors according to age groups

Cardiovascular risk factors		Middle-aged (<65 years) n = 8,263 observations (5,583 participants)					Elderly (≥65 years) n = 12,460 observations (7,387 participants)				
		Seasonal variation	95%	CI	SV change (%)	Peak	Seasonal variation	95%	CI	SV change (%)	Peak
Systolic blood pressure (mmHg)											
Crude		5.24	4.32	6.16	13.8	25-Dec	4.04	3.09	5.00	-3.1	28-Dec
Model 1		4.61	3.69	5.52	0.0	4-Jan	4.17	3.24	5.11	0.0	1-Jan
Model 2	+Lifestyle markers	4.59	3.69	5.48	-0.4	3-Jan	4.01	3.08	4.95	-3.9	0-Jan
Model 3	+Ambient temperature (°C)	1.56	0.61	2.51	-66.1	10-Oct	1.13	-0.58	2.84	-72.9	16-Oct
	+Apparent temperature (°C)	1.99	1.05	2.94	-56.7	18-Sep	1.36	-0.35	3.07	-67.4	26-Sep
	+Sunlight hours	4.88	3.72	6.03	5.9	3-Jan	4.43	3.10	5.76	6.2	1-Jan
	+Precipitation (mm)	4.60	3.66	5.54	0.0	5-Jan	4.21	3.26	5.15	0.8	0-Jan
Diastolic blood pressure (mmHg)											
Crude		2.99	2.46	3.52	2.8	2-Jan	2.04	1.53	2.55	20.3	11-Jan
Model 1		2.91	2.38	3.45	0.0	2-Jan	1.69	1.18	2.20	0.0	17-Jan
Model 2	+Lifestyle markers	2.91	2.38	3.43	-0.2	0-Jan	1.62	1.11	2.13	-4.5	17-Jan
Model 3	+Ambient temperature (°C)	1.09	0.53	1.65	-62.5	24-Oct	0.27	-0.65	1.20	-83.8	12-Jan
	+Apparent temperature (°C)	1.26	0.70	1.81	-56.8	2-Oct	0.25	-0.67	1.18	-85.1	1-Jan
	+Sunlight hours	2.96	2.28	3.64	1.6	1-Jan	1.66	0.94	2.38	-2.0	18-Jan
	+Precipitation (mm)	2.91	2.36	3.46	-0.1	2-Jan	1.70	1.19	2.22	0.6	19-Jan
Total cholesterol (mmol/L)											
Crude		0.11	0.06	0.16	1.3	24-Oct	0.05	0.01	0.10	40.6	10-Oct
Model 1		0.11	0.06	0.16	0.0	17-Oct	0.04	0.0021	0.07	0.0	22-Oct
Model 2	+Lifestyle markers	0.11	0.06	0.16	3.7	16-Oct	0.04	0.00	0.07	1.9	17-Oct
Model 3	+Ambient temperature (°C)	0.14	0.09	0.19	34.4	9-Sep	0.07	0.00	0.13	70.9	27-Aug
	+Apparent temperature (°C)	0.14	0.09	0.19	33.0	11-Sep	0.06	0.00	0.13	68.3	29-Aug
	+Sunlight hours	0.10	0.04	0.16	-9.9	25-Sep	0.05	0.00	0.10	23.7	2-Aug
	+Precipitation (mm)	0.10	0.05	0.15	-7.5	18-Oct	0.03	0.00	0.07	-17.9	25-Oct
Low-density lipoprotein cholesterol (mmol/L)*											
Crude		0.09	0.04	0.15	-5.8	20-Oct	0.07	0.02	0.13	64.9	7-Sep
Model 1		0.10	0.05	0.15	0.0	11-Oct	0.05	-0.005	0.10	0.0	9-Sep
Model 2	+Lifestyle markers	0.11	0.06	0.15	4.4	9-Oct	0.05	0.00	0.10	7.4	5-Sep
Model 3	+Ambient temperature (°C)	0.12	0.07	0.17	16.1	15-Sep	0.03	-0.05	0.12	-26.4	18-Oct
	+Apparent temperature (°C)	0.12	0.07	0.17	19.1	14-Sep	0.03	-0.05	0.12	-24.5	12-Nov
	+Sunlight hours	0.10	0.04	0.16	-3.3	2-Oct	0.05	-0.02	0.12	11.0	17-Aug
	+Precipitation (mm)	0.10	0.05	0.15	0.9	11-Oct	0.04	-0.01	0.09	-0.9	9-Sep
High-density lipoprotein cholesterol (mmol/L)											
Crude		0.01	0.00	0.03	1.5	26-Feb	0.01	-0.01	0.02	-18.8	23-Dec
Model 1		0.01	0.00	0.03	0.0	7-Mar	0.01	0.00	0.02	0.0	14-Jan
Model 2	+Lifestyle markers	0.02	0.00	0.03	32.1	4-Mar	0.01	0.00	0.02	51.7	16-Jan
Model 3	+Ambient temperature (°C)	0.01	0.00	0.03	14.0	29-Feb	0.03	0.01	0.05	358.0	24-Jul
	+Apparent temperature (°C)	0.02		0.03	34.0	24-Feb	0.03	0.01	0.05	355.3	26-Jul
	+Sunlight hours	0.01		0.03	9.7	21-Feb	0.01	-0.01	0.02	-21.3	20-May
	+Precipitation (mm)	0.01		0.03	-13.3	2-Mar	0.01	0.00	0.02	2.8	29-Jan

High-density lipoprotein cholesterol (mmol/L)											
Crude		0.01	0.00	0.03	1.5	26-Feb	0.01	-0.01	0.02	-18.8	23-Dec
Model 1		0.01	0.00	0.03	0.0	7-Mar	0.01	0.00	0.02	0.0	14-Jan
Model 2	+Lifestyle markers	0.02	0.00	0.03	32.1	4-Mar	0.01	0.00	0.02	51.7	16-Jan
Model 3	+Ambient temperature (°C)	0.01	0.00	0.03	14.0	29-Feb	0.03	0.01	0.05	358.0	24-Jul
	+Apparent temperature (°C)	0.02		0.03	34.0	24-Feb	0.03	0.01	0.05	355.3	26-Jul
	+Sunlight hours	0.01		0.03	9.7	21-Feb	0.01	-0.01	0.02	-21.3	20-May
	+Precipitation (mm)	0.01		0.03	-13.3	2-Mar	0.01	0.00	0.02	2.8	29-Jan
Glucose(mmol/L)											
Crude		0.01	0.00	0.03	-9.7	6-Jun	0.01	0.00	0.02	3.6	1-Jan
Model 1		0.01	0.00	0.02	0.0	27-May	0.01	0.00	0.02	0.0	2-Dec
Model 2	+Lifestyle markers	0.01	0.00	0.02	-10.7	24-May	0.01	0.00	0.02	-12.9	20-Nov
Model 3	+Ambient temperature (°C)	0.04	0.03	0.05	200.1	8-Jul	0.01	-0.01	0.03	-20.8	25-Sep
	+Apparent temperature (°C)	0.04	0.03	0.05	183.8	8-Jul	0.01	-0.01	0.03	-6.6	16-Sep
	+Sunlight hours	0.02	0.00	0.03	42.7	4-Jun	0.02	0.01	0.03	60.1	9-Dec
	+Precipitation (mm)	0.01	0.00	0.03	4.9	24-May	0.01	0.00	0.02	-0.2	4-Dec
Waist-to-hip ratio											
Crude		0.01	0.00	0.01	-18.9	11-Jul	0.01	0.00	0.01	-6.7	7-Aug
Model 1		0.01	0.01	0.01	0.0	23-Jun	0.01	0.00	0.01	0.0	7-Aug
Model 2	+Lifestyle markers	0.01	0.01	0.01	7.1	29-Jun	0.01	0.01	0.01	17.6	2-Aug
Model 3	+Ambient temperature (°C)	0.01	0.01	0.02	49.0	4-Jul	0.00	0.00	0.01	-46.2	23-Aug
	+Apparent temperature (°C)	0.01	0.01	0.02	43.1	3-Jul	0.00	0.00	0.01	-58.3	1-Sep
	+Sunlight hours	0.01	0.01	0.01	5.6	22-Jun	0.01	0.00	0.01	-23.5	27-Aug
	+Precipitation (mm)	0.01	0.01	0.01	-1.6	27-Jun	0.01	0.00	0.01	1.1	9-Aug

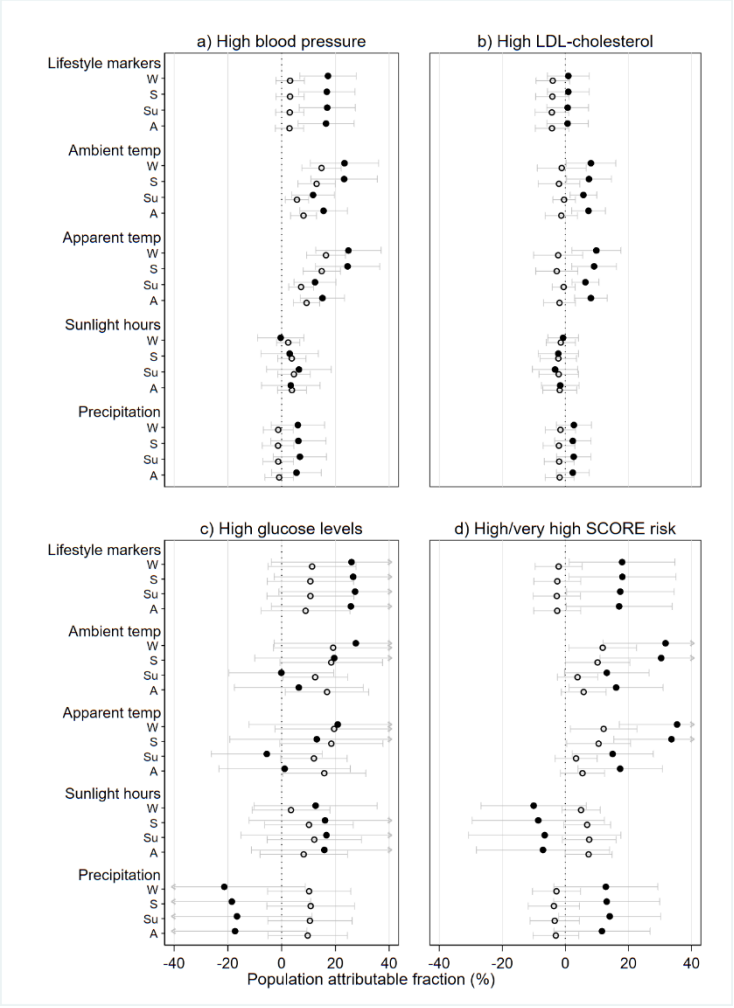
*Sample sizes for LDL-cholesterol estimates: 7,178 observations among 5,387 middle-aged participants and 8,174 observations among 6,769 elderly participants. Crude model: sine and cosine terms. Model 1: cosinor terms, age, sex, smoking status, intake of antihypertensive, statin and antidiabetic medication, income categories, living situation, cohort, date and date squared. Model 2: model 1 plus body mass index (cubic spline, 2df) and zMET-hours/week (cubic spline, 2df) Model 3: model 1 plus meteorological factors (constraint 1: lag days 0-1 and constraint 2: lag days 2-6.

Figure 4. Seasonal variation of cardiovascular risk factors



Adjusted monthly levels of CVRF. Adjusted for cosinor terms, age, sex, smoking status, intake of antihypertensive, statin and antidiabetic medication, income categories, living situation, cohort, date and date squared.

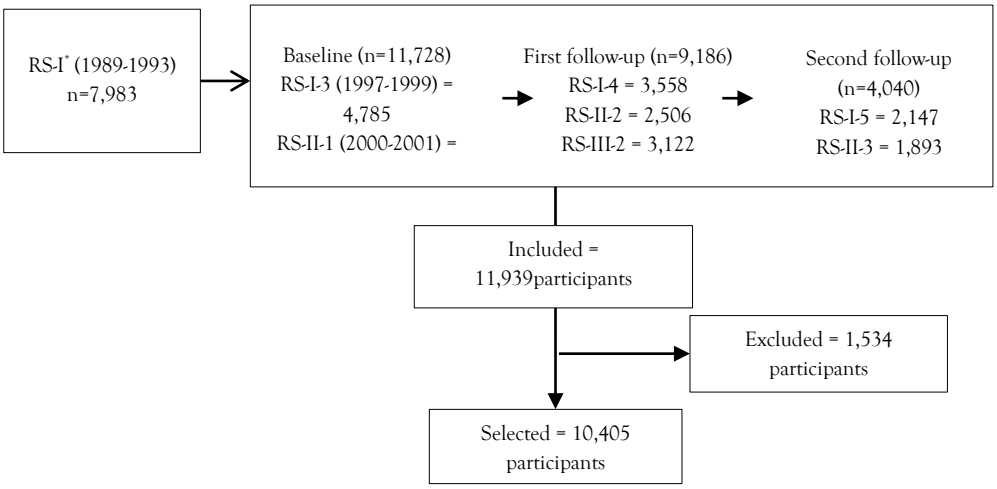
Figure 5. Population attributable fraction to meteorological factors of prevalence per season of selected cardiovascular risk factors



Full circles: Middle-aged. Hollow circles: Elderly. CVRF: Cardiovascular risk factor. High blood pressure: SBP ≥ 140 and/or DBP ≥ 90 mmHg; high LD-cholesterol: ≥1.8, ≥2.6, ≥3.0 mmol/L for population with very high (≥10%) high (≥5%) or low to moderate (<5%) 10-year cardiovascular fatal event according to SCORE chart, respectively; high glucose levels: ≥7 mmol/L, high/very high SCORE risk: 10-year cardiovascular fatal event according to SCORE charts higher than ≥5%. Exposure categories are decile containing BMI ≤24kg/m^2, physical activity ≤0 zMET-hours/week, lowest deciles of ambient and apparent temperature and sunlight hours and highest deciles of precipitation. PAF are adjusted for cosinor terms, age, sex, smoking status, intake of antihypertensive, statin and antidiabetic medication, income categories, living situation, cohort, date and date squared.

SUPPLEMENTARY MATERIAL

Appendix 15. Flow chart of selection of participants



* All observations from the cohort are 28,154 among 14,926 participants. However, we excluded the visits from RS-I-1 and RS-II-2 (2,987 participants [3,185 observations]) for consistency in measurements. Thus, RS-I-3 is considered baseline. Thirteen participants who attended in RS-I-1 or RS-I-2 did not attend in RS-I-3, but did attend in further follow-up visits.
†These exclusions are due to missing values for any of the CVRF in a single visit, except LDL-cholesterol, which were missing for RS-I-4 and RS-II-2 because triglycerides levels were not available. Therefore, the sample sizes for LDL-cholesterol estimates are 7,178 observations among 5,387 middle-aged participants and 8,174 observations among 6,769 elderly participants.

Appendix 16. Measurement procedures of cardiovascular risk factors, physical activity, and body mass index and imputation procedures

Procedures

Hemodynamic risk factors: Blood pressure was measured twice after a resting period of 5 minutes in a single visit using a random-zero sphygmomanometer (cuff size of 32×17 cm) on the right arm of participants in sitting position. The average of the measurements was used in the analyses.

Metabolic risk factors: Fasting blood was drawn during the examination at the research center, and the serum was stored at −80°C. Glucose was measured within one week of sampling using the glucose hexokinase method.[174] Lipid profile measurement were conducted using an automated enzymatic procedure [175] (Hitachi analyzer, Roche Diagnostics, Washington, DC, USA). LDL-cholesterol was calculated using the Friedewald formula [176] [LDL-cholesterol=total-cholesterol–HDL-cholesterol–(0.45*triglycerides)]; 137 observations were excluded because triglycerides was > 4.5mmol/L.[139] LDL-cholesterol were missing for RS-I-4 and RS-II-2, because triglycerides levels were not available.

Anthropometric risk factors: Height, weight and waist circumference were measured with the participants standing without shoes and heavy outer garments, and with emptied out pockets, breathing out gently. Waist circumference was measured at the level midway between the lower rib margin and the iliac crest, hip circumference was recorded as the maximum circumference over the buttocks. Height was measured with a wall-mounted stadiometer, recorded to the nearest 0.1cm; weight was measured with an electronic floor scale, recorded to the nearest 0.1kg; waist and hip circumference were measured using a non-stretchable tape and recorded to the nearest 0.1cm.

Physical activity: Physical activity on RS-I-3 and RS-II-1 was assessed using a validated adapted version of Zutphen Physical Activity Questionnaire,[140] and expressed in METhours/week.[177] Questions on housekeeping activities were added to the original questionnaire that already included questions on walking, cycling, gardening, hobbies, and diverse sports. The LASA Physical Activity Questionnaire (LAPAQ) [117] was used on RS-I-5, RS-II-3 and RS-II-2, which contains questions regarding the frequency and duration of walking, cycling, sports, gardening and housework.

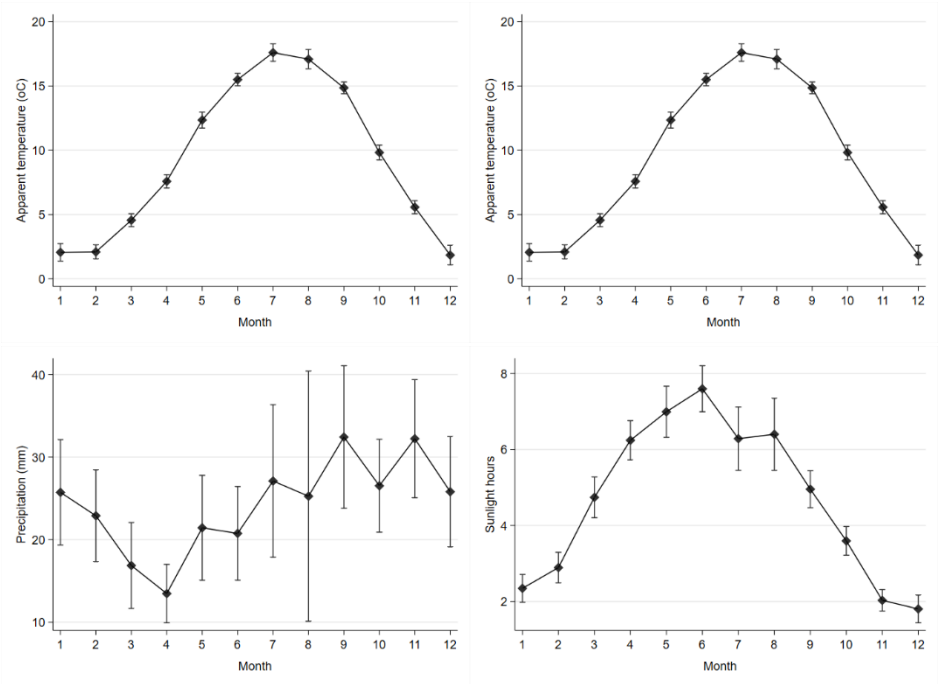
Imputation procedures

Sequential multiple imputation using chained equations was performed to impute missing values of covariates. This approach has been shown to work well in the context of complex longitudinal data, as in the Rotterdam Study.[178] The predictors used to impute the covariates were sex, age, cohort, visit, date, date squared, systolic and diastolic blood pressure, total and HDL-cholesterol, glucose, waist-to-hip ratio, intake of antihypertensive, antidiabetic and statins medication, body mass index, smoking status, physical activity and housing status. To impute dichotomous variables (statin intake, antihypertensive medication intake, antidiabetic medication intake and housing status) we used a logit function. To impute ordered categorical variables (income) we used an ordered logit function. To impute categorical non-ordered variables (smoking behavior) we used a multinomial logit function. To impute continuous variables (physical activity and body mass index), we used a linear function. To ensure reproducibility, we used a random seed (2005). We created five imputed datasets, which were used in all the analysis. Covariates with missing values were: physical activity: 2,159 missing, body mass index: 140 missing, antihypertensive medication intake: 356 missing, antidiabetic medication intake: 250 missing, statins intake: 259 missing; housing: 119 missing, income: 2,140 missing; smoking behavior: 137 missing. Imputations were performed using the *mi impute* command of Stata software.

Appendix 17. Distribution of general characteristics of participants at visit date per season

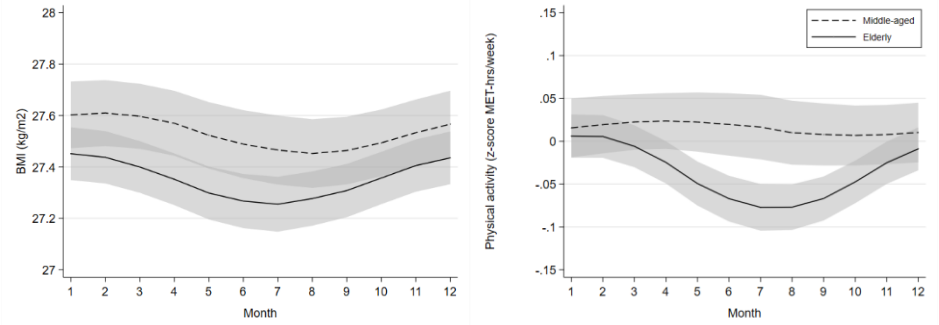
Covariates	Middle-aged (<65 years)													Elderly (≥65 years)										
	Winter (n=1,789)			Spring (n=2,630)			Summer (n=1,792)			Autumn (n=2,052)			p-value	Winter (n=3,037)			Spring (n=3,384)			Summer (n=2,851)			Autumn (n=3,188)			p-value
	p50	IQR		p50	IQR		p50	IQR		p50	IQR			p50	IQR		p50	IQR		p50	IQR		p50	IQR		
Age (years)	60.1	956.4	62.5	59.4	55.9	62.1	59.0	55.3	62.0	60.2	56.5	62.3	<0.01	72.8	68.5	77.8	73.1	68.4	78.2	74.3	69.7	79.5	73.2	68.8	78.3	<0.01
Physical activity (±METs-hours/week)	-0.1	-0.7	0.5	-0.1	-0.7	0.6	-0.2	-0.7	0.5	-0.2	-0.7	0.4	0.20	0.0	-0.5	0.5	-0.1	-0.6	0.4	-0.2	-0.6	0.3	-0.1	-0.6	0.4	<0.01
BMI, kg/m²	27.0	24.6	29.7	26.9	24.5	29.7	27.0	24.4	29.8	26.9	24.4	29.9	0.76	27.1	24.8	30.0	26.9	24.6	29.6	26.7	24.5	29.4	26.9	24.6	29.4	0.02
Hemodynamic risk factors (mmHg)																										
Systolic blood pressure	135.0	123.0	148.0	133.0	122.0	146.0	130.0	118.5	143.5	133.0	121.0	146.5	<0.01	149.0	136.0	164.0	147.0	134.0	162.0	145.0	131.0	161.0	147.0	134.0	162.0	<0.01
Diastolic blood pressure	82.0	75.0	89.0	81.0	74.0	88.0	80.0	72.5	86.5	81.0	73.5	88.0	<0.01	81.0	73.0	89.0	79.5	72.0	87.0	77.5	70.0	86.0	79.0	72.0	87.0	<0.01
Metabolic risk factors (mmol/L)																										
Total-cholesterol	5.6	5.0	6.3	5.7	5.0	6.3	5.6	5.0	6.3	5.7	5.1	6.4	0.01	5.6	4.8	6.2	5.6	4.9	6.3	5.6	4.9	6.3	5.6	4.9	6.3	0.35
LDL-cholesterol	3.5	2.9	4.1	3.6	2.9	4.2	3.5	2.9	4.1	3.6	3.0	4.3	0.15	3.4	2.8	4.1	3.5	2.8	4.1	3.5	2.8	4.1	3.5	2.8	4.1	0.19
HDL-cholesterol	1.4	1.1	1.7	1.4	1.1	1.7	1.4	1.1	1.6	1.4	1.1	1.7	0.63	1.4	1.1	1.7	1.4	1.1	1.7	1.4	1.1	1.7	1.4	1.1	1.7	0.34
Glucose	5.4	5.1	5.9	5.4	5.1	5.9	5.4	5.1	5.9	5.4	5.0	5.9	1.00	5.6	5.2	6.2	5.6	5.2	6.2	5.5	5.2	6.1	5.6	5.2	6.1	<0.01
Anthropometric risk factors																										
Waist-to-hip ratio	0.9	0.8	0.9	0.9	0.8	1.0	0.9	0.8	1.0	0.9	0.8	0.9	0.07	0.9	0.8	1.0	0.9	0.8	1.0	0.9	0.8	1.0	0.9	0.8	1.0	0.58
	n	%		n	%		n	%		n	%			n	%		n	%		n	%		n	%		
Sex[1]																										
Male	44.3	793		43.3	1138		44.4	796		43.2	886		0.78	43.7	1326		43.2	1463		41.6	1186		42.6	1359		0.41
Female	55.7	996		56.7	1492		55.6	996		56.8	1166			56.3	1711		56.8	1921		58.4	1665		57.4	1829		
Medication intake																										
Antihypertensive																										
No	73.0	1306		73.1	1923		74.0	1326		71.1	1459		0.22	51.7	1569		51.2	1733		51.2	1459		51.5	1641		0.98
Yes	27.0	483		26.9	707		26.0	466		28.9	593			48.3	1468		48.8	1651		48.8	1392		48.5	1547		
Antidiabetic																										
No	95.2	1704		95.2	2504		95.7	1715		95.0	1949		0.77	91.4	2775		91.3	3089		91.6	2610		91.9	2931		0.79
Yes	4.8	85		4.8	126		4.3	77		5.0	103			8.6	262		8.7	295		8.4	241		8.1	257		
Statin																										
No	81.4	1456		82.9	2181		82.1	1472		80.3	1648		0.13	76.9	2336		75.8	2566		78.8	2247		75.2	2398		0.01
Yes	18.6	333		17.1	449		17.9	320		19.7	404			23.1	701		24.2	818		21.2	604		24.8	790		
Living situation																										
Community-dweller	100.0	1789		99.9	2628		99.8	1788		100.0	2051		0.26	99.6	3024		99.0	3350		97.4	2777		99.5	3173		<0.01
Non-community dweller	0.0	0		0.1	2		0.2	4		0.0	1			0.4	13		1.0	34		2.6	74		0.5	15		
Smoking status																										
Never	31.6	565		31.3	824		29.6	530		28.7	589		0.06	31.5	957		30.6	1036		31.5	897		32.2	1026		0.31
Current	24.1	431		23.6	620		25.2	451		27.5	563			13.6	412		15.5	525		13.9	396		13.8	439		
Former	44.3	792		45.1	1186		45.3	811		43.9	900			54.9	1668		53.9	1823		54.6	1558		54.1	1724		
Monthly household income at baseline[1,2]																										
<1,700 euros	8.1	145		7.7	203		7.1	127		7.7	159		0.24	22.1	672		24.1	817		24.6	701		25.1	799		<0.01
1,700-3,000 euros	12.1	216		11.4	301		12.2	219		13.5	278			23.6	718		25.8	873		25.7	733		24.3	776		
3,000-4,200 euros	19.7	352		22.3	586		21.9	392		22.2	456			29.5	897		28.0	948		27.5	783		27.2	867		
>4,200 euros	60.1	1076		58.5	1540		58.8	1054		56.5	1159			24.7	750		22.0	745		22.3	635		23.4	746		
Body mass index categories																										
Normal/underweight (<25kg/m²)	28.4	508		30.7	806		31.0	556		31.7	650		0.78	27.2	825		29.5	999		30.2	861		29.9	953		<0.01
Pre-obesity (25-29.9kg/m²)	49.2	880		46.3	1218		46.1	825		44.3	910			47.8	1452		48.5	1640		48.4	1381		49.2	1568		
Obesity (≥30kg/m²)	22.4	401		23.0	605		22.9	411		24.0	493			25.0	760		22.0	746		21.3	609		20.9	667		

Appendix 18. Annual pattern of meteorological factors



Monthly mean and confidence interval of meteorological factors during study period

Appendix 19. Seasonality of lifestyle markers according to age-group



Seasonal variation of BMI = middle-aged: 0.16 kg/m^2 (95%CI 0.04 to 0.28, peak=11-Feb), elderly: 0.20 kg/m^2 (95%CI 0.10 to 0.30, peak=12-Jan). Seasonal variation of physical activity = middle-aged: 0.02 zMET-hours/week (95%CI -0.03 to 0.07, peak=16-Apr), elderly: 0.09 zMET-hours/week (95%CI 0.05 to 0.12, peak=29-Jan). Adjusted monthly levels of CVRF. Adjusted for sine and cosine terms, age, sex, smoking status, intake of antihypertensive, statin and antidiabetic medication, income categories, living situation, cohort, date and date squared. Shade area corresponds to 95% confidence interval

Appendix 20. Seasonality and effect of lifestyle markers and meteorological factors on the seasonality of the cardiovascular risk factors, according to age groups; non-imputed analysis

Cardiovascular risk factors		Middle-aged (<65 years) n = 6,300 observations (4,437 participants)					Elderly (≥65 years) n = 10,046 observations (6,028 participants)				
		Seasonal variation	95%	CI	SV change (%)	Peak	Seasonal variation	95%	CI	SV change (%)	Peak
Systolic blood pressure (mmHg)											
Crude		5.72	4.65	6.79	12.4	1-Jan	3.74	2.69	4.80	-3.0	22-Dec
Model 1		5.09	4.04	6.14	0.0	9-Jan	3.86	2.83	4.89	0.0	28-Dec
Model 2	+Lifestyle markers*	5.05	4.02	6.09	-0.8	7-Jan	3.79	2.76	4.82	-1.8	27-Dec
Model 3	+Ambient temperature (°C)	1.34	0.23	2.44	-73.7	9-Nov	1.39	-0.51	3.29	-64.1	28-Sep
	+Apparent temperature (°C)	1.49	0.39	2.59	-70.8	5-Oct	1.67	-0.24	3.57	-56.8	17-Sep
	+Sunlight hours	5.04	3.71	6.38	-0.9	9-Jan	3.91	2.45	5.37	1.4	29-Dec
	+Precipitation (mm)	5.08	3.99	6.17	-0.2	10-Jan	3.89	2.85	4.93	0.8	27-Dec
Diastolic blood pressure (mmHg)											
Crude		3.06	2.45	3.67	3.0	9-Jan	1.65	1.09	2.22	19.2	5-Jan
Model 1		2.97	2.35	3.59	0.0	9-Jan	1.39	0.82	1.95	0.0	12-Jan
Model 2	+Lifestyle markers *	2.96	2.36	3.56	-0.4	7-Jan	1.31	0.75	1.87	-5.4	11-Jan
Model 3	+Ambient temperature (°C)	0.76	0.11	1.41	-74.4	24-Oct	0.27	-0.76	1.30	-80.3	3-Sep
	+Apparent temperature (°C)	0.98	0.34	1.62	-66.9	21-Sep	0.27	-0.77	1.30	-80.9	20-Sep
	+Sunlight hours	2.90	2.12	3.68	-2.4	10-Jan	1.29	0.49	2.08	-7.1	14-Jan
	+Precipitation (mm)	2.96	2.33	3.60	-0.2	9-Jan	1.39	0.82	1.96	0.4	15-Jan
Total cholesterol (mmol/L)											
Crude		0.10	0.04	0.16	-3.4	3-Oct	0.06	0.01	0.11	37.5	15-Sep
Model 1		0.10	0.05	0.15	0.0	28-Sep	0.04	0.0030	0.08	0.0	3-Oct
Model 2	+Lifestyle markers*	0.10	0.05	0.16	3.6	28-Sep	0.04	0.00	0.08	-0.3	28-Sep
Model 3	+Ambient temperature (°C)	0.18	0.12	0.24	79.7	24-Aug	0.05	-0.02	0.13	24.5	5-Sep
	+Apparent temperature (°C)	0.18	0.12	0.24	78.9	25-Aug	0.05	-0.03	0.12	8.7	17-Sep
	+Sunlight hours	0.10	0.03	0.17	1.7	6-Sep	0.07	0.01	0.13	62.7	26-Jul
	+Precipitation (mm)	0.09	0.04	0.15	-6.1	27-Sep	0.04	0.00	0.08	-16.6	4-Oct
Low-density lipoprotein-cholesterol (mmol/L) †											
Crude		0.09	0.03	0.15	-10.6	2-Oct	0.08	0.01	0.14	88.3	18-Aug
Model 1		0.10	0.05	0.16	0.0	24-Sep	0.04	-0.015	0.10	0.0	21-Aug
Model 2	+Lifestyle markers*	0.11	0.05	0.16	4.2	25-Sep	0.04	-0.01	0.10	6.9	21-Aug

Model 3	+Ambient temperature (°C)	0.17	0.12	0.23	68.0	23-Aug	0.03	-0.07	0.12	-33.1	4-Dec
	+Apparent temperature (°C)	0.18	0.12	0.24	75.0	23-Aug	0.05	-0.05	0.14	11.9	27-Dec
	+Sunlight hours	0.11	0.03	0.18	1.8	12-Sep	0.07	-0.01	0.15	78.4	17-Jul
	+Precipitation (mm)	0.10	0.05	0.16	1.1	24-Sep	0.04	-0.02	0.10	-1.2	20-Aug
High density lipoprotein-cholesterol (mmol/L)											
Crude		0.01	0.00	0.03	-11.7	30-Apr	0.01	-0.01	0.02	-16.5	4-Jan
Model 1		0.02	0.00	0.03	0.0	6-May	0.01	-0.01	0.02	0.0	20-Jan
Model 2	+Lifestyle markers*	0.02	0.00	0.04	22.4	22-Apr	0.01	0.00	0.02	32.0	16-Jan
Model 3	+Ambient temperature (°C)	0.02	0.00	0.04	24.8	16-Mar	0.02	0.00	0.05	226.9	20-Jul
	+Apparent temperature (°C)	0.02	0.00	0.04	38.8	11-Mar	0.02	0.00	0.05	224.7	23-Jul
	+Sunlight hours	0.01	-0.01	0.03	-29.3	2-Apr	0.00	-0.01	0.02	-36.2	29-Apr
	+Precipitation (mm)	0.01	-0.01	0.03	-19.6	15-May	0.01	-0.01	0.02	6.6	5-Feb
Glucose(mmol/L)											
Crude		0.02	0.00	0.03	4.0	8-May	0.01	-0.01	0.02	6.7	26-Dec
Model 1		0.02	0.00	0.03	0.0	13-May	0.01	-0.01	0.02	0.0	22-Nov
Model 2	+Lifestyle markers*	0.01	0.00	0.03	-10.6	11-May	0.01	-0.01	0.02	-12.1	27-Oct
Model 3	+Ambient temperature (°C)	0.05	0.03	0.06	183.0	4-Jul	0.01	-0.01	0.03	72.7	17-Aug
	+Apparent temperature (°C)	0.04	0.03	0.06	171.1	4-Jul	0.01	-0.01	0.03	100.0	16-Aug
	+Sunlight hours	0.02	0.00	0.03	0.3	14-May	0.01	0.00	0.03	107.5	7-Dec
	+Precipitation (mm)	0.02	0.00	0.03	-4.2	15-May	0.01	-0.01	0.02	1.7	25-Nov
Waist-to-hip ratio											
Crude		0.01	0.00	0.01	-21.7	12-Jul	0.01	0.01	0.01	4.8	13-Aug
Model 1		0.01	0.01	0.01	0.0	20-Jun	0.01	0.01	0.01	0.0	13-Aug
Model 2	+Lifestyle markers*	0.01	0.01	0.01	7.2	29-Jun	0.01	0.01	0.01	10.1	9-Aug
Model 3	+Ambient temperature (°C)	0.01	0.01	0.02	42.7	2-Jul	0.00	0.00	0.01	-41.8	4-Sep
	+Apparent temperature (°C)	0.01	0.01	0.02	34.9	1-Jul	0.00	0.00	0.01	-52.9	16-Sep
	+Sunlight hours	0.01	0.01	0.02	11.2	20-Jun	0.01	0.00	0.01	-15.7	27-Aug
	+Precipitation (mm)	0.01	0.00	0.01	-2.7	26-Jun	0.01	0.01	0.01	-0.5	13-Aug

* Sample size in lifestyle markers models are: middle-aged: 6,288 observations (4,433 participants); elderly: 9,963 observations (5,994 participants). †Sample size for LDL-cholesterol is Middle-aged: 5,410 observations (4,316 participants); Elderly: 6,577 observations (5,586 participants); in the lifestyle markers models is: middle-aged 5,404 observations (4311 participants) and elderly 6,571 observations (5,581 participants)

Appendix 22. Seasonality and effect of lifestyle markers and meteorological factors on the seasonality of the cardiovascular risk factors, according to sex and age groups

CVRF	Middle-aged (<65 years)										Elderly (≥65 years)									
	Male n = 3,613 observations (2,450 participants)					Female n = 4,650 observations (3,133 participants)					Male n = 5,334 observations (2,049 participants)					Female n = 7,126 observations (2,773 participants)				
	SV	95%	CI	SV change (%)	Peak	SV	95%	CI	SV change (%)	Peak	SV	95%	CI	SV change (%)	Peak	SV	95%	CI	SV change (%)	Peak
Systolic blood pressure (mmHg)																				
Crude model	5.47	4.15	6.80	7.9	25-Dec	5.01	3.76	6.25	19.4	25-Dec	3.07	1.72	4.42	-4.8	21-Dec	4.93	3.64	6.22	1.0	30-Dec
Model 1	5.07	3.74	6.40	0.0	30-Dec	4.19	2.97	5.41	0.0	8-Jan	3.23	1.90	4.56	0.0	26-Dec	4.88	3.62	6.15	0.0	1-Jan
+Lifestyle markers	5.17	3.87	6.48	2.0	26-Dec	4.17	2.98	5.37	-0.5	8-Jan	3.03	1.70	4.35	-6.3	26-Dec	4.74	3.48	6.00	-2.9	0-Jan
+Ambient temperature (°C)	2.20	0.81	3.58	-56.6	8-Oct	1.16	-0.33	2.65	-72.3	4-Oct	1.46	-0.70	3.61	-54.9	8-Sep	1.31	-1.03	3.65	-73.1	29-Oct
+Apparent temperature (°C)	2.59	1.21	3.96	-49.0	24-Sep	1.73	0.19	3.28	-58.6	7-Sep	1.75	-0.48	3.98	-45.7	4-Sep	1.47	-0.86	3.81	-69.8	4-Oct
+Sunlight hours	5.81	4.12	7.49	14.5	28-Dec	4.04	2.75	5.33	-3.6	8-Jan	3.11	1.77	4.44	-3.8	27-Dec	5.34	3.56	7.12	9.3	1-Jan
+Precipitation (mm)	5.08	3.71	6.45	0.2	29-Dec	4.18	2.91	5.44	-0.3	10-Jan	3.27	1.92	4.62	1.3	25-Dec	4.91	3.63	6.18	0.5	1-Jan
Diastolic blood pressure (mmHg)																				
Crude model	2.99	2.18	3.81	2.2	10-Jan	3.01	2.33	3.69	4.1	27-Dec	1.80	1.05	2.55	38.5	8-Jan	2.15	1.48	2.83	14.7	11-Jan
Model 1	2.93	2.10	3.75	0.0	8-Jan	2.89	2.20	3.58	0.0	27-Dec	1.30	0.55	2.04	0.0	15-Jan	1.88	1.20	2.55	0.0	17-Jan
+Lifestyle markers	2.94	2.13	3.75	0.5	5-Jan	2.88	2.21	3.55	-0.4	27-Dec	1.17	0.44	1.91	-9.5	17-Jan	1.83	1.16	2.50	-2.7	16-Jan
+Ambient temperature (°C)	0.95	0.09	1.80	-67.7	24-Sep	1.31	0.47	2.15	-54.7	4-Nov	0.89	-0.31	2.09	-31.4	17-Jan	0.53	-0.71	1.77	-71.8	24-Jul
+Apparent temperature (°C)	1.22	0.37	2.07	-58.4	11-Sep	1.39	0.52	2.27	-51.9	11-Oct	0.84	-0.41	2.08	-35.5	13-Jan	0.55	-0.69	1.79	-70.8	29-Jul
+Sunlight hours	3.07	2.03	4.11	5.0	7-Jan	2.86	2.13	3.59	-1.1	27-Dec	1.34	0.60	2.09	3.5	14-Jan	1.79	0.84	2.73	-4.8	19-Jan
+Precipitation (mm)	2.91	2.06	3.76	-0.5	7-Jan	2.87	2.16	3.59	-0.6	28-Dec	1.29	0.54	2.05	-0.2	17-Jan	1.90	1.22	2.58	1.3	19-Jan
Total cholesterol (mmol/L)																				
Crude model	0.04	-0.04	0.11	-35.6	27-Oct	0.17	0.10	0.23	10.1	23-Oct	0.06	0.00	0.12	33.7	12-Nov	0.06	0.00	0.11	65.1	22-Sep
Model 1	0.06	-0.02	0.13	0.0	5-Oct	0.15	0.09	0.21	0.0	18-Oct	0.04	-0.0073	0.09	0.0	3-Nov	0.04	-0.01	0.08	0.0	7-Oct
+Lifestyle markers	0.06	-0.01	0.13	10.5	4-Oct	0.16	0.09	0.22	3.3	18-Oct	0.04	-0.01	0.09	-3.2	23-Oct	0.04	-0.01	0.09	5.7	5-Oct
+Ambient temperature (°C)	0.15	0.07	0.22	161.6	15-Aug	0.17	0.10	0.25	15.3	21-Sep	0.05	-0.03	0.14	23.1	20-Sep	0.09	0.00	0.18	148.8	11-Aug
+Apparent temperature (°C)	0.14	0.07	0.22	155.5	16-Aug	0.17	0.09	0.25	15.0	22-Sep	0.05	-0.04	0.14	19.6	25-Sep	0.09	0.00	0.18	150.5	12-Aug
+Sunlight hours	0.06	-0.03	0.15	11.0	20-Aug	0.14	0.07	0.20	-8.7	2-Oct	0.07	0.02	0.12	57.9	17-Jul	0.04	-0.03	0.11	10.1	22-Aug
+Precipitation (mm)	0.04	-0.03	0.12	-24.2	4-Oct	0.15	0.08	0.21	-1.6	18-Oct	0.04	-0.02	0.09	-17.6	7-Nov	0.03	-0.02	0.08	-18.8	8-Oct
Low density lipoprotein-cholesterol (mmol/L)*																				
Crude model	0.04	-0.03	0.12	-35.7	4-Oct	0.14	0.07	0.21	0.1	23-Oct	0.06	-0.02	0.14	17.3	7-Oct	0.09	0.01	0.16	100.4	21-Aug
Model 1	0.07	-0.004	0.14	0.0	12-Sep	0.14	0.07	0.20	0.0	19-Oct	0.05	-0.021	0.12	0.0	23-Sep	0.04	-0.03	0.11	0.0	29-Aug
+Lifestyle markers	0.07	0.00	0.14	7.2	12-Sep	0.14	0.08	0.20	2.6	17-Oct	0.05	-0.02	0.12	3.2	9-Sep	0.05	-0.02	0.12	13.1	31-Aug
+Ambient temperature (°C)	0.08	0.00	0.15	13.2	30-Aug	0.16	0.08	0.24	18.9	17-Sep	0.13	0.02	0.24	159.8	0-Jan	0.10	-0.02	0.22	128.8	3-Aug
+Apparent temperature (°C)	0.07	0.00	0.15	11.3	1-Sep	0.17	0.09	0.25	23.8	14-Sep	0.15	0.03	0.26	196.5	5-Jan	0.09	-0.03	0.21	106.7	5-Aug
+Sunlight hours	0.07	-0.02	0.16	7.6	27-Aug	0.13	0.06	0.20	-4.3	11-Oct	0.06	-0.01	0.13	14.8	11-Aug	0.04	-0.05	0.14	1.8	23-Aug
+Precipitation (mm)	0.07	-0.01	0.14	-2.8	10-Sep	0.14	0.07	0.21	3.6	19-Oct	0.05	-0.02	0.12	2.7	23-Sep	0.04	-0.03	0.11	-2.3	27-Aug
High density lipoprotein-cholesterol (mmol/L)																				
Crude model	0.02	0.00	0.04	6.1	21-Jan	0.01	-0.01	0.03	13.8	14-Apr	0.00	-0.01	0.02	8.7	1-Jul	0.01	0.00	0.03	-8.7	0-Jan
Model 1	0.02	-0.004	0.04	0.0	5-Feb	0.01	-0.01	0.03	0.0	9-Apr	0.00	-0.01	0.02	0.0	17-Jun	0.02	-0.0003	0.03	0.0	11-Jan
+Lifestyle markers	0.02	0.00	0.04	25.5	18-Feb	0.01	-0.01	0.03	-1.9	18-Mar	0.00	-0.01	0.02	-45.1	2-Feb	0.02	0.00	0.03	11.3	16-Jan
+Ambient temperature (°C)	0.01	-0.01	0.03	-54.3	15-Feb	0.02	-0.01	0.04	57.6	2-Mar	0.04	0.02	0.07	849.6	21-Jul	0.02	-0.01	0.06	52.5	27-Jul
+Apparent temperature (°C)	0.01	-0.01	0.03	-54.6	13-Feb	0.02	-0.01	0.05	98.0	25-Feb	0.04	0.02	0.07	853.1	22-Jul	0.02	-0.01	0.06	50.1	30-Jul
+Sunlight hours	0.03	0.00	0.06	79.6	13-Jan	0.01	-0.01	0.04	31.1	4-May	0.03	0.01	0.04	482.3	17-Jun	0.01	-0.01	0.04	-23.8	12-Jan
+Precipitation (mm)	0.02	-0.01	0.04	-5.4	30-Jan	0.01	-0.01	0.03	-12.6	9-Apr	0.01	-0.01	0.02	46.4	24-May	0.02	0.00	0.03	-0.7	15-Jan
Glucose(mmol/L)																				
Crude model	0.02	0.00	0.04	-6.6	10-Jun	0.01	-0.01	0.02	-27.9	23-May	0.01	-0.01	0.03	-24.7	2-Sep	0.03	0.01	0.04	57.1	15-Jan
Model 1	0.02	0.002	0.04	0.0	8-Jun	0.01	-0.01	0.02	0.0	1-May	0.01	0.0001	0.03	0.0	8-Oct	0.02	0.004	0.03	0.0	30-Dec

CVRF	Middle-aged (<65 years)										Elderly (≥65 years)									
	Male n = 3,613 observations (2,450 participants)					Female n = 4,650 observations (3,133 participants)					Male n = 5,334 observations (2,049 participants)					Female n = 7,126 observations (2,773 participants)				
	SV	95%	CI	SV change (%)	Peak	SV	95%	CI	SV change (%)	Peak	SV	95%	CI	SV change (%)	Peak	SV	95%	CI	SV change (%)	Peak
+Lifestyle markers	0.02	0.00	0.04	-16.6	12-Jun	0.01	0.00	0.02	10.8	24-Apr	0.02	0.00	0.03	5.5	26-Sep	0.01	0.00	0.03	-17.9	25-Dec
+Ambient temperature (°C)	0.07	0.05	0.09	218.3	12-Jul	0.02	0.01	0.04	160.7	1-Jul	0.03	0.01	0.06	116.1	15-Aug	0.01	-0.01	0.04	-13.5	24-Dec
+Apparent temperature (°C)	0.06	0.04	0.08	204.9	12-Jul	0.02	0.00	0.04	139.6	30-Jun	0.03	0.01	0.06	124.3	15-Aug	0.01	-0.01	0.04	-28.1	14-Dec
+Sunlight hours	0.03	0.00	0.05	27.6	12-Jun	0.01	0.00	0.03	49.6	19-May	0.02	0.01	0.04	44.8	7-Nov	0.02	0.01	0.04	28.6	28-Dec
+Precipitation (mm)	0.02	0.00	0.04	3.3	6-Jun	0.01	-0.01	0.02	5.4	30-Apr	0.01	0.00	0.03	-12.1	6-Oct	0.02	0.00	0.03	5.1	27-Dec
Waist-to-hip ratio																				
Crude model	0.01	0.00	0.01	-24.1	23-Jun	0.01	0.01	0.01	-5.8	20-Jul	0.01	0.00	0.01	4.4	5-Sep	0.01	0.01	0.01	4.4	18-Jul
Model 1	0.01	0.00	0.01	0.0	3-Jun	0.01	0.01	0.01	0.0	5-Jul	0.01	0.00	0.01	0.0	7-Sep	0.01	0.01	0.01	0.0	22-Jul
+Lifestyle markers	0.01	0.01	0.01	-3.2	21-Jun	0.01	0.01	0.02	7.4	6-Jul	0.01	0.00	0.01	26.3	17-Aug	0.01	0.01	0.01	5.7	21-Jul
+Ambient temperature (°C)	0.01	0.01	0.02	36.0	18-Jun	0.02	0.01	0.02	54.0	13-Jul	0.00	0.00	0.01	-27.4	16-Oct	0.00	0.00	0.01	-54.8	27-Jul
+Apparent temperature (°C)	0.01	0.01	0.02	30.1	16-Jun	0.02	0.01	0.02	49.9	14-Jul	0.00	0.00	0.01	-29.2	19-Oct	0.00	-0.01	0.01	-70.9	27-Jul
+Sunlight hours	0.01	0.00	0.01	-8.1	2-Jun	0.01	0.01	0.02	10.0	4-Jul	0.01	0.00	0.01	4.6	28-Aug	0.01	0.00	0.01	-45.3	22-Aug
+Precipitation (mm)	0.01	0.00	0.01	-3.9	9-Jun	0.01	0.01	0.02	-0.4	9-Jul	0.01	0.00	0.01	-2.5	6-Sep	0.01	0.01	0.01	0.7	25-Jul

Appendix 23. Procedures and PAF estimations

Example of PAF estimations procedure

	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
Deciles			BMI			
			High blood pressure			
0	9.5	10.6	11.5	11.4	1.6	0.0
1	9.0	9.0	8.3	8.2	1.6	0.0
2	10.9	9.1	10.3	11.0	1.5	0.0
3	11.1	10.9	11.2	11.2	1.4	0.0
4	10.5	10.0	9.7	9.0	1.3	0.0
5	11.2	11.9	9.5	10.1	1.2	0.0
6	10.6	8.8	9.8	8.9	1.1	0.1
7	9.0	10.4	10.8	10.1	Ref	
8	9.0	9.9	9.9	10.7	0.9	0.0
9	9.1	9.6	8.9	9.5	0.9	0.1
PAF	16.3	15.6	15.8	15.6		
PAF Total	17.2	16.8	17.0	16.5		

pd(Season$_i$): Proportion of cases that fell on each exposure category, per season. In the example, the numbers below the header "pd(W)" correspond to the percentage of participants with high blood pressure at each category of body mass index during the winter season, among all participants with high blood pressure in winter season.

RR: Corresponds to the adjusted relative risk for each exposure category. In the example, the RR corresponds to the relative risk for each category of body mass index compared to the reference category, which for BMI is at the decile 7, which contained the cut-off of BMI for obesity (24kg/m^2)

Ref: Reference category. Exposure categories are decile containing BMI ≤24kg/m^2, physical activity ≤0 zMET-hours/week, lowest deciles of ambient and apparent temperature and sunlight hours and highest deciles of precipitation.

p-value: Significance of the RR at a confidence level of 95% of the relative risk.

PAF: Population attributable fraction of each of the categories of the exposure. It is calculated as $1 - \sum_{i=0}^{k} \frac{pd_i}{RR_i}$.[146] For example, for winter, it would be calculated as 1-((9.5/1.6)+(9.0/1.6)+(10.9/1.5)....)

PAF Total: Population attributable fraction of the exposures considered in each model (either lifestyle markers or meteorological factors). For the lifestyle markers model, it is calculated as $1 - \left(1 - PAF_{BMI_{season=i}}\right) * \left(1 - PAF_{physical\ activity_{season}}\right)$. For the meteorological factors model, it is calculated as $1 - \left(1 - PAF_{MetFacLag0-1_{season=i}}\right) * \left(1 - PAF_{MetFacLag2-6_{season}}\right)$.

(a) PAF Estimates

Deciles	Middle-aged												Elderly									
	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
	BMI						Physical activity						BMI						Physical activity					
High blood pressure																								
0	9.5	10.6	11.5	11.4	1.6	0.0	10.3	11.5	10.0	8.7	1.1	0.4	10.1	8.7	8.2	7.9	1.1	0.0	7.9	7.9	6.1	5.8	1.0	0.0
1	9.0	9.0	8.3	8.2	1.6	0.0	10.8	11.2	10.3	8.2	1.0	0.7	10.4	9.8	9.2	9.3	1.1	0.0	9.3	8.1	8.0	7.9	1.1	0.0
2	10.9	9.1	10.3	11.0	1.5	0.0	7.8	10.4	9.7	9.3	1.0	0.6	10.4	10.4	9.9	9.7	1.1	0.0	10.7	7.8	7.7	9.6	1.0	0.0
3	11.1	10.9	11.2	11.2	1.4	0.0	10.8	8.9	8.9	10.7	1.0	0.9	10.9	11.5	10.0	11.7	1.0	0.2	12.9	10.2	9.9	11.8	1.0	0.0
4	10.5	10.0	9.7	9.0	1.3	0.0	10.5	10.1	9.8	12.3	Ref		9.8	10.0	9.7	10.6	1.0	0.1	13.7	14.2	13.7	13.8	Ref	
5	11.2	11.9	9.5	10.1	1.2	0.0	9.4	7.3	8.3	9.2	0.9	0.2	10.9	10.6	12.0	10.7	1.0	0.9	11.9	13.5	13.5	12.6	1.0	0.0
6	10.6	8.8	9.8	8.9	1.1	0.1	10.9	9.1	9.7	12.9	1.0	0.9	10.9	10.3	11.4	10.8	1.0	0.4	8.7	11.2	11.6	11.2	1.0	0.0
7	9.0	10.4	10.8	10.1	Ref		9.7	10.8	10.1	9.2	1.0	0.7	9.4	10.6	10.8	10.7	Ref		8.4	9.1	9.1	8.8	1.1	0.0
8	9.0	9.9	9.9	10.7	0.9	0.0	8.5	9.5	10.8	9.3	1.0	0.5	9.3	9.1	9.1	9.3	0.9	0.0	8.5	8.6	8.6	9.4	1.0	0.0
9	9.1	9.6	8.9	9.5	0.9	0.1	11.2	11.2	12.4	10.2	1.0	0.8	8.0	9.1	9.6	9.3	0.9	0.0	7.9	9.3	11.9	9.2	1.1	0.0
PAF	16.3	15.6	15.8	15.6			1.1	1.5	1.4	1.1			1.2	1.0	0.7	0.8			1.9	2.1	2.3	2.1		
PAF Total	17.2	16.8	17.0	16.5									3.1	3.1	3.0	2.9								
High-very high SCORE risk																								
0	7.1	8.5	9.8	8.1	1.2	0.2	8.6	9.5	7.4	6.6	1.1	0.3	7.5	6.4	5.4	5.9	1.0	0.8	7.7	6.8	5.2	5.6	1.0	0.0
1	9.7	8.7	8.2	6.8	1.5	0.0	8.3	9.9	8.4	6.0	1.1	0.2	8.2	8.2	7.2	8.0	1.0	0.7	9.0	6.7	7.3	7.1	1.1	0.0
2	10.6	9.3	9.7	12.1	1.4	0.0	6.4	10.2	8.0	8.5	1.1	0.6	10.4	9.0	8.9	9.0	1.0	0.3	9.8	7.9	7.1	8.8	1.0	0.0
3	10.1	10.9	11.9	12.0	1.4	0.0	9.9	8.6	7.8	9.5	1.2	0.1	11.1	11.7	9.8	11.6	1.0	0.6	12.2	10.1	9.5	10.7	1.0	0.0
4	11.4	10.5	10.3	9.6	1.3	0.0	11.9	9.0	8.7	13.0	Ref		10.1	10.8	10.9	11.2	1.0	0.5	13.9	14.2	13.6	13.2	Ref	
5	12.3	13.1	11.2	10.7	1.2	0.2	10.3	6.6	9.3	9.0	0.9	0.3	12.0	11.3	13.4	11.1	1.0	0.5	11.9	13.4	12.7	12.5	1.0	0.0
6	11.1	9.3	9.5	9.2	1.1	0.5	12.4	9.9	10.7	15.0	1.1	0.3	12.0	11.4	11.7	11.9	0.9	0.1	8.7	11.2	10.7	12.1	1.0	0.0
7	10.0	11.4	11.9	11.0	Ref		10.9	11.1	10.8	10.2	1.0	0.9	10.9	11.1	11.6	11.7	Ref		8.6	9.7	9.3	9.0	1.0	0.0
8	8.5	9.4	10.0	11.3	0.9	0.3	8.0	10.4	11.4	9.9	1.0	0.7	9.2	8.8	9.2	9.9	0.9	0.0	8.1	8.1	9.3	10.0	1.0	0.0
9	9.2	8.9	7.8	9.2	1.0	0.7	13.4	14.9	17.4	12.4	1.1	0.6	8.6	11.2	11.9	9.6	0.9	0.0	10.1	12.0	15.3	11.0	1.0	0.0
PAF	13.6	13.0	13.0	12.7			5.1	5.8	5.0	5.0			-3.5	-3.8	-4.0	-3.9	0.9	0.0	1.4	1.2	1.2	1.2		
PAF Total	18.0	18.1	17.4	17.0									-2.1	-2.6	-2.7	-2.6								
High LDL-cholesterol																								
0	8.4	9.8	9.9	10.2	1.0	0.3	11.0	12.0	10.5	9.5	1.0	0.6	8.9	7.8	7.3	7.3	1.0	0.1	9.3	9.2	6.5	6.5	1.0	0.0
1	8.8	8.2	8.0	7.6	1.1	0.0	11.0	11.8	10.9	8.5	1.0	0.4	9.8	9.1	8.4	8.8	1.0	0.6	10.5	8.9	8.5	8.2	1.0	0.0
2	10.2	8.7	9.7	10.4	1.1	0.0	7.9	10.2	9.3	9.4	1.0	0.4	9.8	9.7	9.5	9.2	1.0	0.8	11.1	8.0	7.8	9.6	1.0	0.0
3	10.9	10.6	11.0	10.8	1.1	0.1	10.7	8.8	8.8	10.3	0.9	0.1	10.9	11.3	9.8	11.4	1.0	0.3	12.2	10.5	9.9	11.8	1.0	0.0
4	10.2	10.0	9.8	8.8	1.1	0.1	10.2	9.7	9.3	11.3	Ref		9.5	9.9	9.6	10.2	1.0	0.9	13.3	13.4	13.3	13.7	Ref	
5	11.0	11.9	9.6	10.2	1.1	0.0	8.2	6.8	8.1	8.7	1.0	0.3	11.3	10.8	12.3	10.5	1.0	0.8	11.5	13.3	12.8	12.0	1.0	0.0
6	11.0	9.0	9.9	9.1	1.0	0.9	10.5	8.9	9.4	12.2	1.0	0.6	11.2	10.5	11.3	11.4	1.0	0.2	8.4	10.8	11.5	11.4	1.0	0.0
7	9.7	10.7	11.2	10.8	Ref		10.0	10.9	10.1	9.6	1.0	0.3	9.8	11.1	11.1	11.2	Ref		7.9	8.8	9.0	8.8	0.9	0.0
8	10.0	10.5	10.7	11.1	1.0	0.9	9.2	9.7	11.1	9.6	1.0	0.7	10.0	9.8	10.0	9.6	0.9	0.0	7.8	7.5	8.3	8.7	0.9	0.0
9	9.9	10.6	10.1	10.9	0.9	0.2	11.2	11.2	12.6	10.9	0.9	0.1	8.9	10.1	10.6	10.4	0.9	0.0	8.0	9.4	12.5	9.2	1.0	0.0
PAF	3.8	3.7	3.6	3.6			-3.0	-2.9	-3.0	-3.0			-1.0	-1.0	-1.1	-1.0			-3.0	-3.1	-3.2	-3.2		
PAF Total	0.9	0.9	0.7	0.7									4.0	4.1	4.3	4.2								
High glucose levels																								
0	16.0	14.5	19.8	20.3	3.4	0.0	9.6	10.4	10.4	8.2	1.1	0.6	17.1	12.7	12.2	10.3	1.7	0.0	7.1	6.9	4.5	4.7	1.0	0.1
1	8.5	11.6	8.8	7.5	2.9	0.0	10.5	11.1	7.5	5.6	0.9	0.8	12.9	12.6	11.6	12.1	1.5	0.0	8.1	7.8	6.5	7.3	0.9	0.1
2	12.6	11.5	10.5	12.1	2.2	0.0	6.5	9.6	10.0	9.1	0.9	0.7	11.7	12.0	11.9	10.9	1.5	0.0	11.2	7.8	7.0	8.5	1.0	0.1
3	11.1	10.3	12.2	10.1	1.7	0.0	9.2	8.2	8.1	9.0	0.7	0.1	10.0	10.9	10.7	13.2	1.3	0.0	12.8	8.2	8.8	12.4	0.9	0.1
4	12.6	11.9	7.7	9.7	1.6	0.0	9.7	8.6	8.4	10.8	Ref		8.3	9.9	8.9	11.4	1.0	0.7	11.1	13.5	12.5	12.8	Ref	
5	8.9	10.1	8.1	8.0	1.4	0.1	11.8	8.1	7.3	9.2	1.0	0.8	8.3	9.5	11.1	9.9	1.2	0.2	10.1	13.2	14.2	12.9	1.0	0.1

	Middle-aged												Elderly											
Deciles	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value		pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value		pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
6	8.5	6.8	8.2	6.7	1.2	0.4		10.6	9.6	9.8	12.8	0.9	0.7	9.8	9.6	10.8	8.4	1.0	0.9		9.6	11.8	10.7	10.6	1.0	0.1
7	8.0	8.0	10.6	8.3	Ref			12.0	10.3	10.7	10.5	1.2	0.3	8.4	9.9	9.5	9.5	Ref			9.4	10.4	9.9	9.4	1.0	0.1
8	6.0	8.5	7.6	10.3	0.8	0.5		7.5	9.0	12.3	11.1	0.9	0.6	8.0	6.5	7.0	8.4	0.6	0.0		10.6	7.8	8.9	9.8	1.1	0.1
9	8.0	6.8	6.4	7.0	0.5	0.0		12.5	15.2	15.5	13.7	1.1	0.6	5.5	6.4	6.4	5.9	0.6	0.0		9.9	12.5	17.0	11.4	1.1	0.1
PAF	28.2	28.7	29.4	28.4				-3.1	-2.9	-2.9	-3.6			11.0	9.9	9.2	8.3				0.3	0.9	1.7	0.7		
PAF Total	26.0	26.6	27.3	25.8										11.3	10.7	10.7	8.9									
Deciles	Ambient temperature (Lag 0-1)						.	Ambient temperature (Lag 2-6)						Ambient temperature (Lag 0-1)						.	Ambient temperature (Lag 2-6)					
	High blood pressure																									
0	0.0	0.7	29.7	14.3	Ref			0.0	0.0	26.9	18.3	Ref		0.0	0.4	25.8	13.5	Ref			0.0	0.0	22.2	17.1	Ref	
1	0.0	0.7	22.2	18.4	1.1	0.2		0.0	0.0	27.1	20.0	1.0	0.8	0.0	1.6	22.4	18.4	1.1	0.1		0.0	0.0	22.8	16.0	1.0	0.0
2	0.0	1.5	17.3	17.1	1.1	0.1		0.0	0.7	18.0	22.4	1.0	0.6	0.0	2.5	23.0	17.1	1.1	0.0		0.0	0.0	23.9	17.7	1.0	0.0
3	2.1	5.8	16.4	21.0	1.2	0.0		0.3	4.1	12.5	15.0	1.1	0.1	1.0	6.9	16.2	16.3	1.1	0.0		1.3	6.4	18.8	18.7	1.0	0.0
4	8.4	12.0	8.5	14.9	1.2	0.1		7.8	8.9	10.9	14.6	1.1	0.1	6.8	12.0	7.0	12.0	1.2	0.0		4.9	13.5	6.7	14.3	1.0	0.0
5	15.8	13.9	4.0	7.9	1.2	0.0		18.8	14.0	3.9	6.0	1.0	1.0	10.3	15.2	3.0	9.4	1.2	0.0		12.0	14.1	4.0	5.6	0.9	0.0
6	18.9	14.5	1.5	2.9	1.2	0.1		17.7	18.6	0.6	1.6	1.1	0.4	13.9	14.7	1.5	6.2	1.1	0.0		16.6	14.7	1.5	6.9	1.0	0.0
7	16.5	17.7	0.3	2.3	1.2	0.0		17.6	18.8	0.0	1.2	1.1	0.6	18.1	16.6	1.1	4.7	1.2	0.0		16.7	18.9	0.0	1.9	1.0	0.1
8	19.2	18.7	0.0	1.0	1.3	0.0		19.8	19.4	0.0	0.9	1.0	0.7	21.7	15.9	0.0	1.5	1.2	0.0		20.2	16.9	0.0	1.4	1.0	0.1
9	19.1	14.6	0.0	0.2	1.3	0.0		17.9	15.6	0.0	0.0	1.0	0.9	28.2	14.0	0.0	1.0	1.3	0.0		28.2	15.5	0.0	0.4	1.0	0.1
PAF	20.0	19.3	8.9	11.8				4.3	5.0	3.1	4.3			16.9	15.3	6.8	9.4				-2.5	-2.8	-1.2	-1.4		
PAF Total	23.4	23.3	11.7	15.6										14.8	13.0	5.7	8.1									
	High very high SCORE risk																									
0	0.0	0.4	28.1	14.6	Ref			0.0	0.0	25.5	18.3	Ref		0.0	0.3	24.8	13.5	Ref			0.0	0.0	21.7	17.2	Ref	
1	0.0	0.5	23.0	21.1	0.9	0.4		0.0	0.0	25.8	21.5	1.2	0.2	0.0	1.6	22.8	19.0	1.1	0.0		0.0	0.0	22.2	16.2	0.9	0.0
2	0.0	2.0	16.1	15.7	1.1	0.4		0.0	0.5	19.6	21.3	1.3	0.1	0.0	2.5	23.3	16.5	1.1	0.1		0.0	0.0	25.9	17.2	1.0	0.0
3	1.5	5.8	19.4	19.9	1.0	1.0		0.4	4.5	12.8	14.8	1.3	0.1	1.0	6.8	17.0	16.5	1.1	0.0		1.8	6.8	18.1	19.6	0.9	0.1
4	9.1	11.2	8.0	14.7	1.0	0.8		7.1	9.1	11.4	14.6	1.2	0.1	7.3	12.0	6.6	12.2	1.1	0.0		5.0	13.6	6.2	13.7	1.0	0.1
5	16.5	14.8	3.5	8.2	1.2	0.3		18.7	14.3	4.2	6.3	1.0	0.9	10.5	15.6	2.6	9.4	1.2	0.0		12.1	13.6	4.2	5.5	0.9	0.1
6	17.6	13.9	1.6	2.6	1.3	0.2		16.2	17.1	0.8	1.7	1.3	0.2	13.8	14.8	1.4	6.2	1.1	0.2		16.5	13.5	1.8	7.2	1.0	0.1
7	16.0	19.1	0.3	2.3	1.3	0.1		18.3	19.2	0.0	0.8	1.1	0.4	17.5	17.1	1.4	4.5	1.2	0.0		16.3	18.6	0.0	1.7	1.0	0.1
8	19.6	18.8	0.0	0.9	1.2	0.4		20.6	21.1	0.0	0.6	1.2	0.4	22.1	15.3	0.0	1.3	1.2	0.0		20.0	17.8	0.0	1.3	1.0	0.1
9	19.7	13.5	0.0	0.1	1.4	0.0		18.7	14.3	0.0	0.0	1.4	0.1	27.8	14.0	0.0	0.9	1.2	0.0		28.4	15.9	0.0	0.5	1.0	0.1
PAF	19.4	17.3	0.6	2.6				15.3	15.8	12.6	13.8			13.8	12.9	7.4	9.3				-2.3	-3.1	-3.8	-3.8		
PAF Total	31.7	30.4	13.1	16.1										11.8	10.2	3.9	5.8									
	High LDL-cholesterol																									
0	0.1	3.7	29.6	9.9	Ref			0.0	2.1	28.5	13.0	Ref		0.1	2.3	30.2	8.2	Ref			0.1	2.0	29.1	10.4	Ref	
1	0.0	2.7	24.5	14.4	1.1	0.1		0.1	2.2	31.1	14.9	1.0	0.8	0.1	3.8	26.3	14.7	1.0	0.2		0.1	2.2	24.0	13.0	1.0	0.0
2	0.0	3.5	18.1	15.2	1.0	0.2		0.1	3.3	17.6	19.2	1.1	0.1	0.1	6.5	21.1	14.0	1.0	0.2		0.1	2.9	24.8	16.2	1.0	0.0
3	2.0	8.0	14.9	17.7	1.0	0.5		0.5	6.2	11.7	14.3	1.1	0.1	1.2	10.8	14.6	15.4	1.1	0.1		0.8	12.0	13.6	16.7	0.9	0.0
4	9.2	12.9	8.4	15.3	1.1	0.1		7.2	10.7	8.0	15.6	1.0	0.4	5.6	14.8	4.6	12.8	1.0	0.7		3.5	13.4	5.6	16.1	0.9	0.0
5	13.2	14.2	3.1	10.8	1.0	1.0		17.3	14.8	2.1	9.5	1.1	0.1	10.3	14.3	1.8	11.3	1.1	0.1		11.2	15.0	2.0	7.9	0.9	0.0
6	20.3	13.7	1.0	5.7	1.0	0.6		15.9	17.3	0.6	5.3	1.0	0.4	13.2	12.8	0.8	8.8	1.0	0.5		14.5	12.7	0.6	9.7	0.9	0.0
7	17.5	16.0	0.0	5.2	1.0	0.8		19.4	17.0	0.2	3.9	1.1	0.3	18.2	13.4	0.5	6.8	1.1	0.1		18.0	16.8	0.1	3.8	0.9	0.0
8	17.7	15.7	0.4	4.0	1.0	0.5		21.2	15.5	0.2	3.0	1.0	0.6	21.8	12.0	0.1	4.5	1.0	0.3		21.2	14.1	0.1	4.4	1.0	0.0
9	20.1	9.7	0.0	1.8	1.1	0.0		18.4	10.9	0.0	1.3	1.0	0.6	29.4	9.2	0.0	3.4	1.1	0.2		30.5	9.0	0.1	1.8	0.9	0.1
PAF	3.9	3.1	3.2	3.4				4.4	4.5	2.6	4.1			5.3	4.7	2.9	4.2				-6.9	-7.1	-3.5	-5.7		
PAF Total	8.1	7.5	5.7	7.3										-1.2	-2.0	-0.4	-1.3									
	High glucose levels																									

	Middle-aged												Elderly									
Deciles	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
0	0.0	2.3	30.8	10.6	Ref		0.0	2.6	28.6	13.7	Ref		0.0	2.3	27.0	7.4	Ref		0.0	2.0	28.9	9.6	Ref	
1	0.0	2.4	26.0	14.4	1.1	0.7	0.0	2.4	29.8	16.4	0.9	0.5	0.1	3.6	27.7	13.6	1.1	0.5	0.1	2.6	24.5	11.3	1.1	0.1
2	0.0	5.2	19.4	14.3	1.1	0.5	0.0	3.1	18.4	20.4	0.9	0.5	0.2	5.3	21.3	14.2	1.0	0.8	0.2	3.0	23.5	14.9	1.2	0.1
3	1.3	7.7	12.2	19.7	1.1	0.5	0.3	7.4	13.2	12.7	1.0	0.8	1.5	10.2	15.6	14.9	0.9	0.4	1.7	11.6	14.2	19.5	1.3	0.1
4	8.1	13.8	8.6	15.7	1.2	0.4	7.1	8.8	7.7	16.9	0.8	0.2	5.7	15.3	5.2	12.3	1.1	0.7	2.9	12.8	5.4	15.7	1.3	0.1
5	13.8	13.5	2.2	10.5	1.7	0.0	17.7	15.8	1.4	7.1	1.0	0.9	11.8	15.2	1.4	11.7	1.0	0.9	10.0	14.4	2.2	8.5	1.4	0.1
6	20.3	13.5	0.6	6.2	1.7	0.0	16.9	18.6	0.6	5.2	0.7	0.2	12.1	12.3	1.0	10.5	0.9	0.5	14.2	13.8	1.1	9.3	1.2	0.1
7	20.1	16.8	0.1	3.9	1.5	0.1	17.0	14.6	0.1	3.7	0.8	0.3	18.1	14.7	0.5	6.2	1.0	0.9	18.9	16.4	0.0	4.4	1.1	0.2
8	14.5	16.4	0.2	3.4	1.9	0.0	22.2	15.3	0.2	3.2	0.9	0.7	22.8	11.5	0.2	4.9	1.0	0.9	22.1	14.6	0.3	4.9	1.3	0.2
9	21.8	8.5	0.0	1.4	1.7	0.1	18.7	11.3	0.0	0.8	1.0	1.0	27.7	9.6	0.0	4.4	1.1	0.6	29.8	8.9	0.0	2.0	1.2	0.2
PAF	38.2	31.8	7.7	17.6			-17.2	-17.9	-8.5	-13.6			0.5	-1.0	0.9	-0.8			18.8	19.3	11.7	17.5		
PAF Total	27.6	19.6	-0.1	6.4									19.2	18.5	12.5	16.9								
Deciles	Apparent temperature (Lag 0-1)						Apparent temperature (Lag 2-6)						Apparent temperature (Lag 0-1)						Apparent temperature (Lag 2-6)					
High blood pressure																								
0	0.0	0.4	29.1	16.9	Ref		0.0	0.0	24.4	22.7	Ref		0.0	0.0	24.7	14.4	Ref		0.0	0.0	19.7	19.1	Ref	
1	0.0	0.5	21.4	17.3	1.1	0.4	0.0	0.0	28.1	17.0	1.0	0.9	0.0	0.7	21.5	20.7	1.1	0.1	0.0	0.0	24.4	14.9	1.0	0.0
2	0.0	1.9	17.4	19.1	1.0	0.6	0.0	0.3	19.3	22.7	1.1	0.1	0.0	3.2	22.6	15.3	1.1	0.1	0.0	0.0	23.9	18.6	1.0	0.0
3	2.2	5.1	17.9	18.4	1.2	0.0	1.6	3.9	12.1	15.7	1.2	0.0	1.3	7.0	17.9	15.5	1.1	0.0	1.9	5.3	17.1	18.0	1.0	0.0
4	9.0	12.1	7.7	14.6	1.1	0.5	7.6	8.3	11.8	13.4	1.3	0.0	6.8	13.2	7.7	10.8	1.2	0.0	4.4	13.2	9.1	14.5	1.0	0.0
5	16.3	15.0	4.5	6.7	1.1	0.2	19.0	15.5	3.7	5.0	1.1	0.3	11.3	13.5	2.7	9.6	1.2	0.0	12.1	13.4	4.3	5.5	1.0	0.0
6	18.4	13.9	1.7	3.4	1.2	0.1	18.1	17.7	0.6	1.4	1.2	0.1	14.6	15.5	1.8	6.6	1.1	0.0	17.2	16.0	1.5	5.7	1.1	0.0
7	17.2	18.1	0.3	2.3	1.2	0.1	16.3	19.6	0.0	1.2	1.1	0.2	16.7	17.5	1.1	4.7	1.2	0.0	16.2	19.5	0.0	1.9	1.0	0.1
8	17.9	18.1	0.0	1.0	1.2	0.0	19.4	19.1	0.0	0.9	1.1	0.3	21.1	15.3	0.0	1.5	1.2	0.0	20.1	17.1	0.0	1.4	1.0	0.1
9	18.9	14.9	0.0	0.2	1.2	0.0	18.0	15.6	0.0	0.0	1.1	0.4	28.2	14.1	0.0	1.0	1.2	0.0	28.2	15.5	0.0	0.4	1.0	0.1
PAF	14.5	13.8	5.7	6.9			12.1	12.5	7.2	8.9			15.5	14.3	6.8	8.9			1.2	0.7	0.4	0.4		
PAF Total	24.9	24.5	12.4	15.2									16.5	14.9	7.2	9.3								
High-very high SCORE risk																								
0	0.0	0.2	27.8	17.4	Ref		0.0	0.0	23.4	23.1	Ref		0.0	0.0	23.4	15.0	Ref		0.0	0.0	18.9	19.3	Ref	
1	0.0	0.5	21.7	19.7	1.0	0.7	0.0	0.0	26.9	17.8	1.1	0.3	0.0	0.7	22.1	20.2	1.1	0.1	0.0	0.0	24.3	14.9	1.0	0.0
2	0.0	2.1	16.3	17.1	1.1	0.5	0.0	0.2	20.1	22.2	1.3	0.0	0.0	3.1	23.0	15.2	1.0	0.3	0.0	0.0	25.2	18.4	1.0	0.0
3	1.6	5.1	20.8	17.8	1.0	0.9	1.4	4.1	12.9	15.5	1.3	0.1	1.3	7.0	18.5	15.8	1.1	0.1	2.3	5.8	16.8	18.6	1.0	0.1
4	10.2	11.4	7.5	14.9	1.0	0.8	7.3	8.8	12.0	13.6	1.3	0.1	7.2	13.3	7.6	10.8	1.2	0.0	4.5	13.1	8.5	14.0	1.0	0.1
5	16.8	15.8	3.6	6.7	1.2	0.3	17.7	15.5	4.0	5.0	1.1	0.7	11.5	13.6	2.4	9.8	1.2	0.0	12.2	13.4	4.5	5.5	0.9	0.1
6	16.8	13.4	2.0	3.0	1.3	0.1	17.5	16.1	0.8	1.3	1.3	0.1	14.6	15.6	1.6	6.5	1.1	0.2	17.2	14.5	1.8	5.8	1.0	0.1
7	16.6	19.4	0.3	2.3	1.3	0.1	17.6	20.1	0.0	0.8	1.2	0.3	16.2	18.0	1.4	4.5	1.2	0.0	15.7	19.3	0.0	1.7	1.0	0.1
8	18.4	18.2	0.0	0.9	1.1	0.4	19.7	20.8	0.0	0.6	1.2	0.2	21.3	14.7	0.0	1.3	1.1	0.0	19.8	18.0	0.0	1.3	1.0	0.1
9	19.6	13.9	0.0	0.1	1.5	0.0	18.8	14.3	0.0	0.0	1.5	0.0	27.8	14.1	0.0	0.9	1.2	0.0	28.3	15.9	0.0	0.5	1.0	0.1
PAF	19.1	17.3	2.0	3.7			20.2	19.8	13.2	14.2			13.4	12.3	5.6	7.6			-1.5	-2.1	-2.3	-2.3		
PAF Total	35.4	33.6	15.0	17.4									12.1	10.5	3.4	5.4								
High LDL-cholesterol																								
0	0.1	3.2	29.7	11.9	Ref		0.0	1.5	28.6	16.5	Ref		0.1	1.8	29.9	9.5	Ref		0.1	1.5	27.7	12.0	Ref	
1	0.0	2.7	23.1	14.1	1.0	0.2	0.1	2.3	30.7	12.7	1.0	0.4	0.1	2.8	25.7	15.8	1.0	0.8	0.1	2.4	24.5	12.8	1.0	0.0
2	0.1	3.6	18.2	17.6	1.1	0.1	0.1	3.3	17.6	18.8	1.1	0.1	0.1	7.0	20.2	13.0	1.0	0.6	0.1	2.9	23.8	16.4	1.0	0.0
3	1.9	7.8	16.2	14.6	1.0	0.2	1.0	6.5	11.4	15.7	1.1	0.0	1.4	11.9	16.0	14.6	1.0	0.7	1.2	9.7	13.7	16.8	1.0	0.0
4	10.4	12.2	8.0	15.0	1.1	0.1	7.7	10.0	8.8	14.8	1.0	0.8	5.9	15.1	5.1	11.9	1.0	0.8	3.0	14.8	7.4	15.7	1.0	0.0
5	13.5	14.0	3.2	11.4	1.0	0.7	17.7	15.5	1.9	8.6	1.1	0.0	10.5	13.8	1.6	12.2	1.0	1.0	11.7	14.7	2.1	7.6	1.0	0.0
6	19.4	13.9	1.1	4.6	1.0	0.7	15.6	16.5	0.6	5.1	1.1	0.2	13.6	12.6	0.9	8.5	1.0	0.8	15.3	13.8	0.6	8.7	1.0	0.0
7	16.8	17.3	0.0	5.0	1.0	1.0	18.2	17.9	0.2	3.5	1.1	0.3	17.9	13.8	0.5	6.8	1.0	0.5	17.7	16.9	0.1	3.9	0.9	0.0
8	18.1	15.6	0.4	4.0	1.0	0.5	21.8	15.6	0.2	3.0	1.1	0.4	20.7	12.0	0.1	4.4	1.0	1.0	20.1	14.3	0.1	4.3	1.0	0.0

	Middle-aged												Elderly											
Deciles	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
9	19.8	9.7	0.0	1.8	1.1	0.0	18.0	10.9	0.0	1.3	1.1	0.3	29.7	9.2	0.0	3.4	1.0	0.7	30.5	9.0	0.1	1.8	1.0	0.1
PAF	4.2	3.5	3.2	3.7			5.9	5.8	3.2	4.6			1.0	0.8	0.4	0.6			-3.3	-3.6	-1.0	-2.4		
PAF Total	9.8	9.1	6.3	8.1									-2.3	-2.7	-0.5	-1.9								
	High glucose levels																							
0	0.0	2.1	32.0	12.3	Ref		0.0	1.5	28.0	18.2	1.0	0.0	0.0	1.7	26.1	8.8	Ref		0.0	1.6	27.3	11.4	Ref	
1	0.0	2.4	23.9	14.9	1.1	0.6	0.0	2.7	30.0	13.2	0.8	0.2	0.1	3.2	28.1	15.4	1.0	0.9	0.1	3.0	25.4	10.6	1.2	0.1
2	0.1	4.9	17.9	16.6	1.0	1.0	0.0	3.3	18.6	19.8	0.9	0.6	0.2	5.7	19.8	12.4	1.0	0.9	0.2	2.6	22.0	16.2	1.2	0.1
3	1.2	8.2	14.6	16.6	1.1	0.8	1.1	7.7	13.7	13.9	1.0	0.9	1.4	10.7	16.4	14.7	0.8	0.1	2.2	9.6	14.9	18.7	1.3	0.1
4	9.5	13.1	8.3	15.2	1.2	0.5	6.8	8.1	7.5	16.0	0.8	0.2	6.2	15.3	6.5	11.2	1.0	1.0	2.5	13.5	7.2	15.4	1.4	0.1
5	14.3	12.7	2.2	10.9	1.6	0.0	18.0	16.8	1.4	6.5	0.9	0.6	12.1	14.5	1.1	11.8	1.0	0.7	10.4	15.1	1.8	7.8	1.4	0.1
6	18.8	14.3	0.7	4.8	1.6	0.0	17.3	17.8	0.6	5.1	0.7	0.2	12.4	13.1	1.3	10.8	0.9	0.3	15.1	14.4	1.1	8.7	1.4	0.1
7	19.2	18.1	0.1	3.8	1.5	0.1	15.8	15.3	0.1	3.5	0.7	0.1	17.8	14.8	0.5	5.9	0.9	0.6	18.5	16.7	0.0	4.4	1.1	0.2
8	15.2	15.2	0.2	3.4	1.9	0.0	22.5	15.5	0.2	3.2	0.8	0.5	21.1	11.4	0.2	4.6	1.0	0.7	21.4	14.8	0.3	4.9	1.4	0.2
9	21.7	9.1	0.0	1.4	1.6	0.1	18.4	11.3	0.0	0.8	1.0	0.9	28.7	9.6	0.0	4.4	1.0	0.9	29.6	8.9	0.0	2.0	1.3	0.2
PAF	36.0	29.3	4.7	13.4			-23.6	-22.9	-10.7	-14.2			-4.8	-6.6	-3.7	-6.0			23.2	23.6	15.1	20.6		
PAF Total	20.9	13.1	-5.5	1.1									19.5	18.5	12.0	15.9								
Deciles	Sunlight hours (Lag 0-1)						Sunlight hours (Lag 2-6)						Sunlight hours (Lag 0-1)						Sunlight hours (Lag 2-6)					
	High blood pressure																							
0	0.0	5.6	25.2	5.8	1.1	0.1	0.0	7.4	26.8	3.8	1.0	0.5	0.0	11.3	27.1	5.7	1.0	0.2	0.0	12.1	27.8	4.2	1.1	0.0
1	0.5	10.7	14.6	10.6	1.1	0.1	0.0	6.7	15.9	8.2	1.0	0.7	0.6	14.8	19.2	11.5	1.0	0.5	0.0	11.5	19.5	13.3	1.1	0.0
2	2.7	12.7	16.6	8.8	1.1	0.1	0.4	9.7	15.7	13.3	1.0	1.0	4.6	10.6	11.8	10.5	1.0	0.2	1.6	11.8	18.2	11.1	1.0	0.0
3	3.8	11.4	10.8	12.7	1.0	0.9	3.5	9.0	14.3	16.8	1.0	1.0	3.2	10.7	11.8	15.2	1.0	0.8	2.2	5.4	11.0	17.3	1.0	0.0
4	8.3	11.8	10.0	12.2	1.1	0.3	4.8	13.0	12.4	11.6	1.0	0.8	8.5	10.0	8.2	10.1	1.0	0.5	6.6	9.6	9.9	11.9	1.1	0.0
5	11.7	10.2	9.6	14.1	1.0	0.5	7.6	13.9	8.2	13.4	1.0	0.6	11.7	9.5	6.9	9.5	1.0	0.3	6.2	12.0	4.2	12.2	1.0	0.0
6	12.5	9.0	3.7	13.8	1.1	0.4	11.8	13.2	4.7	12.8	1.0	0.5	12.0	7.5	6.4	10.5	1.0	0.6	12.4	11.3	5.7	9.9	1.0	0.0
7	18.0	10.5	4.9	7.5	1.1	0.2	18.4	9.4	0.8	9.1	0.9	0.1	17.0	7.5	4.1	9.5	1.0	0.7	21.6	9.0	1.4	8.9	1.0	0.0
8	16.8	10.3	4.3	7.8	1.1	0.2	22.4	10.9	1.4	6.1	0.9	0.1	18.3	10.2	4.1	9.4	1.0	0.3	22.9	9.9	2.4	6.4	1.0	0.0
9	25.6	7.8	0.3	6.7	Ref		31.1	6.8	0.0	4.9	Ref		24.1	8.0	0.4	8.2	Ref		26.6	7.5	0.0	4.8	Ref	
PAF	4.4	6.2	8.1	5.9			-5.0	-3.4	-1.8	-2.6			1.6	1.3	0.5	1.6			0.8	2.5	4.1	2.3		
PAF Total	-0.4	3.0	6.4	3.4									2.4	3.8	4.6	3.8								
	High-very high SCORE risk																							
0	0.0	4.2	24.3	5.5	0.9	0.4	0.0	6.3	27.4	3.6	1.0	1.0	0.0	10.9	25.7	5.3	1.0	0.5	0.0	11.9	28.1	3.9	1.1	0.0
1	0.4	10.0	14.4	10.3	0.9	0.6	0.0	5.6	14.6	8.6	1.1	0.4	0.7	15.2	18.7	11.0	1.0	0.7	0.0	11.5	18.9	12.7	1.1	0.0
2	2.7	10.6	16.0	9.8	0.9	0.4	0.4	8.1	15.1	13.1	1.1	0.3	4.3	10.0	12.5	9.5	1.1	0.1	1.6	11.8	18.2	10.7	1.1	0.0
3	4.9	12.5	12.3	13.6	0.9	0.1	3.5	7.9	15.3	16.1	1.0	0.8	3.2	10.7	12.0	15.3	1.0	0.9	2.4	5.5	10.5	17.6	1.1	0.0
4	8.5	12.3	10.4	11.5	0.9	0.2	5.5	12.9	13.3	10.5	1.0	0.9	7.8	9.9	8.3	10.0	1.0	0.6	6.3	9.2	9.8	12.1	1.1	0.0
5	11.8	11.4	9.8	13.1	1.0	0.6	8.1	14.5	6.9	13.1	1.0	0.9	11.5	9.6	6.8	9.4	1.0	0.4	6.2	12.1	4.2	12.5	1.1	0.0
6	11.3	9.0	4.0	14.1	1.0	0.8	10.9	14.3	5.1	13.4	1.1	0.2	12.2	7.6	6.7	10.5	1.0	0.4	12.8	11.5	5.9	10.7	1.0	0.0
7	16.5	12.1	5.1	7.0	1.0	0.9	17.6	9.6	0.9	9.7	0.9	0.2	16.7	7.9	4.5	10.0	1.0	0.4	21.7	9.2	1.9	9.2	1.0	0.0
8	17.1	10.6	3.5	8.7	0.9	0.5	21.6	13.0	1.4	6.3	0.9	0.1	19.3	10.3	4.3	9.7	1.0	0.5	22.6	9.7	2.6	5.7	1.0	0.0
9	26.7	7.2	0.3	6.4	Ref		32.5	7.7	0.0	5.8	Ref		24.4	7.9	0.5	9.3	Ref		26.5	7.5	0.0	5.1	Ref	
PAF	-5.1	-8.3	-10.2	-8.4			-4.8	-0.3	3.3	1.2			2.2	1.8	1.1	2.0			2.8	5.2	6.5	5.5		
PAF Total	-10.1	-8.6	-6.6	-7.1									5.0	6.9	7.6	7.4								
	High LDL-cholesterol																							
0	0.1	8.7	21.0	4.0	1.0	0.6	0.1	9.7	22.0	3.2	1.0	0.4	0.3	13.4	25.1	4.0	1.0	0.4	0.5	14.0	23.5	3.4	1.0	0.0
1	0.7	10.8	14.3	8.5	1.0	0.4	0.2	10.2	16.2	5.8	0.9	0.2	2.2	15.7	15.7	9.9	1.0	0.4	0.7	13.2	19.2	10.7	1.0	0.0
2	3.0	13.9	15.6	7.2	1.0	0.4	1.4	11.1	14.6	9.8	1.0	0.5	5.8	10.6	13.2	8.7	1.0	0.7	2.6	14.0	16.4	9.1	1.0	0.0

	Middle-aged												Elderly									
Deciles	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
3	4.5	11.7	12.5	10.2	0.9	0.1	2.9	10.1	16.5	13.9	1.0	0.7	4.0	13.2	11.1	11.2	1.0	0.6	2.0	7.4	14.1	14.1	1.0	0.0
4	8.2	12.3	12.0	11.8	1.0	0.7	5.4	14.0	12.5	11.9	1.0	0.4	7.9	9.2	9.3	9.7	1.0	0.9	6.7	10.3	10.5	10.6	1.0	0.0
5	11.6	9.9	9.6	14.4	1.0	0.4	9.4	11.7	9.5	12.8	1.0	0.8	13.6	8.5	7.9	9.8	1.0	0.7	7.7	10.3	5.4	12.0	1.0	0.0
6	12.8	7.4	5.2	13.4	1.0	0.5	13.2	11.0	5.2	12.9	1.0	0.5	11.0	7.5	7.0	11.2	1.0	0.8	11.8	10.3	6.1	9.7	1.0	0.0
7	18.4	10.8	5.2	10.7	1.0	0.8	18.4	7.9	1.9	11.1	1.0	0.5	16.3	7.0	5.9	12.3	1.0	0.9	20.9	6.9	1.5	12.9	1.0	0.0
8	16.1	7.7	3.7	9.4	1.0	0.8	20.1	9.2	1.5	8.5	1.0	0.4	18.5	8.6	3.9	12.8	1.0	0.7	20.8	7.9	3.3	9.3	1.0	0.0
9	24.6	6.8	0.8	10.2	Ref		28.9	5.3	0.2	10.1	Ref		20.5	6.2	0.9	10.3	Ref		26.2	5.6	0.1	8.1	Ref	
PAF	-0.6	-1.1	-1.4	-1.1			-0.1	-1.1	-1.9	-0.6			0.4	-0.4	-0.8	0.1			-1.8	-1.8	-1.3	-1.9		
PAF Total	-0.7	-2.2	-3.3	-1.6									-1.5	-2.2	-2.1	-1.8								
	High glucose levels																							
0	0.1	9.4	22.0	4.5	1.1	0.6	0.1	9.2	22.4	2.9	1.2	0.3	0.3	12.9	23.1	3.1	1.0	0.8	1.0	13.4	20.0	3.3	1.1	0.1
1	1.0	9.2	13.9	8.2	1.0	0.8	0.1	10.2	16.6	7.4	1.0	0.8	2.2	15.7	18.6	9.2	1.1	0.2	0.5	15.7	20.4	9.8	1.1	0.1
2	3.1	11.6	16.0	8.0	1.0	0.9	0.8	11.6	14.7	9.3	1.1	0.6	4.8	10.7	13.6	8.4	1.1	0.5	2.5	12.4	17.0	8.1	1.2	0.1
3	5.4	9.0	13.4	11.1	1.0	0.8	3.4	9.4	18.0	15.3	1.2	0.4	4.7	15.2	10.4	11.5	1.0	0.9	2.9	6.5	12.8	14.3	1.0	0.1
4	9.3	15.5	11.2	11.4	1.0	0.8	7.6	13.7	12.4	10.6	1.2	0.3	7.6	9.5	8.2	11.7	1.0	0.9	6.2	11.0	12.9	10.5	1.2	0.1
5	12.7	9.5	8.4	14.6	1.0	0.8	9.3	12.7	7.2	14.6	1.3	0.1	13.7	8.7	8.5	10.2	1.0	0.8	7.2	9.7	5.7	13.8	1.1	0.1
6	12.9	9.5	5.9	13.7	1.1	0.5	11.5	12.8	5.2	13.5	1.2	0.3	9.4	6.7	5.4	10.6	1.0	0.7	11.1	9.2	6.5	9.6	1.1	0.1
7	17.7	8.7	4.1	9.5	1.1	0.4	18.4	7.5	1.8	9.9	1.1	0.5	18.7	6.9	7.1	12.2	1.0	1.0	21.1	7.1	1.0	13.9	1.1	0.1
8	14.8	10.2	4.6	8.8	1.0	1.0	21.6	6.9	0.9	8.5	1.1	0.7	17.1	8.0	3.6	12.9	0.9	0.4	20.0	8.5	3.1	9.1	1.0	0.1
9	22.9	7.4	0.5	10.2	Ref		27.1	5.9	0.9	8.2	Ref		21.5	5.8	1.3	10.1	Ref		27.3	6.4	0.5	7.5	Ref	
PAF	3.9	3.9	4.1	3.8			9.1	12.8	13.1	12.6			-0.7	1.9	2.6	0.9			4.1	8.4	9.8	7.4		
PAF Total	12.6	16.1	16.7	15.9									3.5	10.2	12.2	8.2								
Deciles	Precipitation (Lag 0-1)					.	Precipitation (Lag 2-6)						Precipitation (Lag 0-1)					.	Precipitation (Lag 2-6)					
	High blood pressure																							
0	11.2	5.9	8.9	16.0	Ref		9.8	6.7	10.2	17.3	Ref		10.8	5.1	9.4	12.2	Ref		11.1	4.3	6.2	15.6	Ref	
1	12.1	9.5	10.5	8.7	1.0	0.7	16.8	8.1	12.1	11.1	1.1	0.4	9.8	9.1	9.1	10.1	1.0	0.5	12.2	6.3	8.0	5.8	1.0	0.0
2	11.0	8.9	8.0	10.7	1.1	0.3	15.2	9.9	9.3	10.2	1.1	0.3	10.3	9.7	9.4	8.7	1.0	0.9	8.7	7.3	7.6	13.5	1.0	0.0
3	9.9	12.1	11.3	12.1	1.0	0.7	11.1	8.9	11.2	8.4	1.2	0.0	11.5	9.5	7.7	9.9	1.0	0.7	9.8	9.5	11.8	10.2	1.0	0.0
4	10.7	10.1	9.3	7.9	1.0	0.5	8.9	9.8	11.5	6.6	1.1	0.4	13.3	8.3	11.1	9.5	1.0	0.6	13.8	11.2	9.6	7.8	1.0	0.0
5	13.9	10.9	7.7	8.6	0.9	0.3	12.6	10.5	6.9	10.0	1.0	0.6	11.4	9.9	6.5	7.5	1.0	0.5	13.2	10.4	10.8	8.6	1.0	0.0
6	9.3	8.3	5.7	6.6	1.1	0.3	11.3	11.3	8.3	9.6	1.1	0.1	7.1	8.6	6.7	6.3	1.0	1.0	7.6	10.5	10.4	7.9	1.0	0.0
9	21.9	34.4	38.7	29.6	1.0	0.3	5.9	9.4	7.9	10.1	1.0	0.9	25.7	39.9	39.9	35.7	1.0	0.8	8.2	12.5	12.8	11.0	1.0	0.0
0	0.0	0.0	0.0	0.0	1.0	0.0	2.1	9.1	4.6	5.8	1.0	0.8	0.0	0.0	0.0	0.0	1.0	0.0	4.7	8.3	7.2	3.6	1.0	0.0
0	0.0	0.0	0.0	0.0	1.0	0.0	6.2	16.4	17.9	10.9	1.1	0.3	0.0	0.0	0.0	0.0	1.0	0.0	10.6	19.5	15.6	16.1	1.0	0.0
PAF	1.3	1.9	2.0	1.7			4.8	4.5	4.9	3.9			-0.6	-0.7	-0.6	-0.7			-0.6	-0.6	-0.7	-0.3		
PAF Total	6.0	6.2	6.8	5.5									-1.3	-1.3	-1.3	-0.9								
	High-very high SCORE risk																							
0	10.6	7.3	8.6	15.9	Ref		8.7	6.9	10.1	17.4	Ref		11.2	5.1	10.3	12.3	Ref		10.7	4.5	6.6	16.4	Ref	
1	12.0	10.8	11.9	9.0	1.0	0.9	17.0	8.6	11.2	10.3	1.1	0.2	10.1	8.8	9.0	10.4	0.9	0.1	12.2	5.7	7.7	4.8	1.0	0.0
2	11.5	9.0	8.0	11.6	1.2	0.1	16.7	11.5	9.1	11.0	1.0	0.8	9.9	9.9	10.3	9.2	1.0	0.7	8.5	7.2	8.8	13.4	1.1	0.0
3	10.2	11.9	10.9	11.5	1.1	0.3	10.8	9.2	14.1	8.3	1.1	0.3	11.7	9.5	7.6	10.4	0.9	0.1	10.8	9.8	11.5	10.8	1.0	0.0
4	11.2	9.4	9.7	7.7	1.1	0.3	7.4	10.9	9.9	6.2	1.2	0.1	13.0	8.0	11.2	9.6	1.0	0.3	12.8	11.5	9.4	8.0	1.0	0.0
5	11.3	12.0	7.8	8.2	1.1	0.4	11.3	9.9	6.3	11.6	1.0	1.0	10.8	10.4	5.8	7.6	1.0	0.4	14.1	9.8	10.7	9.3	1.0	0.0
6	9.4	7.8	5.6	7.0	1.0	0.8	11.1	11.4	8.8	8.6	1.1	0.4	7.3	8.0	6.5	6.0	1.0	0.7	7.4	10.4	10.7	8.1	1.0	0.0
9	23.8	31.8	37.6	29.2	1.2	0.0	6.1	9.7	7.8	10.5	0.9	0.4	25.9	40.3	39.3	34.5	0.9	0.0	8.2	13.5	12.1	10.7	1.0	0.0
0	0.0	0.0	0.0	0.0	1.0	0.0	3.5	7.4	5.9	5.8	1.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	4.9	8.3	7.6	3.7	1.0	0.0
0	0.0	0.0	0.0	0.0	1.0	0.0	7.3	14.6	16.6	10.3	1.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	10.3	19.2	14.8	14.9	1.0	0.0
PAF	9.1	10.1	10.5	9.5			4.1	3.3	4.0	2.3			-4.7	-5.3	-5.1	-4.9			1.8	1.6	1.6	1.8		

	Middle-aged												Elderly									
Deciles	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value	pd(W)	pd(S)	pd(Su)	pd(A)	RR	p-value
PAF Total	12.8	13.1	14.1	11.6									-2.8	-3.6	-3.4	-3.0								
			High LDL-cholesterol																					
0	10.5	6.7	10.4	15.7	Ref		10.5	6.1	12.7	15.8	Ref		10.1	5.5	11.8	13.0	Ref		10.0	4.0	9.2	14.1	Ref	
1	11.3	9.2	11.1	8.5	1.0	0.5	15.8	7.4	13.0	13.1	1.1	0.0	10.4	9.1	11.0	9.3	1.0	0.7	11.8	6.3	8.0	7.7	1.0	0.0
2	12.7	9.9	7.1	12.1	1.0	0.9	15.1	10.7	11.0	10.4	1.0	0.7	10.8	10.3	9.1	10.0	1.0	0.4	9.2	8.2	9.5	13.8	1.0	0.0
3	10.3	11.4	10.5	11.9	1.0	0.9	12.5	9.4	9.4	8.8	1.0	0.2	10.4	9.6	6.9	10.4	1.0	0.3	11.7	10.2	11.4	10.5	1.0	0.0
4	12.4	11.4	11.6	8.6	1.0	0.7	9.6	9.6	10.6	7.0	1.0	0.3	12.3	8.5	9.9	9.7	1.0	0.5	14.1	11.8	9.1	9.7	1.0	0.0
5	10.7	9.1	7.4	8.7	1.0	0.1	10.8	9.5	6.8	10.9	1.0	0.3	10.6	9.6	5.3	8.3	1.0	0.9	10.2	10.1	8.7	8.2	1.1	0.0
6	9.2	7.9	4.4	7.0	1.0	0.9	9.8	12.0	8.5	10.0	1.0	0.5	8.5	7.1	6.9	5.4	1.0	0.2	9.2	9.5	8.9	7.1	1.0	0.0
9	22.9	34.4	37.4	27.5	1.0	0.6	6.1	8.5	7.3	10.2	1.0	0.6	26.9	40.3	39.0	33.9	1.0	0.1	8.3	11.4	11.8	11.1	1.0	0.0
0	0.0	0.0	0.0	0.0	1.0	0.0	2.2	9.9	4.8	3.8	1.0	0.6	0.0	0.0	0.0	0.0	1.0	0.0	5.8	8.8	7.4	2.9	1.0	0.0
0	0.0	0.0	0.0	0.0	1.0	0.0	7.5	16.9	15.9	10.1	1.0	0.6	0.0	0.0	0.0	0.0	1.0	0.0	9.7	19.6	16.0	14.8	1.0	0.0
PAF	0.9	1.0	1.0	0.9			1.8	1.4	1.7	1.5			-2.3	-2.7	-2.6	-2.4			0.7	0.6	0.6	0.6		
PAF Total	2.7	2.3	2.6	2.3									-1.6	-2.1	-2.0	-1.8								
			High glucose levels																					
0	9.6	8.2	10.3	15.8	Ref		8.7	6.1	12.5	16.7	Ref		10.7	6.2	9.7	14.0	Ref		10.4	3.7	7.3	14.7	Ref	
1	13.1	7.8	9.1	9.9	1.0	0.8	15.1	6.3	11.9	12.1	1.0	0.9	9.8	6.2	9.7	14.0	1.0	0.7	11.8	6.9	7.6	7.6	1.2	0.1
2	12.2	10.5	8.4	12.1	0.9	0.3	14.3	10.3	8.5	9.3	0.8	0.1	11.2	8.1	10.7	8.1	1.0	0.9	9.9	9.2	10.3	13.3	1.1	0.1
3	9.3	12.3	11.0	12.3	1.0	1.0	15.0	11.4	11.2	9.8	0.7	0.0	11.1	10.0	9.9	10.7	1.0	0.6	10.7	8.6	14.0	12.6	1.1	0.1
4	11.7	11.4	11.7	6.7	0.8	0.3	12.0	11.0	8.8	8.2	1.0	0.9	12.8	9.9	6.3	9.4	1.0	0.9	13.9	9.5	7.7	7.9	1.1	0.1
5	10.8	10.2	6.1	8.8	1.0	1.0	10.0	8.6	8.2	9.5	1.1	0.7	10.8	9.4	11.0	11.9	1.1	0.2	10.9	10.0	10.2	8.4	1.1	0.1
6	10.3	8.3	5.2	8.3	0.8	0.2	10.0	12.3	9.9	11.3	0.9	0.4	6.3	9.8	5.0	9.0	1.1	0.3	9.0	12.1	8.7	6.7	1.0	0.1
9	22.9	31.3	38.2	26.1	0.9	0.4	6.8	8.8	6.2	10.3	0.8	0.2	27.3	6.9	8.2	5.0	1.1	0.5	8.4	12.0	11.4	11.1	1.1	0.1
0	0.0	0.0	0.0	0.0	1.0	0.0	1.2	8.8	5.5	3.0	0.9	0.8	0.0	39.6	39.2	31.9	1.0	0.0	6.0	10.2	7.7	3.1	1.1	0.1
0	0.0	0.0	0.0	0.0	1.0	0.0	6.9	16.3	17.2	9.8	1.2	0.3	0.0	0.0	0.0	0.0	1.0	0.0	8.9	17.8	15.0	14.4	1.1	0.1
PAF	-9.4	-9.4	-9.2	-8.3			-10.9	-8.4	-6.7	-8.3			1.9	2.7	2.3	2.0			8.4	8.3	8.4	7.8		
PAF Total	-21.3	-18.5	-16.6	-17.3									10.2	10.8	10.5	9.7								

2.2.2 Seasonality of insulin resistance, glucose, and insulin among middle-aged and elderly population

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ABSTRACT

Introduction: There are discrepancies in the seasonality of insulin resistance (IR) across the literature, probably due to age-related differences in the seasonality of lifestyle factors and thermoregulation mechanisms.

Objective: To estimate the seasonality of IR according to the homeostatic model assessment-insulin resistance (HOMA-IR), glucose, and insulin levels; and to examine the role of lifestyle markers (body mass index (BMI) and physical activity) and meteorological factors, according to age.

Methods: Seasonality was examined using cosinor analysis among middle-aged (45 to 65 years) and elderly (≥65 years) participants of a population-based Dutch cohort. We analyzed 13,622 observations from 8,979 participants (57.6% women) without diagnosis of diabetes and fasting glucose <7mmol/L. BMI was measured, physical activity was evaluated using a validated questionnaire, and meteorological factors (daily mean ambient temperature, mean relative humidity, total sunlight hours, and total precipitation) were obtained from local records. Seasonality estimates were adjusted for confounders.

Results: Among the middle-aged, seasonal variation estimates were: 0.11units (95%CI 0.03, 0.20) for HOMA-IR, 0.28μIU/mL (-0.05, 0.69) for insulin, and 0.05mmol/L (0.01, 0.09) for glucose. These had a summer peak and lifestyle markers explained the pattern. Among the elderly, seasonal variations were: 0.29units (0.21, 0.37) for HOMA-IR, 0.96μIU/mL (0.58, 1.28) for insulin, and 0.01mmol/L (-0.01, 0.05) for glucose. These had a winter peak and ambient temperature explained the pattern.

Conclusion: Impaired thermoregulation mechanisms could explain the winter peak of IR among elderly people without diabetes. The seasonality of lifestyle factors may explain the seasonality of glucose.

INTRODUCTION

The seasonal winter peak of glucose, glycosylated hemoglobin, and type 1 diabetes diagnosis has been widely described in several populations.[5,179,180] In contrast, there are discrepancies in the seasonality of insulin resistance or insulin levels, which could be attributed to age differences across populations studied. For example, studies reporting a seasonal variation of insulin resistance were more likely to include elderly people,[181-186] whereas those reporting no seasonal variation were performed mostly in middle-aged and young populations.[187,188]

Differences between age groups in the seasonality of insulin resistance can be explained by age-related differences of underlying mechanisms, including lifestyle and environmental factors. Lifestyle factors (e.g. diet and physical activity) and lifestyle markers (e.g. body composition and body mass index (BMI)) have a well-described seasonal variation.[5,169] However, factors that change with age, such as marital status, onset of comorbidities, and housing status (community dweller versus other) could lead to different patterns in the seasonality of physical activity [17] and diet.[169] Additionally, the elderly are more susceptible to thermal challenges, such as exposure to low ambient temperature, due to age-related impairment of thermoregulation mechanisms [138,189], some of which are related to insulin resistance.[162] In consequence, age-related differences in the seasonality of lifestyle and in the influence of meteorological factors could lead to different seasonal patterns of insulin resistance.

Nevertheless, to our knowledge, no previous study has evaluated the role of individual and environmental factors in the seasonality of insulin resistance with an age-specific approach. Therefore, we examined the seasonal variation of insulin resistance, according to the homeostatic model assessment-insulin resistance (HOMA-IR), and that of glucose and insulin levels, among middle-aged and elderly participants of a Dutch population-based cohort. Additionally, we examined the role of lifestyle markers and of meteorological factors in the seasonal patterns.

METHODS

Study design

This study is a cross-sectional analysis based on the Rotterdam Study, a population-based prospective cohort comprised in 1989 inviting all elderly people living in the Ommoord district in Rotterdam, the Netherlands.[23] The Rotterdam Study aims to investigate factors that determine the occurrence of cardiovascular, neurological, ophthalmological, endocrinological, and psychiatric disease in elderly population. The study is composed by three cohorts (RS-I: 7,893 participants aged 55 years or above; RS-II: 3,011 participants aged over 55 years of age or who moved into the district; and RS-III: 3,932 participants aged 45 years and over). Follow-up visits are performed every five years.[23] The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Out of 40,846 visits obtained from 14,926 participants, fasting insulin and glucose levels were determined in 15,928 visits (10,259 participants) (Appendix 25, page 103). We further excluded those with diabetes diagnosis or taking medication for diabetes at the time of a visit, and

those with glucose levels above 7mmol/L. Analyses were performed with data obtained in 13,622 visits (observations) among 8,979 participants (Appendix 25, page 103).

Assessment of HOMA-IR, glucose and insulin

HOMA-IR was estimated with the formula $HOMA - IR = \frac{Insulin\left(\frac{\mu IU}{mL}\right) - Glucose\left(\frac{mmol}{L}\right)}{22.5}$.[190] The HOMA-IR has been shown to correlate well with the euglycemic hyperinsulinemic clamp method,[191] which is the gold standard for insulin resistance. Fasting blood serum was drawn during the examination at the research center. Blood collection was performed by venipuncture at the center and the blood was stored at -80°C in 5mL aliquots. Glucose was measured within one week of sampling using the glucose hexokinase method.[174] Serum insulin levels were determined in samples that had been kept frozen from baseline (1997–2001) until usage in 2008 and were measured on a Roche Modular Analytics E170 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) by electrochemiluminescence immunoassay technology. From 2009 onwards, fasting insulin and glucose levels were measured using a COBAS 8000 Modular Analyzer (Roche Diagnostics). Clinical chemistry measurements were done at the Erasmus MC AKC laboratory.

Assessment of meteorological factors

Average ambient temperature (°C), average relative humidity (%), accumulated precipitation (mm) and total sunlight hours in Rotterdam, at participants' blood sampling date and the five days before, were obtained from the Koninklijk Nederlands Meteorologisch Instituut (KNMI, Royal Dutch Meteorological Institute).[67] The monitor is located approximately at 8km from Ommoord district (coordinates: 51° 58' North latitude 04° 27' East longitude). Date of blood sampling was classified in seasons according to the light definition, centered at equinoxes (winter: November 6 to February 4, spring: February 5 to May 6, summer: May 7 to August 5, autumn: August 6 to November 5).[32]

Assessment of lifestyle markers

Physical activity was assessed on the third visit of RS-I and on the first visit of RS-II using a validated adapted version of Zutphen Physical Activity Questionnaire,[140] and expressed in metabolic equivalent of task (MET)-hours/week.[116] Questions on housekeeping activities were added to the original questionnaire that already included questions on walking, cycling, gardening, hobbies, and diverse sports. The LASA (Longitudinal Aging Study Amsterdam) Physical Activity Questionnaire (LAPAQ) [117] was used on the fifth visit of RS-I, the third visit of RS-II, and the first and second visit of RS-III, which contains questions regarding the frequency and duration of walking, cycling, sports, gardening and housework. BMI was calculated as weight divided by height squared (kg/m^2). Height and weight were measured with participants standing without shoes and heavy outer garments and with emptied out pockets, breathing out gently. Height was measured with a wall-mounted stadiometer and recorded to the nearest 0.1 cm. Weight was measured with an electronic floor scale and recorded to the nearest 0.1 kg.

Assessment of covariates

Data on covariates were collected through home interviews or measured in the Rotterdam Study research center by trained research assistants.[23] History of cardiovascular disease, chronic obstructive pulmonary disease, and cancer at the visit date was obtained from medical records. Smoking behavior was requested via questionnaires and categorized as never, current or former. Housing condition was classified as community-dweller vs. non-community-dweller (i.e., service flat, nursing home). Alcohol intake was obtained using the AUDIT tool[91] and was categorized as <2.5, 2.5-4.4, and ≥4.5 glass/day. Blood pressure was measured twice after a resting period of five minutes in a single visit using a random-zero sphygmomanometer (cuff size of 32×17 cm) on the right arm of participants in sitting position; the average of the measurements was used in the analyses. Lipid profile measurement were conducted using an automated enzymatic procedure [175] (Hitachi analyzer, Roche Diagnostics, Washington, DC, USA). Waist circumference was measured at the level midway between the lower rib margin and the iliac crest using a non-stretchable tape and recorded to the nearest 0.1cm.

Fat mass index (FMI) was determined by dividing total fat mass by height squared in meters (grams/m^2). Total fat mass was available for 7,182 participants and was assessed by DXA using a Prodigy TM total body-fan beam densitometer (GE Lunar Corp, Madison, WI, USA) following manufacturer protocols; scans were analyzed with enCORE software V13.6 using pre-determined regions of interest. Data on diet quality were available for 8,766 participants and is expressed as the adherence to 2015 Dutch dietary guidelines for fourteen food groups.[108,109] Diet quality data was obtained using a self-administered semi-quantitative food frequency questionnaire (FFQ) with 170 food items among participants of the first visit of RS-II [108] and with an extended FFQ based on 389 self-administrated food items among remaining participants.[103,104]

Participants were classified as having metabolic syndrome if they had two out of four of the following factors, modified from Alberti et al:[192] abdominal obesity (waist circumference ≥88 among men or ≥102cm among women), hypertriglyceridemia (triglycerides ≥1.7mmol/L or taking statins), low HDL-cholesterol (HDL-cholesterol ≤1.3 among women or ≤1.0 among men, or taking statins), and hypertension (systolic blood pressure ≥130mmHg, diastolic blood pressure ≥85mmHg, or taking antihypertensive medication).

Statistical methods

All analyses are stratified as follows: middle-aged below <65 years-old and elderly above ≥65 years-old. To account for potential bias associated with missing data, we used multiple imputation procedures (n=5 imputations) to impute missing values of covariates. We applied sequential multiple imputation using chained equations with outcomes and covariates as predictors (a full description of the imputation procedure is provided in the Appendix 26 (page 104)). General characteristics of the population according to age-group and season on the imputed dataset are presented using descriptive statistics. Categorical variables were described with absolute frequency and percentage; the distribution was compared using Chi-squared test. Continuous variables were described with median and interquartile range (25^{th} and 75^{th} percentile); the distribution was compared using the Kruskal-Wallis test.

We examined the seasonality of HOMA-IR, glucose, and insulin using generalized linear mixed model with log link, due to their skewed distribution. Seasonality was examined with cosinor analysis using the date of blood sampling as time parameter. We assumed a sinusoidal seasonal variation with a period of one year. Model 1 (crude model) included the cosinor terms and the date (linear and quadratic, to account for long-term temporal trends). Model 2 (adjusted model) additionally included age, sex, visit, smoking behavior, alcohol intake, housing status, prevalence of cardiovascular disease, comorbidities, antihypertensive and statin intake, systolic blood pressure (mmHg), and total cholesterol (mmol/L). The cosinor terms were used to calculate the seasonal variation, which corresponds to the peak-to-nadir variation of the outcome throughout the annual period. The percentage of seasonal variation compared to the adjusted average (POA) was calculated as (seasonal variation/adjusted average)*100. Procedures for estimation of the seasonal variation are provided elsewhere.[5,69] Confidence intervals around seasonal variation were calculated under the assumption of normality, using bootstrap (100 samples).

Additional models were fitted to estimate the effect of BMI and physical activity (Model 3, lifestyle model) and of meteorological factors (Model 4, meteorological factors models) on the seasonality of the outcomes. The effect was operationalized as the reduction of the seasonal variation of Model 3 and Model 4, separately, compared to the seasonal variation in Model 2, and was expressed in percentage. BMI was included as a cubic spline with three degrees-of-freedom. Meteorological factors at the blood sampling date and the five days before were included in the models with one constraint between day 0 (sampling day) and day 1 and other between day 2 to day 5.

To test for possible effect modification, we repeated our main analysis according to age groups and sex. We examined the seasonality of HOMA-IR, glucose, and insulin according to metabolic syndrome (yes vs. no) and obesity (BMI $<$ vs. $\geq$ 30kg/m^2). Two sensitivity analyses were performed in subsamples of the population with available data of dietary patterns (kilocalories intake and diet quality) and of FMI, separately, by examining the change of the seasonal variation after including these covariates. A third sensitivity analysis was performed by excluding participants with one or more comorbidities. For all analysis, we used Stata version 14.1 SE (StataCorp LP, College Station, Texas).[72]

RESULTS

Age and sex distribution was 58.9 years (IQR=55.3, 61.7) and 57% women among the middle-aged, and 73.1 years (IQR=68.6, 78.2) and 59% women among the elderly. Table 11 (page 98) shows the characteristics of participants at date of visit according to season and age-group. Median systolic blood pressure was significantly higher in winter than in summer both among middle-aged (133 vs. 129mmHg, p<0.001) and elderly participants (147 vs. 143.8mmHg, p<0.001). Elderly community-dweller participants were more likely to attend the study in winter than in summer, compared to non-community dweller (91.2% vs. 82.8%, p<0.001).

Seasonality of HOMA-IR, glucose, and insulin

Among middle-aged participants, HOMA-IR was 0.11 units (95%CI 0.03, 0.20) higher in summer than in winter, representing about 10.5% of the average HOMA-IR. Among elderly, HOMA-IR was 0.29 units (95% CI 0.21, 0.37) higher in winter than in summer, representing about 29.2% of the average HOMA-IR (Figure 6 (page 101), (Table 12 (page 100)). These patterns were reflected by the increment of insulin by 0.28 μIU/mL in summer (95%CI -0.05, 0.69; POA=11.8%) among the middle-aged and 0.96 μIU/mL in winter ((95%CI 0.58, 1.28), POA=41.2%) among the elderly. Glucose was stable throughout the year, although the seasonality was somewhat larger among middle-aged than among elderly participants (0.05mmol/L (0.01, 0.09; POA=3.2%) vs. 0.01 (-0.01, 0.05; POA=0.8%)) (Table 12 (page 100)).

Effect of lifestyle and meteorological factors on the seasonality of HOMA-IR, glucose, and insulin

Lifestyle markers explained most of the seasonality of HOMA-IR among middle-aged participants (seasonal variation change= -39%). Among elderly, ambient temperature explained the largest part of the seasonality of HOMA-IR (-88%), followed by sunlight hours (-24%) and lifestyle markers (-20%). Among the middle-aged, glucose seasonality was explained by relative humidity (-56%), followed by lifestyle markers (-18%); whereas insulin seasonality was partly explained by lifestyle markers (-23%). Among the elderly, glucose seasonality was not influenced by lifestyle markers or meteorological factors; whereas insulin seasonality was mostly explained by ambient temperature (-89%), followed by sunlight hours (-24%), lifestyle markers (-16%), and relative humidity (-13%). Analyses based on the non-imputed dataset are shown on Appendix 26 (page 104).

Stratified and sensitivity analyses

Middle-aged men had a larger seasonal variation than middle-aged women for HOMA-IR (0.22 vs. 0.10units), glucose (0.07 vs. 0.05mmol/L), and insulin (0.87 vs. 0.41μIU/L). Lifestyle factors explained a smaller part of HOMA-IR seasonality among middle-aged men (-30%) than among women (-48%). Lifestyle markers and relative humidity explained a larger part of glucose seasonality among middle-aged men (-23% and -62%) than among women (-12% and -41%). Among elderly, seasonality estimates were similar according to sex for HOMA-IR (men=0.30 vs women=0.27units), glucose (0.03 vs 0.02mmol/L), and insulin (0.98 and 0.91μIU/L). Lifestyle markers explained a larger part of HOMA-IR seasonality among elderly men (-33%) than among women (-8%), but the influence of ambient temperature and sunlight hours was larger among elderly women (-72% and -37%) than among men (-49% and -10%) (Appendix 27, page 106).

No large differences in the seasonality of HOMA-IR, glucose, and insulin levels were observed in the stratified analysis according to metabolic syndrome or obesity. Among the middle-aged, the seasonality of glucose was partly explained (-12%) by diet quality, but not by FMI. None modified the seasonality estimates among elderly. Among the middle-aged, seasonality estimates remained similar after the exclusion of participants with comorbidities, except for insulin that increased to 0.36μIU/L (POA=15.1%). Among the elderly, HOMA-IR seasonality remained the same, but glucose seasonality increased to 0.03mmol/L (POA=1.8%) and insulin seasonality increased to 1.04 (POA=44.7%). (Appendix 28 (page 108) and Appendix 29 (page 109))

DISCUSSION

In this large population-based Dutch cohort, seasonality of insulin resistance according to HOMA-IR was larger among elderly than among middle-aged participants. Among the elderly, the seasonality of HOMA-IR reflected that of insulin, with peak in winter, and ambient temperature mostly explained the pattern. In contrast, among the middle-aged, the modest seasonality of HOMA-IR reflected that of glucose, with peak in summer, and lifestyle markers mostly explained the pattern. Age-related differences in thermoregulation could explain the winter peak of insulin resistance among the elderly.

The winter peak of insulin resistance among the elderly was mostly explained by ambient temperature. This finding agrees with previous studies showing that sustained exposure to lower ambient temperature increases energy expenditure and insulin resistance.[189,193] Nevertheless, this finding was not observed in middle-aged participants. This could be explained by the age-related impairment of thermoregulation, which may alter the cold perception among elderly, exposing them more frequently to sustained exposure to low ambient temperature.[160,161] Consequently, the winter peak of insulin resistance may reflect a sustained cold exposure, probably due to the failure to undertake preventive behavioral measures, like proper thermal isolation in buildings and clothing. In addition, the nature of the thermoregulation impairment among elderly may systematically magnify the insulin resistance under cold exposure. Indeed, exposure to low ambient temperature activates physiological thermoregulation mechanisms aimed to produce and preserve heat.[138] These mechanisms involve the sympathetic nervous system and local signaling pathways. However, for reasons not completely understood, the sympathetic pathway is diminished among elderly, creating a discrepancy that in the long term may reduce the NO bioavailability.[138] The reduced NO bioavailability creates a state of insulin resistance, as insulin uses NO to induce vasodilation in insulin sensitive tissues,[194] some of which are involved in heat preservation through skin vasoconstriction and blood flow redistribution. Therefore, an impaired response to cold exposure would elicit a reactive hyperinsulinemia.[195] Moreover, the ability of thermoregulation mechanisms to cope with the seasonal variation of ambient temperature may be further hampered by the higher prevalence of comorbidities and of medication intake among the elderly.

Among middle-aged participants, the seasonality of insulin resistance reflected the seasonality of glucose, which was explained by lifestyle markers, i.e. BMI and physical activity, in agreement with previous evidence.[181] We found that relative humidity also explained a large part of the seasonality of glucose among the middle-aged. We hypothesize that relative humidity may influence the seasonality of glucose through diet behavior. For example, low levels of relative humidity during summer, when ambient temperature increases, may explain the summer peak of sugar-containing beverages, ice-creams, and alcohol intake.[169] Indeed, diet quality explained about 12% of the seasonality of glucose among the middle-aged in our sensitivity analysis and the seasonality of diet quality was explained by relative humidity in both age-groups (results not shown). We are not aware of studies addressing the role of meteorological factors in diet behavior, but these are required to examine this association. The fact that relative humidity did not explain the glucose seasonality among the elderly suggests that its potential influence was outweighted by the large seasonality of insulin, which contributed to maintain constant levels of

glucose.[181] Additionally, the influence of lifestyle factors on the seasonality estimates was smaller among elderly, reflecting the smaller seasonality of their lifestyle factors. Similarly, lifestyle factors had a smaller influence on the seasonality estimates among women than among men. This finding can be attributed to the higher prevalence of comorbidities among elderly and a healthy behavior consciousness both among elderly and women, which would make them less prone to changes in lifestyle behavior on a seasonal basis.

Our findings have multiple relevant implications. First, the winter peak of insulin resitance among the elderly provide a plausible mechanism for the seasonality of cardiovascular risk and of proinflamatory and pro-coagulant factors [5,196,197] and, arguably, also of cardiovascular mortality.[6,32,198] Indeed, the reactive winter hyperinsulinemia has been associated with a phenomenon of selective insulin resistance, which may worsen the endothelial dysfunction[162] and increase the production of proinflamatory and prothrombotic mediators,[195] such as endothelin-1.[162] Future studies are required to confirm this hypothesis among susceptible population, such as elderly. Second, fact that ambient temperature did not influence the seasonality of glucose in our population contradicts previous evidence suggesting that low ambient temperature exposure [199] could modify glucose metabolism through brown adipose tissue (BAT) activation.[200,201] However, it is possible that under non-experimental conditions, factors such as diet counteract the effect of BAT activation. Therefore, population-based studies addressing the role of BAT on glucose metabolism are required to weight its therapeutic value to prevent and control diabetes. Third, although we were unable to determine the actual mechanisms of exposure to low ambient temperature, our findings suggest that ambient temperature is a leading cause of the winter-related insulin resistance in susceptible population. Clinicians and clinical guidelines should address the exposure to low ambient temperature by recommending and pursuing measures to warrant proper thermal isolation in home buildings and in clothing. Nevertheless, our understanding of the mechanisms to low ambient temperature exposure needs to be improved to account for the clustering of associated factors, such as energetic poverty and frailty. Finally, our findings suggest that season-led recommendations on dietary patterns and physical activity would contribute to reduce the burden of seasonal hyperglycaemia.

Our study has several strenghts. First, in contrast with previous studies, we had up to two measurements for most of our participants, which contributes to reducing the random variability of the outcomes across seasons. Second, we adjusted for several counfounders, which reduces the seasonality that could be explained by the non-random participation of the cohort population. Third, to our knowledge, we are the first to examine the efect of lifestyle markers and meteorological factors in the seasonality of HOMA-IR, glucose and insulin. However, we also have to acknowledge several limitations. First, because our population was predominantly an European white population with high levels of physical activity, comorbidities, and high BMI, our findings must be confirmed in other settings. Second, lifestyle markers did not modify the seasonality of HOMA-IR and insulin among middle-aged participants when we replicated the analysis in the non-imputed dataset. This discrepancy can be explained because HOMA-IR and insulin levels were higher in the population with missing data on BMI and/or physical activity than in those with complete data. HOMA-IR and insulin at lower levels could be less susceptible

to the variation of BMI and physical activity than at higher levels, and consequently, the influence of these lifestyle markers could be underestimated in the non-imputed dataset.

Conclusion

In this large population-based Dutch cohort, seasonality of insulin resistance, according to HOMA-IR, was larger among elderly than among middle-aged participants. Among middle-aged participants, the modest seasonality of insulin resistance reflected that of glucose, with a summer peak, and lifestyle factors explained the pattern. Among elderly, the seasonality of insulin resistance reflected that of insulin, with a winter peak, and ambient temperature explained the pattern. Insulin resistance seasonality among elderly appears susceptible to adverse climatic conditions, due to increased exposure to low ambient temperature and age-related impairment of thermoregulation, thus providing a plausible mechanism for the winter peak of cardiovascular risk through endothelial dysfunction. Taking into account that susceptible population is rising given the worldwide ageing trend, it is an urgent matter from the clinical and public health perspective to address environmental risk factors, such as exposure to low ambient temperature, as a relevant mechanism of insulin resistance, with potential implications on cardiovascular risk.

Table 11. Distribution of population characteristics at measurement date according to season*

Covariates	Middle-aged (<65 years) (n=6,657 observations)					Elderly (≥65 years) (n=6,965 observations)				
	Winter (n=1,517)	Spring (n=2,091)	Summer (n=1,523)	Autumn (n=1,526)	p-value	Winter (n=1,735)	Spring (n=1,903)	Summer (n=1,605)	Autumn (n=1,722)	p-value
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
Age, years	59.3 (55.6; 61.8)	58.8 (55.5; 61.7)	58.3 (54.6; 61.7)	58.8 (55.3; 61.6)	<0.001	72.4 (68.4; 78.0)	72.9 (68.1; 77.9)	73.4 (69.2; 79.0)	73.5 (69.1; 78.2)	<0.001
BMI, kg/m^2	26.8 (24.4; 29.3)	26.6 (24.2; 29.2)	26.6 (24.2; 29.3)	26.6 (24.1; 29.3)	0.63	26.6 (24.5; 29.4)	26.5 (24.3; 29.2)	26.4 (24.2; 28.9)	26.4 (24.3; 28.9)	0.29
SBP, mmHg	133.0 (122.0; 146.5)	132.0 (121.0; 145.0)	129.0 (117.8; 142.0)	130.0 (118.1; 143.5)	<0.001	147.0 (133.0; 162.6)	144.0 (131.3; 159.0)	143.8 (129.0; 159.6)	145.0 (132.0; 161.0)	<0.001
Total cholesterol, mmol/L	5.7 (5.0; 6.3)	5.7 (5.1; 6.4)	5.6 (5.0; 6.3)	5.7 (5.1; 6.4)	0.03	5.7 (4.9; 6.3)	5.6 (5.0; 6.3)	5.7 (4.9; 6.3)	5.7 (5.0; 6.4)	0.34
Fat mass index, %	4.6 (3.4; 5.4)	4.7 (3.4; 5.4)	4.5 (3.4; 5.4)	4.7 (3.4; 5.4)	0.10	4.9 (3.9; 5.7)	4.9 (3.8; 5.6)	4.8 (3.8; 5.6)	5.0 (3.9; 5.7)	0.19
Kilocalories intake, kcal/day	2,192.6 (1,826.1; 2,675.6)	2,134.2 (1,808.4; 2,594.5)	2,134.8 (1,725.6; 2,594.8)	2,151.2 (1,810.9; 2,622.1)	0.36	1,987.3 (1,623.4; 2,359.8)	1,934.4 (1,607.3; 2,288.3)	1,924.6 (1,591.6; 2,306.2)	1,949.6 (1,589.0; 2,318.8)	0.06
Diet quality score	7.0 (6.0; 8.0)	7.0 (5.0; 8.0)	7.0 (5.0; 8.0)	7.0 (5.0; 8.0)	1.00	7.0 (6.0; 8.0)	7.0 (6.0; 8.0)	7.0 (5.0; 8.0)	7.0 (6.0; 8.0)	1.00
Physical activity, z-score MET-hours/week	-0.1 (-0.7; 0.7)	-0.1 (-0.7; 0.7)	-0.1 (-0.7; 0.6)	-0.2 (-0.7; 0.6)	0.41	-0.1 (-0.7; 0.7)	-0.1 (-0.7; 0.7)	-0.2 (-0.8; 0.5)	-0.2 (-0.7; 0.5)	<0.001
Glucose metabolism†										
HOMA-IR	2.4 (1.7; 3.5)	2.5 (1.7; 3.6)	2.5 (1.7; 3.6)	2.4 (1.7; 3.6)	0.58	2.4 (1.7; 3.5)	2.4 (1.7; 3.6)	2.3 (1.6; 3.4)	2.3 (1.6; 3.3)	<0.001
Glucose, mmol/L	5.3 (5.0; 5.7)	5.4 (5.0; 5.8)	5.4 (5.0; 5.7)	5.3 (5.0; 5.7)	<0.001	5.5 (5.1; 5.9)	5.5 (5.1; 5.8)	5.4 (5.1; 5.8)	5.4 (5.1; 5.8)	<0.001
Insulin, µIU/mL	10.1 (7.2; 14.5)	10.2 (7.3; 14.5)	10.4 (7.3; 14.4)	10.4 (7.2; 14.5)	0.60	10.1 (7.1; 14.0)	10.1 (7.2; 14.5)	9.4 (6.6; 13.5)	9.4 (6.6; 13.4)	<0.001
	n (%)	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	n (%)	
Sex										
Male	659 (43.4)	877 (41.9)	668 (43.9)	628 (41.2)	0.38	723 (41.7)	79 (42.0)	665 (41.4)	702 (40.8)	0.90
Female	858 (56.6)	1,214 (58.1)	855 (56.1)	898 (58.8)		1,012 (58.3)	1,104 (58.0)	940 (58.6)	1,020 (59.2)	
Community-dweller										
No	18 (1.2)	31 (1.5)	24 (1.6)	22 (1.5)		153 (8.8)	139 (7.3)	276 (17.2)	220 (12.8)	
Yes	1,499 (98.8)	2,060 (98.5)	1,499 (98.4)	1,504 (98.5)	0.81	1,582 (91.2)	1,764 (92.7)	1,329 (82.8)	1,502 (87.2)	<0.001
Prevalent CVD										
No	1,415 (93.3)	1,954 (93.4)	1,433 (94.1)	1,419 (93.0)	0.65	1,366 (78.7)	1,505 (79.1)	1,256 (78.3)	1,386 (80.5)	0.42
Yes	102 (6.7)	137 (6.6)	90 (5.9)	107 (7.0)		369 (21.3)	398 (20.9)	349 (21.7)	336 (19.5)	
Comorbidities										
No	1,391 (91.7)	1,891 (90.4)	1,384 (90.8)	1,393 (91.3)	0.60	1,392 (80.2)	1,540 (80.9)	1,287 (80.2)	1,351 (78.5)	0.31
Yes	126 (8.3)	200 (9.6)	139 (9.2)	133 (8.7)		343 (19.8)	363 (19.1)	318 (19.8)	371 (21.5)	

Smoking status										
Never	481 (31.7)	649 (31.0)	455 (29.9)	439 (28.8)	0.34	594 (34.2)	634 (33.3)	515 (32.1)	581 (33.7)	0.24
Current	377 (24.8)	508 (24.3)	391 (25.6)	418 (27.4)		230 (13.3)	305 (16.0)	226 (14.1)	249 (14.5)	
Former	659 (43.5)	935 (44.7)	677 (44.5)	669 (43.8)		911 (52.5)	964 (50.6)	864 (53.8)	892 (51.8)	
Alcohol intake										
<2.5glass/day	1,116 (73.6)	1,602 (76.6)	1,129 (74.1)	1,145 (75.1)	0.15	1,489 (85.8)	1,621 (85.2)	1,377 (85.8)	1,434 (83.3)	0.14
2.5-4.4 glass/day	306 (20.2)	367 (17.6)	278 (18.6)	284 (18.6)		197 (11.4)	232 (12.2)	190 (11.8)	242 (14.1)	
≥4.5glass/day	94 (6.2)	121 (5.8)	116 (7.6)	96 (6.3)		48 (2.8)	49 (2.6)	38 (2.4)	46 (2.7)	
Medication intake										
Antihypertensive										
No	1,171 (77.3)	1,599 (76.5)	1,190 (78.1)	1,150 (75.4)	0.33	977 (56.3)	1,038 (54.6)	882 (55.1)	963 (56.0)	0.70
Yes	346 (22.8)	492 (23.5)	333 (21.9)	376 (24.6)		758 (43.7)	865 (45.4)	723 (44.9)	759 (44.0)	
Statin										
No	1,280 (84.4)	1,802 (86.2)	1,303 (85.5)	1,256 (82.3)	0.01	1,407 (81.1)	78.2 (1491)	1,307 (81.5)	1,368 (79.3)	0.05
Yes	237 (15.6)	289 (13.8)	220 (14.5)	270 (17.7)		328 (18.9)	21.6 (412)	296 (18.5)	356 (20.7)	

* Descriptive estimates based on imputed dataset. † Adjusted for cosinor terms, age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities, medication intake (antihypertensive and statins), cholesterol levels and systolic blood pressure. n=absolute frequency. BMI: Body mass index. CVD: cardiovascular disease. COPD: Chronic obstructive pulmonary disease. HOMA-IR: Homeostatic model assessment - insulin resistance. IQR = Interquartile range (25^{th} to 75^{th} percentile). SBP: Systolic blood pressure

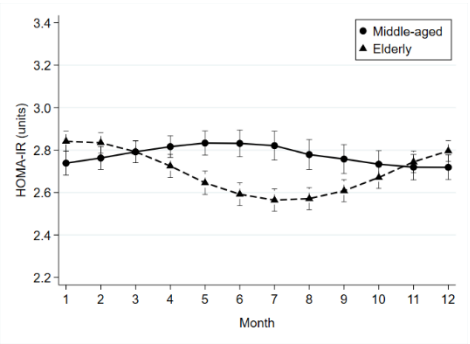
Table 12. Seasonal variation of HOMA-IR, glucose and insulin according to age group

Outcome	Model	Middle-aged (<65 years, n=6,657observations)				Elderly (≥65 years, n=6,965observations)			
		Seasonal variation	SV change (%)	POA	Peak	Seasonal variation	SV change (%)	POA	Peak
HOMA-IR, units	Model 1	0.09	-12	9.2	30-Apr	0.29 **	0	29.2	27-Jan
	Model 2	0.11	Ref	10.5	30-May	0.29 **	Ref	29.2	20-Jan
	Model 3: + BMI and PA	0.06	-39	6.4	2-May	0.23 **	-20	23.7	12-Jan
	Model 4: + ambient temperature	0.20	87	19.6	30-Jun	0.03	-88	3.5	12-Jan
	+ relative humidity	0.12	13	11.8	30-May	0.27 **	-7	27.2	23-Jan
	+ precipitation	0.11	0	10.4	31-May	0.29 **	0	29.1	20-Jan
	+ sunlight	0.15	42	14.8	4-Jun	0.22 **	-24	22.2	30-Jan
Glucose, mmol/L	Model 1	0.02	-67	1.1	4-Aug	0.02	73	1.4	7-Dec
	Model 2	0.05 **	Ref	3.2	22-Jun	0.01	Ref	0.8	30-Nov
	Model 3: + BMI and PA	0.04 **	-18	2.7	20-Jun	0.02	13	0.9	28-Sep
	Model 4: + ambient temperature	0.09 **	58	5.1	8-Jul	0.03	91	1.6	17-Aug
	+ relative humidity	0.02	-56	1.4	22-Jul	0.04	195	2.5	15-Dec
	+ precipitation	0.06 **	2	3.3	20-Jun	0.02	14	0.9	21-Nov
	+ sunlight	0.08 **	52	4.9	21-Jun	0.02	41	1.2	11-Dec
Insulin, μIU/mL	Model 1	0.37	32	15.6	30-Mar	0.91 **	-5	39.1	24-Jan
	Model 2	0.28	Ref	11.8	21-Apr	0.96 **	Ref	41.2	18-Jan
	Model 3: + BMI and PA	0.22	-23	9.1	26-Mar	0.81 **	-16	34.8	13-Jan
	Model 4: + ambient temperature	0.80	182	33.2	5-Jul	0.10	-89	4.5	26-Aug
	+ relative humidity	0.44	58	18.6	6-May	0.84 **	-13	35.9	24-Jan
	+ precipitation	0.34	21	14.3	18-Apr	0.97 **	0	41.3	19-Jan
	+ sunlight	0.54	91	22.5	22-May	0.73 **	-24	31.2	28-Jan

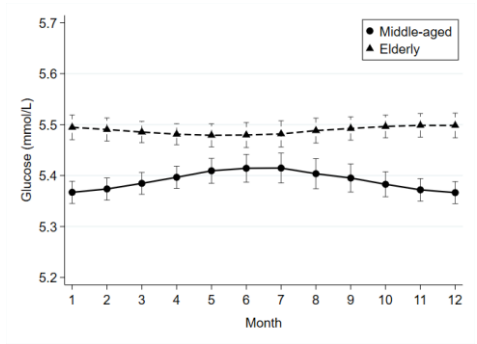
Model 1: Cosinor terms and date (linear and quadratic). Model 2: Model 1 + age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities (chronic obstructive pulmonary disease, cancer), medication intake (antihypertensive and statin), cholesterol levels and systolic blood pressure. SV: Seasonal variation. BMI: body mass index (cubic spline with 3df). PA: Physical activity (z-score of MET-hours/week). **means at least one cosinor term significant at 0.025 level. *means at least one cosinor term significant at 0.05 level. POA: Proportion of seasonal variation vs. adjusted average levels of the outcome.

Figure 6. Seasonal pattern of HOMA-IR, glucose and insulin according to age group

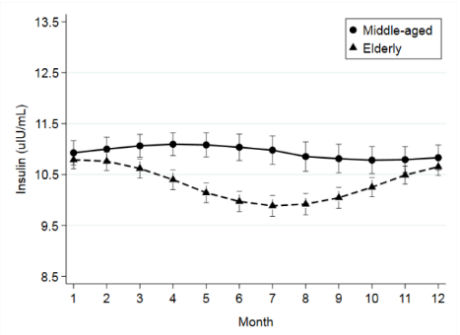
(a) HOMA-IR



(b) Glucose



(c) Insulin



The solid line corresponds to the seasonal pattern among middle-aged participants and the dashed line the seasonal pattern among elderly participants. The range around estimates corresponds to 95% confidence intervals. The patterns are adjusted for cosinor terms, date (linear and quadratic), age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities (chronic obstructive pulmonary disease, cancer), medication intake (antihypertensive and statin), cholesterol levels and systolic blood pressure.

SUPPLEMENTARY MATERIAL

Appendix 24. Overview of the Rotterdam Study

Sub-cohort	Visits					
RS-I	1 n=7,983	2 n=6,315	3 n=4,785 ★	4 n=3,558		5 n=2,147 ★+●[2]
RS-II			1 n=3,011 ★+●[1]	2 n=2,506		3 n=1,893 ★+
RS-III					1 n=3,932 ★+●[2]	2 n=3,122 ★+●[2]
Timeline ->	1989-1993	1993-1995	1996-2001	2002-2005	2006-2008	2009-2013

RS: Rotterdam Study

Included visits

Not included visits

★Overall study population

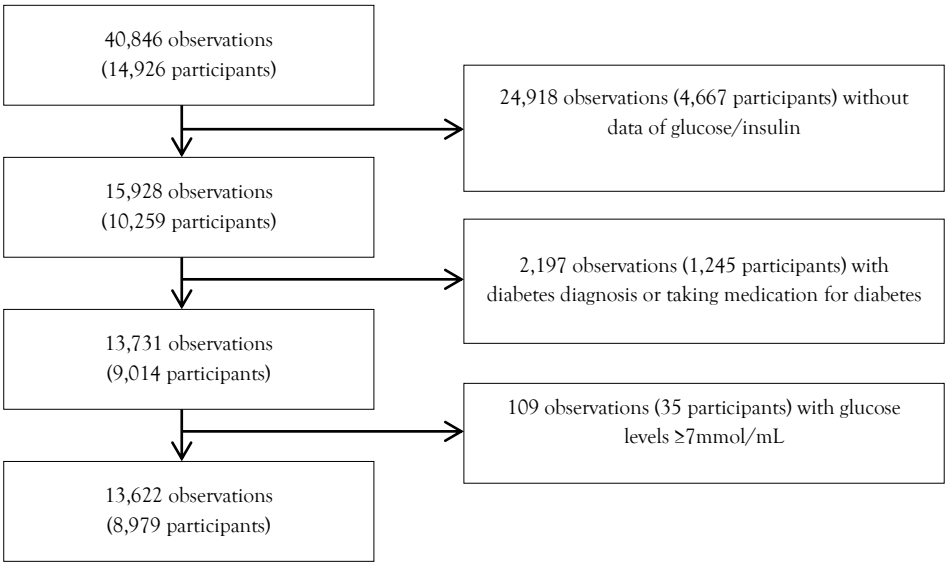
Population of sensitivity analysis

+ Fat mass index data

●[1] Diet quality data, assessed with 170 food items FFQ

●[2] Diet quality data, assessed with 339 food items FFQ

Appendix 25. Flowchart of data selection



Appendix 26. Seasonal variation of HOMA-IR, glucose and insulin according to age group – Not imputed dataset

Outcome	Model	Middle-aged (<65 years-old, n=6,657 observations)					Elderly (≥75 years-old, n=6,965 observations)				
		Seasonal variation	SV change (%)	POA	Peak	Observations	Seasonal variation	SV change (%)	POA	Peak	Observations
HOMA-IR, units	Model 1	0.09	8	9.2	30-Apr	6,657	0.29 **	8	29.2	27-Jan	6,965
	Model 2	0.09	Ref	8.6	22-May	6,482	0.27 **	Ref	27.0	20-Jan	6,536
	Model 3:+ BMI and PA	0.11	26	11.1	20-Apr	5,401	0.17 **	-37	17.3	23-Jan	5,996
	Model 4:+ ambient temperature	0.17	92	16.5	28-Jun	6,482	0.01	-96	1.0	11-Jan	6,536
	+ relative humidity	0.09	6	9.1	23-May	6,482	0.25 **	-8	24.9	24-Jan	6,536
	+ precipitation	0.08	-4	8.3	25-May	6,482	0.27 **	-1	26.8	18-Jan	6,536
	+ sunlight	0.11	31	11.2	28-May	6,482	0.20	-26	19.8	1-Feb	6,536
Glucose, mmol/L	Model 1	0.02	-60	1.1	4-Aug	6,657	0.02	47	1.4	7-Dec	6,965
	Model 2	0.05 **	Ref	2.7	26-Jun	6,482	0.02	Ref	1.0	7-Nov	6,536
	Model 3:+ BMI and PA	0.04	-14	2.3	15-May	5,401	0.03	87	1.8	30-Aug	5,996
	Model 4:+ ambient temperature	0.08 *	77	4.8	12-Jul	6,482	0.03	108	2.0	21-Aug	6,536
	+ relative humidity	0.02	-60	1.1	31-Aug	6,482	0.04	124	2.2	4-Dec	6,536
	+ precipitation	0.05 **	2	2.7	25-Jun	6,482	0.02	14	1.1	3-Nov	6,536
	+ sunlight	0.07 **	49	4.0	24-Jun	6,482	0.01	-28	0.7	30-Oct	6,536
Insulin, µIU/L	Model 1	0.37	44	15.6	30-Mar	6,657	0.91 **	4	39.1	24-Jan	6,965
	Model 2	0.26	Ref	10.8	10-Apr	6,482	0.88 **	Ref	37.7	18-Jan	6,536
	Model 3:+ BMI and PA	0.33	29	14.1	15-Apr	5,401	0.61 **	-31	26.2	25-Jan	5,996
	Model 4:+ ambient temperature	0.65	149	27.0	3-Jul	6,482	0.22	-75	9.5	1-Aug	6,536
	+ relative humidity	0.39	52	16.4	30-Apr	6,482	0.75 **	-15	32.2	26-Jan	6,536
	+ precipitation	0.30	16	12.5	10-Apr	6,482	0.88 **	0	37.7	18-Jan	6,536
	+ sunlight	0.43	68	18.2	15-May	6,482	0.67 **	-24	28.7	28-Jan	6,536

Model 1: Cosinor terms and date (linear and quadratic). Model 2: Model 1 + age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities (COPD, cancer), medication intake (antihypertensive and statin), cholesterol levels and systolic blood pressure. BMI: body mass index (cubic spline with 3df). PA: Physical activity (z-score of MET-hours/week). **means at least one cosinor term significant at 0.025 level. *means at least one cosinor term significant at 0.05 level. POA: Proportion of seasonal variation vs. adjusted average level of the outcome.

Imputation procedures: Sequential multiple imputation using chained equations was performed to impute missing values of covariates. This approach has been shown to work well in the context of complex longitudinal data, as in the Rotterdam Study.[178] The predictors used to impute the covariates were sex, age, HOMA-IR (log scale), presence of comorbidities, cohort, previous CVD, cholesterol levels, income, smoking behavior, BMI (cubic spline with 3df) and BMI continuous, statins intake, antihypertensive intake, housing status, alcohol intake, waist-to-hip ratio, physical activity (z-score of MET-hours/week), and systolic blood pressure. To impute dichotomous variables (income, statin intake, antihypertensive intake, housing status and comorbidities) we used a logit function. To impute ordered categorical variables (alcohol) we used an ordered logit function. To impute categorical non-ordered variables (smoking behavior) we used a multinomial logit function. To impute BMI (splines and continuous in mutually excluding functions), waist-to-hip ratio, physical activity and

systolic blood pressure, we used a linear function. To ensure reproducibility, we used a random seed (2005). We created five imputed datasets, which were used in all the analysis. Covariates with missing values were: smoking behavior: 69 missing, alcohol intake: 153 missing, housing status: 58 missing, comorbidities: 89 missing, antihypertensive intake: 306 missing, statins intake: 259 missing, systolic blood pressure: 87 missing, BMI: 71 missing, physical activity: 1,678 missing. Imputations were performed using the *mi impute* command of Stata software.

Effect of imputation in the analyses: There are differences in the effect of lifestyle markers (physical activity and BMI) in the seasonality of glucose among middle-aged participants. To explore the cause of this difference, we examined if there were differences in the age or the distribution of the outcomes between those with missing data of BMI and/or physical activity compared to those with complete data. We found that compared with those observations with complete data, those with missing BMI and/or physical activity were younger (median=56.2 years (IQR=51.5-59.6) vs. 59.3(53.0, 62.0)), had higher HOMA-IR (2.7 (1.8, 4.1) vs. 2.4 (1.7, 3.5)) and insulin (11.5 μIU/mL (7.8, 16.8) vs. 10.1 (7.2, 14.1), but similar glucose levels (5.3 (5.0, 5.7) vs. 5.4 mmol/L (5.0, 5.7)). Also, imputed physical activity levels were higher (0.02z-score MET-hours/week (-0.64, 0.73) vs. -0.13 (-0.68, 0.62)), whereas imputed and observed BMI were similar (27.3kg/m^2 (24.6, 30.1) vs. 26.5 (24.2, 29.2)).

Appendix 27. Seasonal variation of HOMA-IR, glucose and insulin according to age group and sex

Outcome	Model	Middle-aged (<65 years-old)					Elderly (≥65 years-old)				
		Seasonal variation		SV change (%)	POA	Peak	Seasonal variation		SV change (%)	POA	Peak
Women		n = 3,825 observations					n = 4,076 observations				
HOMA-IR, units	Model 1	0.04		-56	4.4	23-Jul	0.30	**	10	29.8	19-Jan
	Model 2	0.10		Ref	10.0	27-Jul	0.27	**	Ref	27.1	16-Jan
	Model 2 + BMI and PA	0.05		-48	5.3	5-Jul	0.25	**	-8	25.1	3-Jan
	Model 2 + ambient temperature	0.17		76	17.6	2-Aug	0.08		-72	7.7	8-Jan
	Model 2 + relative humidity	0.09		-10	9.1	6-Aug	0.26	**	-5	25.8	18-Jan
	Model 2 + precipitation	0.10		1	10.2	30-Jul	0.27	**	0	27.3	17-Jan
	Model 2 + sunlight	0.13		36	13.6	15-Jul	0.17		-37	17.1	31-Jan
Glucose, mmol/L	Model 1	0.03		-41	1.7	20-Sep	0.02		222	1.4	24-Nov
	Model 2	0.05	*	Ref	2.9	7-Jul	0.01		Ref	0.4	5-Nov
	Model 2 + BMI and PA	0.04		-12	2.6	30-Jun	0.02		145	1.1	23-Sep
	Model 2 + ambient temperature	0.11	**	132	6.8	19-Jul	0.03		249	1.5	6-Aug
	Model 2 + relative humidity	0.03		-41	1.7	11-Aug	0.05		644	3.2	13-Dec
	Model 2 + precipitation	0.05	*	3	3.0	1-Jul	0.01		17	0.5	30-Oct
	Model 2 + sunlight	0.10	**	104	6.0	27-Jun	0.01		78	0.8	13-Dec
Insulin, µIU/L	Model 1	0.30		-26	12.7	5-Jul	0.98	**	7	41.7	24-Jan
	Model 2	0.41		Ref	17.3	15-Jul	0.91	**	Ref	38.9	18-Jan
	Model 2 + BMI and PA	0.30		-27	12.7	3-Jul	0.85	**	-7	36.4	8-Jan
	Model 2 + ambient temperature	1.10	**	171	46.8	25-Jul	0.14		-84	6.1	7-Jan
	Model 2 + relative humidity	0.49		20	20.8	7-Jul	0.84	**	-8	35.9	22-Jan
	Model 2 + precipitation	0.41		0	17.3	11-Jul	0.93	**	2	39.6	20-Jan
	Model 2 + sunlight	0.71		73	29.9	3-Jul	0.60		-34	25.5	3-Feb
Men		n = 2,832 observations					n = 2,889 observations				
HOMA-IR, units	Model 1	0.23	*	1	20.8	10-Apr	0.30	**	-2	29.8	5-Feb
	Model 2	0.22	*	Ref	20.6	24-Apr	0.30	**	Ref	30.5	23-Jan
	Model 2 + BMI and PA	0.16		-30	14.6	25-Mar	0.20		-33	20.5	30-Jan
	Model 2 + ambient temperature	0.29		30	26.8	30-May	0.04		-87	3.9	23-Jul
	Model 2 + relative humidity	0.27	*	19	24.6	1-May	0.28	*	-7	28.4	26-Jan

	Model 2 + precipitation	0.23	*	2	21.0	24-Apr	0.30	**	-1	30.1	19-Jan
	Model 2 + sunlight	0.25	*	13	23.3	2-May	0.27		-10	27.5	26-Jan
Glucose,	Model 1	0.03		-54	1.8	1-Jun	0.03		3	1.6	5-Dec
mmol/L	Model 2	0.07	*	Ref	3.9	4-Jun	0.03		Ref	1.6	26-Nov
	Model 2 + BMI and PA	0.05		-23	3.0	4-Jun	0.02		-37	1.0	25-Sep
	Model 2 + ambient temperature	0.06		-5	3.7	5-Jun	0.04		46	2.3	31-Aug
	Model 2 + relative humidity	0.03		-62	1.5	9-Jun	0.03		8	1.7	6-Dec
	Model 2 + precipitation	0.07	*	0	3.9	4-Jun	0.03		20	1.9	18-Nov
	Model 2 + sunlight	0.06		-8	3.6	4-Jun	0.03		23	1.9	28-Nov
Insulin,	Model 1	1.03	**	18	42.0	4-Mar	0.90	**	-8	38.8	24-Jan
µIU/L	Model 2	0.87	*	Ref	35.8	9-Mar	0.98	**	Ref	42.3	16-Jan
	Model 2 + BMI and PA	0.74		-15	30.5	18-Feb	0.72	**	-27	31.0	26-Jan
	Model 2 + ambient temperature	0.69		-21	28.2	15-May	0.50		-49	21.6	6-Aug
	Model 2 + relative humidity	0.98	**	12	40.0	29-Mar	0.80		-19	34.2	24-Jan
	Model 2 + precipitation	0.97	**	11	39.7	11-Mar	0.98	**	-1	41.9	13-Jan
	Model 2 + sunlight	0.89	**	1	36.2	3-Apr	0.87		-11	37.5	19-Jan

Model 1: Cosinor terms and date (linear and quadratic). Model 2: Model 1 + age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities (COPD, cancer), medication intake (antihypertensive and statin), cholesterol levels and systolic blood pressure. BMI: body mass index (cubic spline with 3df). PA: Physical activity (z-score of MET-hours/week). **means at least one cosinor term significant at 0.025 level. *means at least one cosinor term significant at 0.05 level. POA: Proportion of seasonal variation vs. adjusted average level of the outcome.

Appendix 28. Stratified analyses

Strata	Outcome	Middle-aged (<65 years, n=6,657observations)					Elderly (≥65 years, n=6,965observations)				
		Seasonal variation¶		POA	Peak	Observations	Seasonal variation¶		POA	Peak	Observations
Metabolic syndrome†											
No	HOMA-IR, units	0.14		14.7	18-May	5,308	0.29	**	32.0	24-Jan	4,995
	Glucose, mmol/L	0.06	**	3.9	1-Jun		0.02		1.1	6-Dec	
	Insulin, μIU/mL	0.45	*	19.4	18-Apr		1.06	**	47.2	28-Jan	
Yes	HOMA-IR, units	0.05		4.0	30-Oct	1,349	0.36	**	29.2	28-Jan	1,970
	Glucose, mmol/L	0.07		4.2	1-Sep		0.01		0.4	3-Apr	
	Insulin, μIU/mL	0.04		1.4	1-Nov		0.95		37.1	30-Jan	
Obesity‡											
No	HOMA-IR, units	0.10		10.6	29-Apr	5,296	0.20	**	21.7	13-Jan	5,592
	Glucose, mmol/L	0.05	*	2.9	5-Jun		0.03		1.7	22-Oct	
	Insulin, μIU/mL	0.35		15.2	22-Mar		0.75	**	33.3	20-Jan	
Yes	HOMA-IR, units	0.19		13.4	5-Jul	1,334	0.38	*	28.2	23-Jan	1,329
	Glucose, mmol/L	0.08		4.6	25-Jul		0.07		3.8	19-Apr	
	Insulin, μIU/mL	0.89		31.9	5-Jul		0.83		31.1	13-Jan	
Comorbidities											
No	HOMA-IR, units	0.11		11.1	17-May	5,649	0.29	**	28.9	16-Jan	4,416
	Glucose, mmol/L	0.05	**	3.0	22-Jun		0.03		1.8	8-Feb	
	Insulin, μIU/L	0.36		15.1	2-May		1.04	**	44.7	17-Jan	

†Metabolic syndrome defined as 2 out of four items, including abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, hypertension. ‡Obesity defined as BMI ≥30kg/m^2. ¶Estimates are adjusted for cosinor terms and date (linear and quadratic) + age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities (COPD, cancer), medication intake (antihypertensive and statin), cholesterol levels and systolic blood pressure. POA: Proportion of seasonal variation vs. adjusted average levels of the outcome. ** means at least one cosinor term significant at 0.025 level. * means at least one cosinor term significant at 0.05 level.

Appendix 29. Sensitivity analyses

Outcome	Model	Middle-aged (<65 years)				Elderly (≥65 years)			
		Seasonal variation		SV change (%)	Peak	Seasonal variation		SV change (%)	Peak
Subanalysis (A): Diet quality (kilocalories intake + diet quality score)		n = 3,584 observations				n = 5,182 observations			
HOMA-IR, units	Model 2	0.15		Ref	30-Mar	0.23	**	Ref	16-Jan
	Model 2 + diet quality	0.15		1	24-Mar	0.23	**	-1	16-Jan
Glucose, mmol/L	Model 2	0.04		Ref	3-Jun	0.05		Ref	8-Nov
	Model 2 + diet quality	0.04		-12	31-May	0.05		-1	8-Nov
Insulin, µIU/mL	Model 2	0.66	**	Ref	27-Mar	0.84	**	Ref	21-Jan
	Model 2 + diet quality	0.66	**	0	23-Mar	0.83	**	-1	21-Jan
Subanalysis (B): fat mass index		n = 3,924 observations				n = 3,258 observations			
HOMA-IR, units	Model 2	0.19	**	Ref	19-Jun	0.31	**	Ref	13-Jan
	Model 2 + fat mass index	0.19	**	-1	18-Jun	0.31	**	0	13-Jan
Glucose, mmol/L	Model 2	0.04		Ref	11-Jul	0.002		Ref	6-Jan
	Model 2 + fat mass index	0.04		-2	10-Jul	0.002		4	21-Dec
Insulin, µIU/mL	Model 2	0.40		Ref	14-Jun	1.16	**	Ref	13-Jan
	Model 2 + fat mass index	0.39		-4	11-Jun	1.16	**	0	14-Jan

Model 2 include cosinor terms and date (linear and quadratic) + age, sex, visit, smoking behavior, alcohol intake, housing status, prevalent cardiovascular disease, comorbidities (COPD, cancer), medication intake (antihypertensive and statin), cholesterol levels and systolic blood pressure.

2.2.3 Seasonality of cognitive performance in the general population: the Rotterdam Study

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ABSTRACT
Objective: To assess the seasonality of cognitive performance in the general population.

Methods: Within the prospective, population-based Rotterdam Study, we assessed cognitive performance in 8,560 participants free from dementia at baseline and subsequent visits between 1999 and 2016. Using multiple measurements in cosinor linear mixed models, we assessed the annual seasonality of global cognition. Cognition was constructed with principal component analysis based on four different cognitive tests in 16,707 observations. In addition, we assessed seasonality patterns for each standardized test separately.

Results: In this study, the median age was 67.1 years and 57.2% were women. Global cognition had a significant annual seasonal variation (SV) with lowest values in winter and highest values in summer months (3.2 times lower in winter than in summer, SV: 0.04 standard deviation (SD), 95% confidence interval (CI): 0.01 to 0.06). Individual tests with large SV were: manual dexterity test (1.35 times lower in winter than in summer, 0.11 SD, 95%CI: 0.08 to 0.15), and verbal fluency test (1.29 times lower in early-spring than in autumn, 0.04 SD, 95%CI: 0.01 to 0.07). These SVs were less pronounced for the Stroop interference task (0.03 SD, 95%CI: 0.00 to 0.06) and the letter digit substitution test (0.02, 95%CI: -0.01 to 0.05). Largest variations in seasonality were seen for individuals with a higher score on global cognition, those with higher Mini-Mental State Examination (MMSE) scores and those with higher educational levels.

Conclusions: Cognitive performance shows an annual seasonal variation pattern in the general population, characterized by lower performance during winter and higher performance during summer. Larger seasonal variation in cognitive performance may be a sign of preserved cognitive function while ageing, yet future studies are warranted to confirm these observations.

INTRODUCTION

With ageing populations worldwide, reliable assessment of cognitive performance is becoming increasingly important to detect subsequent decline. Cognitive performance is largely determined by an individual's educational attainment, although vascular and lifestyle factors also play an important role.[202-206] More recently, several clinical conditions and environmental factors that influence cognitive performance have been identified,[35] some of which could express their effects depending on the season. For instance, seasonal affective and bipolar disorders[207] and allergies[208] are associated with lower cognitive performance during their symptomatic periods of the seasonal pattern. Among the most widely studied environmental factors influencing cognition are ambient temperature and sunlight. Both low and high temperature are associated with lower Mini-Mental Score Examination (MMSE) score[209] whereas sunlight may harbor indirect effects on cognition via mood disorders[207] and vitamin D insufficiency [210,211]

However, regardless of extensive evidence on these seasonal factors influencing cognition, less is known about the seasonal variation of cognitive function in the general population.[36,37] Some studies have shown differences in attention and information processing[212,213] during winter, however these findings come from studies with 100 or less participants [36,212-214] or were performed in selected populations (i.e., individuals with bipolar disorder).[207] A seasonal variation in cognitive function could help to better understand the influence of environmental determinants of cognition, as well as the interpretation of cognitive tests across seasons in the general population. In this large cohort study, we studied the seasonality of cognitive functioning among community-dwelling individuals.

METHODS

Study design

This study was embedded in the Rotterdam Study, a population-based prospective cohort conducted in the Ommoord district in Rotterdam, the Netherlands.[23] Briefly, the first cohort of this study comprised of 7,983 participants aged 55 years old and above, between 1997 and 1999. Between 2000 and 2001, 3,011 new participants who were 55 years and older or moved into the district since the start of the study, were included in a second cohort (RS-II). A third cohort (RS-III) was comprised between 2006 and 2008 with 3,932 new participants aged 45 and above. The overall response rate across cohorts was 72%. Study participants were invited at the research center every three to four years.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Study population

For this study, we used baseline and follow-up visits of all three cohorts. A cognitive test battery was implemented in the study protocol in 1997. We therefore considered the third visit of the first cohort (RS-I) as baseline. We excluded participants who did not provide informed consent to

access medical records or hospital discharge letters (compromising 184 observations on cognitive tests from 121 participants). We further excluded participants who were not or insufficiently screened at baseline for dementia or suffered from dementia while performing the cognitive test (n=896 observations, from 587 participants). We finally excluded observations from individuals with insufficient data on the subsequent tests to calculate a global cognition score (n=9,760 observations from 2,683 participants) (the flowchart of population selection is provided in Appendix 37 (page 142)).

Assessment of cognitive performance

Both at baseline and during follow-up, participants underwent the Mini-Mental State Examination (MMSE) during home interviews and an extensive cognitive test battery at the research center. This cognitive test battery consisted of the following four tests: Letter-Digit Substitution Task (LDST, participants have to write down as many numbers underneath the corresponding letters as possible in 60 seconds), Stroop test (reading task of printed words, task of naming colours and interference task, which combines both: participants are asked to name the colors of color names printed in incongruous ink color), Verbal Fluency Test (VFT, animal categories), and Purdue Pegboard Test (PPT, placing as many as possible metal pins into one of the 25 holes in a pegboard in 30 seconds). In 3,313 individuals with 18,173 visits, 15-Word Learning Tests (15-WLT; including three immediate trials to remember these words (mean used for analysis), a 10-minute delayed retrieval task and a recognition task) were additionally obtained.

Covariates

Data collection for each research visit included a standardized home interview and two visits at the research center for clinical examination and blood sampling. Sex, age and ethnicity were obtained during the baseline home-interview, ethnicity was categorized as Caucasian or non-Caucasian. Participants' level of education and monthly household income at baseline were assessed by trained interviewers. Level of education was categorized as low (primary education), low/intermediate (lower/intermediate general education or lower vocational education), high general (intermediate vocational or higher general education) and university (higher vocational education or university). Participants were screened for depressive symptoms using the Center for Epidemiology Studies Depression Scale (CES-D Scale).[90] Alcohol intake was obtained using the AUDIT tool[91] in grams/day. Smoking behavior was categorized as never, current or former and requested via questionnaires. Housing condition was classified as community-dweller and non-community-dweller (i.e., service flat, nursing home). History of cardiovascular disease at the visit date was obtained from medical records. At the research center, height and weight were measured with the participants standing without shoes and heavy outer garments, and with emptied pockets, breathing out gently. Height was measured with a wall-mounted stadiometer, recorded to the nearest 0.1cm; weight was measured with an electronic floor scale, recorded to the nearest 0.1 kg. Participants were categorized as having a normal weight or being underweight (<25 kg/m^2), overweight (25-30 kg/m^2) and obese (≥30 kg/m^2). Systolic blood pressure was measured twice after a resting period of five minutes during a single visit using a random-zero sphygmomanometer on the right arm of participants in sitting position. The average of the

measurements was used in the analyses. Cholesterol and high-density lipoprotein cholesterol (HDL) levels were measured using an automated enzymatic procedure.[175] Glucose was measured within one week of blood sampling using the glucose hexokinase method.[174] Because only housing status was readily available at analysis date at the sixth visit of RS-I and the fourth of RS-II, we assumed that the remaining covariates did not change with respect to the previous visit. Alcohol intake was assumed to be the arithmetic mean of the previous visits, as long as there were three data points for estimation.

Data analyses

We used 5-fold multiple imputation with chained equations to impute missing covariates, using both covariates and outcomes as predictors. A full description of imputation procedures is shown in Appendix 37 (page 142). To examine the influence of imputation procedures on main analyses, we also performed a full-case analysis (shown in Appendix 35 (page 140)). General characteristics of the population in the imputed dataset are presented in absolute frequency and percentage for categorical variables and as median and interquartile range (25th and 75th percentile) for continuous variables. Additionally, we compared the general characteristics of the participants according to their season of examination. Continuous variables were compared with the Kruskal-Wallis test. Categorical variables were compared with Chi-squared test. Seasons were classified according to the light definition, centered at equinoxes:[32] winter (November 6th to February 4th), spring (February 5th to May 6th), summer (May 7th to August 5th), and autumn (August 6th to November 5th).

We calculated global cognition as the first compound of an unrotated principal component analysis (PCA) that incorporates tasks from all four cognitive function tests (LDST, Stroop, VFT, and PPT). Although three Stroop subtasks are available, we only used data from the most complicated task of the Stroop to prevent highly correlated tasks distorting factor loadings in the PCA when calculating the g-factor of global cognition. We examined the seasonality of this g-factor and of the standardized score (z-score) of each component of the factor (log-Stroop interference (because of non-normal distribution), LDST, VFT, PPB both hands) using linear mixed model with random intercept for each participant to account for the correlation between visits. Date of study visit was included in the model transformed into its cosinor terms (i.e. sine and cosine)[101,119] with an assumed annual seasonality. The coefficients of the cosinor terms were used to calculate the seasonal variation, which corresponds to the maximal difference between the highest level (peak) and the lowest level (nadir) of the levels of the corresponding outcome throughout the annual period.[119] Detailed descriptions to estimate the seasonal variation are provided elsewhere.[101,119] Confidence intervals around seasonal variation were calculated using the delta-method.[215]

In all of the following analyses, we adjusted seasonality estimates for non-random participation of study population throughout the year. We constructed two models for analyses. First, we fitted a crude model, which contained age, sex, and cosinor terms. In additional model, we selected several potential confounders based on the basis of literature, including education level, systolic blood pressure, total cholesterol levels, HDL-cholesterol levels, glucose levels, use of antihypertensive, antidiabetic or statin medication, depressive symptoms, body mass index,

smoking behavior, alcohol intake, housing status, number of follow-up visit, date of the visit, and date of visit squared.

We repeated all analyses using as outcome the g-factor that included the delayed WLT test in the principal analysis calculation (n=13,826 observations) and the delayed WLT test itself. Next, we examined if the fit of the model was improved by flexibilizing the annual variation assumption, by including in the model cosinor terms for a biannual variation (biannual harmonics). The fit of the models (annual seasonality and annual seasonality with biannual harmonics) was compared for each outcome using the Bayesian Information Criterion (BIC). A priori, we considered the fit as significantly improved if it was reduced by more than 10 units.[216]

We also tested to what extent each component of the g-factor influenced seasonality, by re-calculating the g-factor while excluding one of the components at a time and the seasonality of the remainder was estimated.

In stratified analyses, we examined potential effect modification by age (middle-aged (>65 years), young-elderly (65-75 years), old-elderly (≥75 years), sex, presence or absence of cardiovascular disease (defined as at least one of the following: history of cardiovascular disease or use of antihypertensive, statin or diabetes medication), depressive symptoms (CES-D scale ≥16), education (primary, low/intermediate, high general, and university), MMSE (< 26 or ≥26), low or high g-factor (below or above zero), and working status (occupied or not occupied).

Finally, we conducted a number of sensitivity analyses to test the robustness of our findings. First, we excluded participants who were diagnosed with dementia within five years after the study visit. Second, we calculated the seasonality of the g-factor and components of the g-factor only in the years with the most extreme variations of the average monthly ambient temperature to test the potential influence of ambient temperature on the seasonality of the g-factor. Ambient temperature was obtained from the Royal Dutch Meteorological Institute (KNMI).[67] The monitor is located approximately at 8km from the study district. Third, we visually inspected the annual variation of all outcomes without pre-specifying any seasonality assumptions, as is done with the use of cosinor terms (such as annual or bi-annual patterns). To do this, we converted the date of visit of each participant into the day of the year of the corresponding year. Next, we repeated the analyses using flexible splines with seven knots over the year,[217] instead of the cosinor terms. The obtained predicted values of the outcome and the average predicted value per week were plotted. All analyses were performed in Stata version 14.1 SE (StataCorp LP, College Station, Texas).[72]

RESULTS

Characteristics of the overall population at study visit are provided in Table 13 (page 120). Median age was 67.1 years (interquartile range (IQR): 60.0-75.2). There were more women (57%) than men and most of the participants attained low to intermediate education (41.1%) level of education. Participants who attended the research center in winter were on average less than one year older than those in summer (68.1 vs. 66.9 years, p<0.01). Other covariates were largely similar across seasons when attending the corresponding examination round (
Appendix 30 (page 125)).

Seasonality of global cognitive functioning

Global cognition constructed with factor analysis had an annual seasonal variation with lowest values observed in early February, (seasonal variation (SV): 0.04 standard deviation, 95% confidence interval (CI): 0.02 to 0.07) (Table 14 (page 121) and Figure 7 (page 123)). This variation was also observed in middle-aged participants, among whom the seasonality was larger than among the overall population (SV: 0.07 SD, 95%CI: 0.04 to 0.10), with nadir in late October (Table 14 (page 121)). Overall, participants with a higher g-factor had a larger seasonality of the score than their respective counterparts, such as women (0.05 SD, 0.02 to 0.08, nadir in mid-January), participants with low CES-D scale (0.04 SD, 0.01 to 0.06, nadir in mid-January), with a g-factor above the average (0.03 SD, 0.01 to 0.06, nadir in late-January), and those with an MMSE score of 26 and above (0.05 SD, 0.02 to 0.07, nadir in early-February) (Table 14 (page 121)). Compared to the age and sex adjusted model, the SV of the g-factor was smaller in the fully adjusted model (SV: 0.07 SD, 95%CI: 0.05 to 0.01 vs. 0.04 SD, 95%CI: 0.02 to 0.07), indicating that up to 64.6% of the observed seasonality of the g-factor was explained by confounders included in the full model (Appendix 31 (page 127)).

Within a subsample of the observations in which the delayed WLT test was obtained (n=13,833, 83%), we recalculated the g-factor, showing similar seasonal variation to that of the g-factor without the test (SV: 0.04 SD, 95% CI: 0.02 to 0.06), although the nadir was shifted from early-February to late-October (Appendix 32 (page 128)). The seasonality of the delayed WLT test itself was small and non-significant (SV: 0.02 SD, 95%CI: -0.01 to 0.06).

According to the BIC value, there is no evidence of better model fit for any of the cognitive tests when we included the biannual harmonics in the annual models (Appendix 33 (page 138)). The seasonality of Stroop Interference and Color naming test and LDST in the crude model was largely explained by the covariates, as the seasonal variation was significantly smaller when covariates were included in the model (Appendix 31 (page 127)).

Stratified analyses

The PPT both hands test had a significant seasonality in every subgroup examined, except in the old-elderly population and in those with working status occupied (Appendix 32 (page 128)). The magnitude of the seasonality was higher among those with a higher g-factor. The VFT also showed a significant seasonality among most of the subgroups analyzed, except among old-elderly, those with primary and intermediate-high education, g-factor above the average, and MMSE score equal or above 26.

Seasonality of cognitive function based on individual tests

The seasonality of the g-factor was mostly driven by the seasonality of the PPT for both hands, which showed the largest variation with lowest values in early January (SV: 0.08 SD, 95%CI: 0.05 to 0.11) and the VFT, which had the lowest value in late January (SV: 0.04 SD, 0.01 to 0.07) (Table 15 (page 122) and Figure 7 (page 123)). Indeed, after excluding the PPB for both hands from the g-factor calculation, the seasonality of the g-factor was smaller (SV: 0.03 SD, 95%CI: 0.01 to 0.06) (Appendix 34 (page 139)).

Sensitivity analyses

First, after excluding participants with a dementia diagnosis within the first five years after their last visit, the g-factor seasonality remained largely unchanged and statistically apparent (SV: 0.04, 95%CI: 0.02 to 0.07) (Table 14 (page 121)). The seasonality of the cognitive tests also remained similar after excluding these participants (Appendix 32 (page 128)). Second, in analyses among participants who attended the years with the most extreme variations in monthly average ambient temperature, the seasonality of the VFT test becomes smaller, whereas the seasonality of immediate, delayed and recognition tests becomes larger and significant, all with nadir in late-February (Appendix 36 (page 141)). Third, we found no evidence of substantial differences in the seasonal variation between the model using flexible splines and the annual plus harmonics test (Appendix 38 (page 143)).

DISCUSSION

In this study, we found an annual seasonality pattern of cognitive performance with up to three times lower global cognition in winter compared to summer among community-dwelling individuals. In addition, a lower seasonal variation was consistently observed among those individuals who had lower global cognition, attained lower levels of education and had lower MMSE score. These findings highlight the potential role of seasonal factors on cognitive performance and suggest that a smaller seasonal variation in cognitive performance may yield information on cognitive deterioration.

To our knowledge, this is the first study to examine the seasonality of cognitive function in middle-aged and elderly individuals from a large, population-based cohort. So far, few studies have examined the seasonality of cognitive function, reporting heterogeneous results. For instance, among 80 healthy cognitively unimpaired individuals from the United States (US) (mean age 20.9 years SD = 2.0) who underwent cognitive tests at different times of the year while measuring brain activity using electroencephalographic (EEG) P300 waves, a higher activity was observed during spring and summer compared to autumn and winter.[212] The P300 wave is an electroencephalographic measure of brain response under processes of decision making.[218,219] In contrast, another study from the US, among 32 healthy participants (mean age 26.2 years SD = 5.1), showed that auditory and visual P300 and visual slow waves were highest in winter or spring. These participants were also measured at two different seasons.[213] More recently, a cross-sectional fMRI study in young healthy adults showed that brain areas involved in attention tasks had a stronger brain activity in summer than in winter.[214] Also, brain areas involved in working memory tasks had a seasonal variation, which was slightly smaller and shifted about three months later than those in involved in attention tasks.[214] Nevertheless, they did not observe seasonality in a visual psychomotor vigilance task (sustained attention). Among 100 community-dwelling people from Tromso, about 300 kilometers above the Arctic Circle, no seasonality was observed in the performance of cognitive tests such as Stroop, time estimation, short-term memory (Sternberg task), or face recognition.[36] However, they found a better performance in speed of reaction task, dot numerosity, mapping, and word memory during winter and in fluency during summer.[36] In a longitudinal study with allergic patients, these had a slight reduction in the speed of cognitive processing and some individuals had difficulties in working memory, whereas no variation was

observed in non-allergic individuals during the ragweed season.[208] These findings may suggest that brain pathways involved in cognitive functioning are influenced by different environmental conditions depending on the season, leading to seasonal variation in some cognitive tests, but not in others.

In this study, the seasonal variation of cognitive function was mostly explained by a lower performance in a manual dexterity test (the Purdue Pegboard Task) and in a verbal fluency test during winter. Mechanistic and biological mechanisms can explain these findings. Among mechanistic, cold exposure may explain the lower test performance on the Purdue Pegboard Task in winter. For this test, as many as possible cylindrical metal pins have to be put into small holes on a board within 30 seconds – an effort that may be affected by ambient temperature, such as having cold hands. It is unlikely that ambient temperature also influences the test performance on the VFT test, where individuals have to come up with as many as possible animal names within one minute. However, evidence coming from 594 elderly men within the Veterans Affairs Normative Aging Study showed a decreased risk of low MMSE score when ambient temperature was between 10-15°C, whereas the risk increased at temperatures above and below this range.[209] As such, it is possible that when ambient temperature is too low or too high, participants may feel themselves uncomfortable and subsequently become inattentive, what could influence the test performance.[209] Among biological mechanisms, one study found higher activation of frontal, dorsolateral, prefrontal and parietal cortex under warmer temperatures during sustained attention task among patients with multiple sclerosis.[220] It is also suggested that ambient temperature may influence brain functions (e.g. learning and memory) through alterations on hippocampal neural activities.[209] Unfortunately, we did not have information on indoor temperature. This would be useful to disentangle the contribution of either mechanism because, for example, the potential influence of ambient temperature may be affected by the use of heaters and air conditioning inside the visit center building. Therefore, future studies examining the influence of temperature in cognitive performance while controlling for indoor temperature and thermal adaptation are required to test these hypotheses.

Other potential mechanisms explaining the seasonality of cognition could be sunlight exposure and seasonal mood changes. The circadian rhythm associated to sunlight exposure may underlie the seasonality of cognition.[214] However, no association was observed between the seasonality of EEG P300 wave length amplitude and sunlight time.[213] The vitamin D insufficiency has been associated with lower cognition performance,[210,211] thus, the seasonality of vitamin D might also relate the seasonal variation of sunlight exposure with cognition seasonality. Nevertheless, it is unclear if vitamin D levels could influence the cognition performance on a short-term basis or the extent at what the levels should fluctuate to induce such seasonality on cognition. Changes in mood may also contribute to the seasonality of cognition, as these are highly influenced by seasonal factors.[221,222] For instance, a case-control study among bipolar patients, their first-degree relatives, and unrelated controls,[207] found that individuals from families of patients performed worse during winter compared to controls on measures of visual and verbal attention, working memory, verbal ability, verbal fluency and executive functioning. In a study aimed to test the seasonality of cognitive function among participants with seasonal affective disorder, the patients showed improved attention during summer, when compared to

controls.[37] However, one study performed in Turkey among hemodialysis patients did not observe seasonality in MMSE score, but they found a significant seasonality in depression symptoms and quality of life.[223] We observed a seasonal variation of cognition independently of mood status, but it was statistically significant only among those who did not report the presence of depressive symptoms; thus, further studies are required to specifically address this potential mechanism.

Larger seasonal variation was consistently observed among participants with higher cognitive performance. We hypothesize this could be a sign of healthier or better preserved cognitive function, where healthy participants may have a better capability to cope with environmental changes across seasons.[224,225] Cognitively impaired individuals also exhibited a seasonal variation in cognition, but of smaller amplitude. Additionally, we observed that the inclusion of covariates in the statistical model had a larger impact in the magnitude of the seasonal variation among those more cognitively impaired than among the unimpaired. The inclusion of covariates in the model is aimed to account for the uneven distribution of participants' characteristics across the year, what may lead to a spurious seasonal variation of cognition. This finding is in concert with our hypothesis that among cognitively impaired individuals, the cognitive performance remains relatively stable on their pathophysiological (neurodegenerative) level throughout the year, whereas cognitively more unimpaired individuals could be able to fluctuate across seasons, relying on their cognitive reserve.

Because decline in cognition may lead to poor decision- making ability and executive functioning,[226,227] a seasonal variation in cognitive function could help to understand the nature of other seasonality phenomena among ageing populations. For example, the winter increase of cardiovascular disease[5] and the mortality excess related to heat waves could be partly attributed to reduced awareness of cool or warm feeling and execution of preventive measures. Our findings underscore the potential effects of seasonality on cognition and highlight that lack of seasonality across cognitive tests may be indicative for potential cognitive decline. Yet, as this is the first attempt to generalize findings from small and cross-sectional studies to the general population using a large cohort study, further studies are required to examine whether the effect estimates have a direct translation to clinical practice. It would be interesting to further study the potential role of seasonality effects involved in physiological pathways that constitute cognitive performance and maintain cognitive reserve.

Strengths of this study include the repeatedly and consistent cognitive test assessments in a large sample size of community-dwelling individuals. This reduces the between-subject heterogeneity that could explain the seasonal patterns. Additionally, we took a large set of potential confounders into consideration and we examined several sensitivity analyses to test the robustness of findings. Additionally, we also provided evidence that participants do not attend the research at random independently of the season, because up to 60% of the crude seasonal variation of the g-factor was explained by participants' characteristics. Nevertheless, some limitations deserve attention. First, we were not able to examine the seasonality of the cognition performance for the same individual at different seasons, because these volunteers attend the center at their best convenience during the year. For instance, individuals who had a demanding job may have visited the research center more often during holidays (e.g., summer times). These cognitively active individuals may have subsequently performed better on their cognitive tests,

confounding our results. However, there were no large differences in the proportion of occupied participants attending the research center across seasons, in fact, those who were occupied tended to attend the center more often in winter compared to summer (76.5% vs. 74.6%, p = 0.10). Moreover, results were unaffected after adjusting for several covariates, including educational level, household income and cardiovascular risk factors, yet we cannot exclude any further residual confounding. Second, by design, the generalizability of our findings is limited to white middle-aged and elderly population and may not generalizable to settings with smaller environmental differences across seasons, such as ambient temperature and sunlight hours.

In conclusion, we observed a seasonal variation in cognitive function, with lowest values in winter months. This pattern is largely explained by the seasonality of a manual dexterity and a verbal fluency test. These findings were more pronounced among individuals with higher cognitive functioning; indicating that seasonal variation in cognitive performance may provide information about healthy brain function ageing. Future studies are warranted to better understand the potential influence of the seasonality on cognitive performance, and to establish whether the season of a cognitive assessment may be of clinical importance.

Table 13. Characteristics of the participants at date of study visit

Covariates	Median	IQR	
Age, years	67.1	60.0	75.2
Depression (CES-D scale)	10.0	2.0	13.0
Alcohol intake, grams/day	6.4	1.0	14.0
Systolic blood pressure, mmHg	142.0	128.0	157.0
Total cholesterol, mmol/L	5.6	4.9	6.3
HDL-cholesterol, mmol/L	1.4	1.2	1.7
Glucose, mmol/mL	5.5	5.1	6.0
	n	%	
Sex			
Men	7,156	42.8	
Women	9,551	57.2	
Ethnicity			
Other	678	4.1	
White	16,029	95.9	
Living situation			
Community-dweller	16,654	99.7	
Non-community dweller	53	0.3	
Education			
Low	1,433	8.6	
Low/intermediate	6,872	41.1	
High general	5,022	30.1	
University	3,381	20.2	
Smoking status			
Never	5,162	30.9	
Current	2,935	17.6	
Former	8,611	51.5	
Body mass index			
<25 kg/m^2	4,782	28.6	
25-30 kg/m^2	8,006	47.9	
≥30 kg/m^2	3,919	23.5	
History of cardiovascular disease			
No	14,804	88.6	
Yes	1,903	11.4	
Medication use			
Antihypertensive			
No	10,006	59.9	
Yes	6,701	40.1	
Antidiabetic			
No	15,530	93.0	
Yes	1,177	7.0	
Statin			
No	12,781	76.5	
Yes	3,926	23.5	

Table 14. Seasonality of g-factor in overall population and stratified

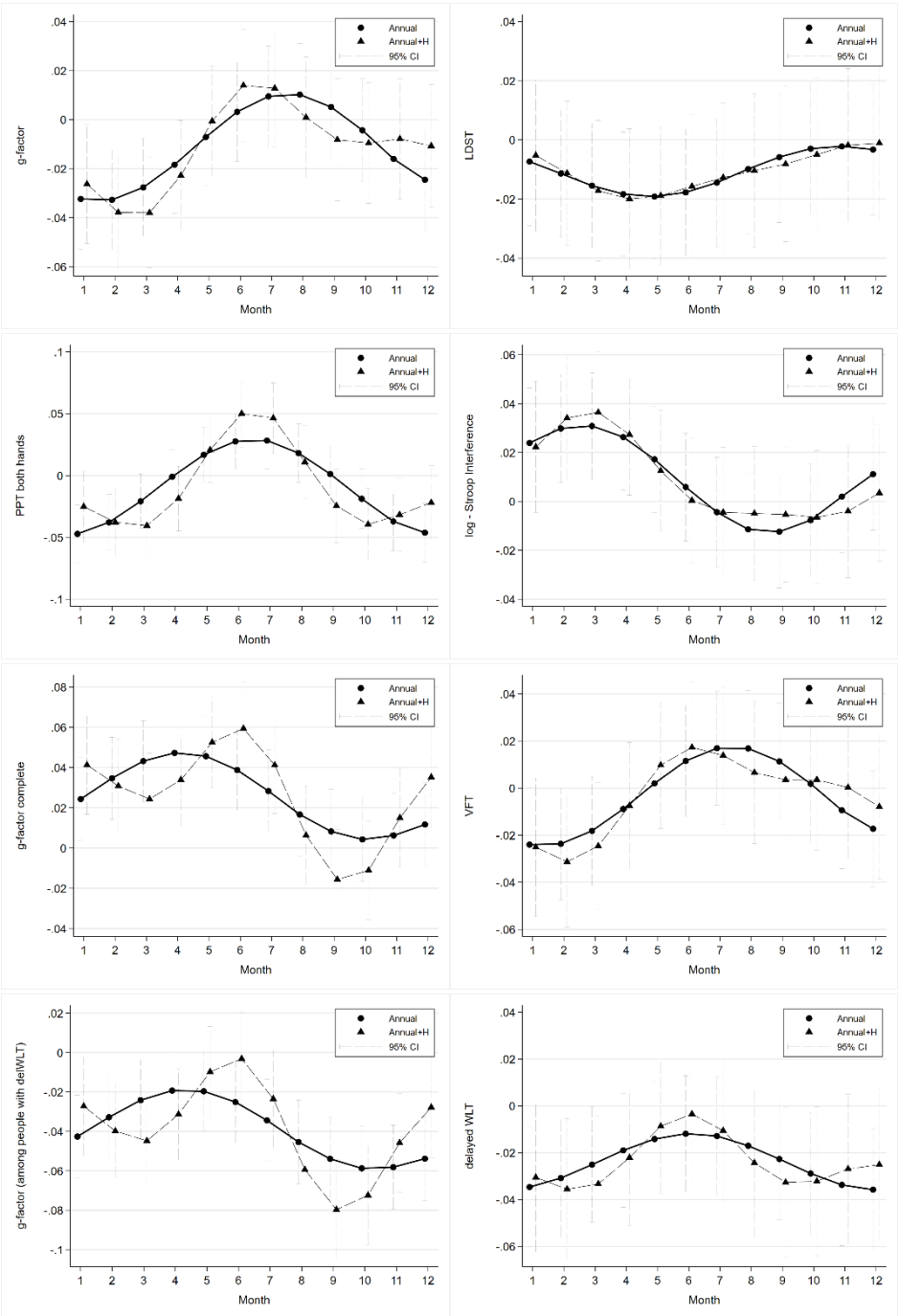
Strata	Observations	Average g-factor	Seasonal variation	95%	CI	Nadir
All	16,707	-0.01	0.04	0.02	0.07	1-Feb
Age-group						
Middle-aged	7,123	0.34	0.07	0.04	0.10	28-Oct
Young-elderly	5,310	0.06	0.05	0.00	0.09	5-Mar
Old elderly	4,274	-0.69	0.04	-0.02	0.10	6-Mar
Sex						
Men	7,156	-0.08	0.04	0.00	0.07	29-Feb
Women	9,551	0.04	0.05	0.02	0.08	15-Jan
CES-D						
<16	14,357	0.02	0.04	0.01	0.06	20-Jan
≥16	2,155	-0.16	0.07	-0.01	0.16	22-Apr
Education						
Low	1,412	-0.78	0.01	-0.09	0.10	2-Jun
Low/intermediate	6,788	-0.12	0.07	0.03	0.10	24-Jan
High general	4,966	0.04	0.05	0.01	0.09	11-Mar
University	3,346	0.46	0.03	-0.01	0.08	7-Dec
g-factor						
Below average	7,826	-0.83	0.02	-0.02	0.05	13-Mar
Above average	8,881	0.72	0.03	0.01	0.06	28-Jan
MMSE						
≥26	13,723	0.14	0.05	0.02	0.07	1-Feb
<26	2,933	-0.73	0.04	-0.03	0.12	15-Feb
Dementia diagnosis < 5 years						
Excluded	16,354	0.02	0.04	0.02	0.07	3-Feb
Working status						
Occupied	2,806	0.37	0.04	-0.04	0.12	19-Oct
Not occupied	8,751	-0.26	0.04	0.00	0.07	24-Nov

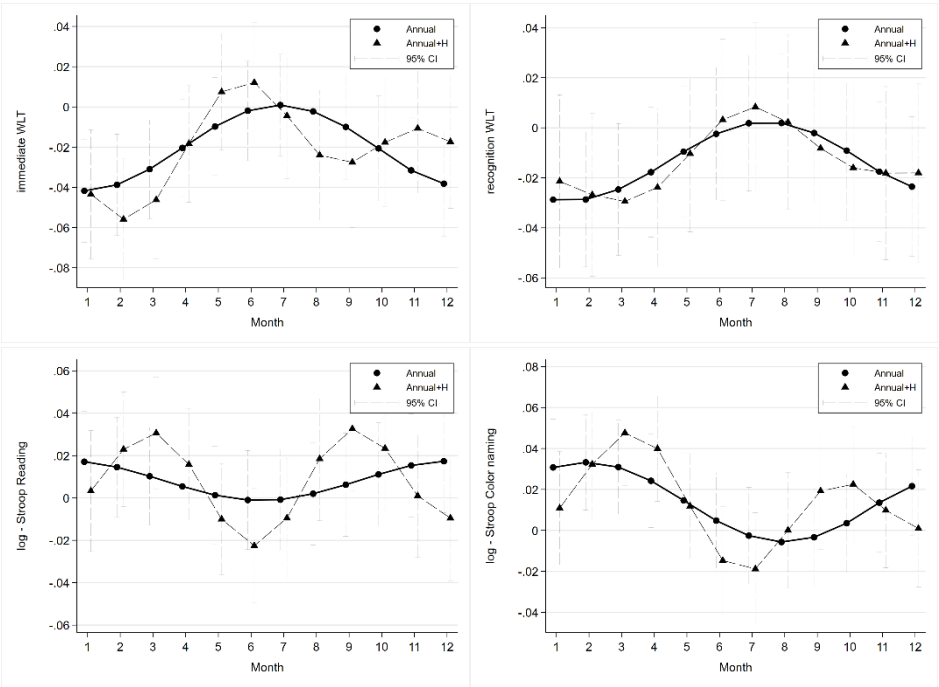
Table 15. Seasonality of cognitive tests

Cognitive tests*	Observations	Average g-factor	Seasonal variation	95%	CI	Nadir
g-factor	16,707	-0.01	0.04	0.02	0.07	1-Feb
LDST	16,707	-0.01	0.02	-0.01	0.04	10-May
log-Stroop Interference	16,707	0.01	0.04	0.02	0.07	5-Mar
VFT	16,707	0.00	0.04	0.01	0.07	28-Jan
PPT - both hands	16,707	-0.01	0.08	0.05	0.11	0-Jan
g-factor + delayed WLT	13,833	-0.04	0.04	0.02	0.06	26-Oct
g-factor of participants with WLT	13,833	0.03	0.04	0.02	0.07	20-Oct
Delayed WLT	13,833	-0.02	0.02	-0.01	0.06	19-Dec
Immediate WLT	13,837	-0.02	0.04	0.01	0.08	13-Jan
Recognition WLT	13,930	-0.01	0.03	-0.01	0.07	29-Jan
log-Stroop Color naming	16,706	0.01	0.04	0.01	0.07	15-Feb
log-Stroop Reading	16,704	0.01	0.02	-0.01	0.05	26-Dec

*All cognitive tests, except g-factor, are standardized. LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

Figure 7. Seasonal variation of g-factor and standardized cognitive tests





LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test. Annual + H means annual variation plus biannual harmonics. Monthly averages are adjusted for cosinor terms, age (linear and quadratic), ethnicity, SBP, cholesterol, HDL-cholesterol, glucose, antihypertensive, antidiabetic, statins intake, CES-D scale, BMI (categories), smoking behavior, alcohol intake, housing status, education, cohort, date (linear and quadratic).

SUPPLEMENTARY MATERIAL

Appendix 30. Characteristics of the participants at date of study visit, per season

Covariates	Winter (n=3,696)			Spring (n=4,963)			Summer (n=4,382)			Autumn (n=3,666)			p-value
	Median	IQR		Median	IQR		Median	IQR		Median	IQR		
Age, years	66.9	60.2	75.1	66.7	59.2	74.7	68.1	60.6	75.9	66.8	60.1	75.0	<0.01
Systolic blood pressure, mmHg	143.0	129.0	158.0	140.7	127.0	156.0	141.7	127.7	157.2	143.0	129.0	158.4	<0.01
Total cholesterol, mmol/L	5.6	4.9	6.3	5.5	4.8	6.2	5.6	4.9	6.3	5.6	4.9	6.3	0.04
HDL-cholesterol, mmol/L	1.4	1.2	1.7	1.4	1.1	1.7	1.4	1.2	1.7	1.4	1.2	1.7	0.14
Glucose, mmol/mL	5.5	5.1	6.1	5.5	5.1	6.0	5.5	5.1	6.0	5.5	5.1	6.0	1.00
CES-D scale	10.0	3.0	13.0	10.0	2.0	12.5	10.0	2.0	13.0	10.0	2.0	12.5	1.00
Alcohol intake, grams/day	7.2	0.8	13.9	6.4	0.7	13.0	6.4	1.2	13.5	6.6	1.4	14.9	0.08
Physical activity, z-score MET-hours/week)	-0.2	-0.8	0.5	-0.2	-0.7	0.6	-0.2	-0.7	0.5	-0.2	-0.8	0.5	
	n	%		n	%		n	%		n	%		
Sex													
Men	1,615	43.7		2,125	42.8		1,817	41.5		1,599	43.6		0.15
Women	2,081	56.3		2,838	57.2		2,565	58.5		2,067	56.4		
Ethnicity													
Other	145	3.9		234	4.7		156	3.6		143	3.9		0.04
White	3,551	96.1		4,729	95.3		4,226	96.4		3,523	96.1		
Living situation													
Community-dweller	3,688	99.8		4,950	99.7		4,361	99.5		3,655	99.7		0.15
Non-community dweller	8	0.2		13	0.3		21	0.5		11	0.3		
Prevalent CVD													
No	3,282	88.8		4,416	88.0		3,857	88.0		3,249	88.6		0.51
Yes	414	11.2		547	11.0		525	12.0		417	11.4		
Education													
Low	279	7.5		439	8.8		392	9.0		322	8.8		0.68
Low/intermediate	1,559	42.2		1,987	41.0		1,811	41.3		1,515	41.3		
High general	1,106	29.9		1,536	30.9		1,305	29.8		1,075	29.3		
University	751	20.3		1,002	20.2		874	19.9		754	20.6		
Smoking status													
Never	1,144	30.9		1,547	31.2		1,343	30.6		1,128	30.8		0.28

Current	611	16.5	900	18.1	749	17.1	674	18.4	
Former	1,941	52.2	2,516	50.7	2,290	52.3	1,864	50.8	
Body mass index									
<25 kg/m^2	1,033	27.9	1,421	28.6	1,311	29.9	1,017	27.7	0.20
25-30 kg/m^2	1,794	48.5	2,333	47.0	2,075	47.4	1,804	49.2	
≥30 kg/m^2	869	23.5	1,208	24.3	996	22.7	845	23.1	
Medication use									
Antihypertensive									
No	2,210	59.8	2,088	60.2	2,634	60.1	2,173	59.3	0.83
Yes	1,486	40.2	1,975	39.8	1,748	39.9	1,493	40.7	
Antidiabetic									
No	3,423	92.6	4,605	92.8	4,092	93.4	3,410	93.0	0.54
Yes	273	7.4	358	7.2	290	6.6	256	7.0	
Statin									
No	2,801	75.8	3,857	77.9	3,349	76.4	2,774	75.7	0.09
Yes	895	24.2	1,106	22.3	1,033	23.6	892	24.3	

Appendix 31. Seasonality of g-factor and cognitive tests – Crude models (adjusted for cosinor terms, age and sex).

			Annual seasonal variation					Annual + harmonics seasonal variation				
Cognitive test	**Observations**	**Average**	**Seasonal variation**	**95%**	**CI**	**Nadir**	**%Change SV**	**Seasonal variation**	**95%**	**CI**	**Nadir**	**%Change SV**
g-factor	16,707	-0.07	0.07	0.05	0.10	21-Feb	64.6	0.07	0.03	0.10	31-Mar	65.3
LDST	16,707	-0.06	0.04	0.02	0.07	12-Apr	138.6	0.04	0.00	0.08	30-Apr	104.9
log-Stroop Reading	16,704	0.04	0.03	0.00	0.06	12-Feb	41.3	0.05	0.01	0.10	31-Mar	43.7
log-Stroop Color naming	16,706	0.05	0.06	0.03	0.08	24-Feb	47.3	0.05	0.01	0.09	31-Mar	39.1
log-Stroop Interference	16,707	0.05	0.08	0.05	0.10	7-Mar	71.4	0.08	0.04	0.12	31-Mar	52.2
VFT	16,707	-0.03	0.06	0.03	0.09	13-Feb	35.3	0.06	0.02	0.11	1-Mar	28.4
PPB both hands	16,707	-0.04	0.08	0.05	0.11	20-Jan	4.5	0.03	-0.02	0.07	31-Mar	17.7
g-factor + delWLT	13,833	-0.07	0.04	0.01	0.07	5-Mar	3.9	0.07	0.03	0.11	31-Mar	104.9
Delayed WLT	13,833	-0.04	0.05	0.01	0.08	5-Mar	97.1	0.06	0.01	0.11	31-Mar	491.8
Immediate WLT	13,837	-0.03	0.07	0.03	0.11	25-Feb	62.2	0.11	0.05	0.16	1-Mar	97.3
Recognition WLT	13,930	-0.02	0.05	0.01	0.09	2-Mar	56.4	0.05	-0.01	0.10	31-Mar	161.9
g-factor of participants with WLT data	13,833	0.00	0.03	0.00	0.05	11-Feb	-35.3	0.05	0.01	0.09	31-Mar	15.8

%Change SV indicates the change of the seasonal variation in the crude model (adjusted for cosinor terms, sex, and age) with respect to the seasonal variation estimated in the fully adjusted model. It was estimated as ((seasonal variation crude model – seasonal variation adjusted model)/seasonal variation adjusted model)*100. A positive change indicates that the seasonal variation was larger in the crude model than in the fully adjusted model, suggesting that covariates explained the crude seasonal variation. A negative change indicates that the seasonal variation was larger in the fully adjusted model than in the crude model, and may also indicate that differences in the population characteristics across seasons led to underestimate the seasonality in the crude model. LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

Appendix 32. Seasonality of cognitive tests, stratified by subgroups

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
g-factor										
All	16,707	-0.01	0.04	0.02	0.07	1-Feb	0.04	0.01	0.07	1-Apr
Age-group										
Middle-aged	7,123	0.34	0.07	0.04	0.10	28-Oct	0.07	0.02	0.12	27-Oct
Young-elderly	5,310	0.06	0.05	0.00	0.09	5-Mar	0.02	-0.05	0.10	1-May
Old elderly	4,274	-0.69	0.04	-0.02	0.10	6-Mar	0.07	0.02	0.12	2-Mar
Sex										
Men	7,156	-0.08	0.04	0.00	0.07	29-Feb	0.06	0.01	0.11	31-Mar
Women	9,551	0.04	0.05	0.02	0.08	15-Jan	0.05	0.00	0.10	31-Jan
CES-D scale										
<16	14,357	0.02	0.04	0.01	0.06	20-Jan	0.03	-0.01	0.07	24-Mar
≥16	2,155	-0.16	0.07	-0.01	0.16	22-Apr	0.13	0.01	0.26	30-Apr
Education										
Low	1,412	-0.78	0.01	-0.09	0.10	2-Jun	0.07	-0.07	0.22	28-Aug
Low/intermediate	6,788	-0.12	0.07	0.03	0.10	24-Jan	0.06	0.01	0.11	1-Mar
High general	4,966	0.04	0.05	0.01	0.09	11-Mar	0.06	-0.01	0.12	31-Mar
University	3,346	0.46	0.03	-0.01	0.08	7-Dec	0.03	-0.04	0.09	27-Oct
G-factor										
Below average	7,826	-0.83	0.02	-0.02	0.05	13-Mar	0.04	-0.01	0.09	18-Mar
Above average	8,881	0.72	0.03	0.01	0.06	28-Jan	0.03	0.00	0.07	22-Mar
MMSE										
≥26	13,723	0.14	0.05	0.02	0.07	1-Feb	0.03	0.00	0.07	6-Apr
<26	2,933	-0.73	0.04	-0.03	0.12	15-Feb	0.04	-0.07	0.15	6-Apr
Dementia diagnosis < 5 years										
Excluded	16,354	0.02	0.04	0.02	0.07	3-Feb	0.04	0.01	0.07	2-Mar
Working status										
Occupied	2,806	0.37	0.04	-0.04	0.12	19-Oct	0.05	-0.05	0.16	27-Sep
Not occupied	8,751	-0.26	0.04	0.00	0.07	24-Nov	0.05	-0.01	0.10	26-Dec
std-LDST										
All	16,707	-0.01	0.02	-0.01	0.04	10-May	0.02	-0.02	0.06	19-May
Age-group										
Middle-aged	7,123	0.28	0.03	-0.01	0.07	9-Sep	0.05	-0.01	0.10	27-Oct
Young-elderly	5,310	0.05	0.05	0.00	0.10	25-Jun	0.07	-0.01	0.15	14-Jun
Old elderly	4,274	-0.56	0.04	-0.02	0.10	23-May	0.03	-0.03	0.08	10-Jul

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Sex										
Men	7,156	-0.05	0.00	-0.03	0.04	14-Jul	0.02	-0.04	0.08	27-Sep
Women	9,551	0.01	0.03	0.00	0.06	14-May	0.02	-0.03	0.07	30-May
CES-D scale										
<16	14,357	0.02	0.01	-0.02	0.04	10-Jun	0.00	-0.04	0.05	13-Jul
≥16	2,155	-0.17	0.11	0.02	0.19	21-May	0.18	0.05	0.31	9-May
Education										
Low	1,412	-0.79	0.06	-0.03	0.16	29-Apr	0.10	-0.04	0.24	31-Mar
Low/intermediate	6,788	-0.12	0.03	-0.01	0.07	12-Mar	0.04	-0.02	0.10	31-Mar
High general	4,966	0.06	0.04	0.00	0.08	7-Jul	0.07	0.00	0.14	27-Sep
University	3,346	0.44	0.00	-0.05	0.06	29-Sep	0.05	-0.03	0.12	26-Dec
G-factor										
Below average	7,826	-0.69	0.04	0.00	0.08	25-May	0.06	0.01	0.11	22-Aug
Above average	8,881	0.59	0.01	-0.02	0.04	14-Aug	0.02	-0.02	0.07	22-Jul
MMSE										
≥26	13,723	0.12	0.01	-0.02	0.03	23-Mar	0.01	-0.03	0.05	29-May
<26	2,933	-0.65	0.09	0.02	0.17	4-May	0.12	0.01	0.23	28-Jul
Dementia diagnosis < 5 years										
Excluded	16,354	0.02	0.02	-0.01	0.04	18-May	0.02	-0.01	0.06	19-May
Working status										
Occupied	2,806	0.32	0.04	-0.05	0.12	2-Jul	0.08	-0.05	0.20	28-Aug
Not occupied	8,751	-0.21	0.05	0.01	0.08	12-Aug	0.07	0.01	0.12	27-Oct
std-log-Stroop Reading										
All	16,704	0.01	0.02	-0.01	0.05	26-Dec	0.04	-0.01	0.08	14-Oct
Age-group										
Middle-aged	7,122	-0.19	0.01	-0.03	0.06	1-Feb	0.13	0.06	0.19	27-Oct
Young-elderly	5,308	-0.04	0.01	-0.05	0.06	4-Mar	0.02	-0.06	0.11	5-Jan
Old elderly	4,274	0.38	0.02	-0.05	0.09	29-Aug	0.04	-0.02	0.10	15-Mar
Sex										
Men	7,155	-0.02	0.01	-0.03	0.05	15-Aug	0.09	0.02	0.15	27-Sep
Women	9,549	0.03	0.03	-0.01	0.07	11-Jun	0.01	-0.04	0.07	1-Mar
CES-D scale										
<16	14,354	-0.02	0.02	-0.01	0.05	14-Jul	0.05	0.00	0.09	6-Apr
≥16	2,155	0.11	0.11	0.01	0.20	29-Feb	0.19	0.04	0.33	6-Nov
Education										

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Low	1,412	0.61	0.06	-0.05	0.18	8-Sep	0.15	-0.03	0.33	31-Mar
Low/intermediate	6,787	0.09	0.03	-0.01	0.08	28-Jun	0.02	-0.05	0.09	1-Mar
High general	4,965	-0.05	0.03	-0.03	0.08	30-Jul	0.03	-0.05	0.11	31-Mar
University	3,345	-0.32	0.04	-0.02	0.11	4-Mar	0.08	-0.02	0.17	27-Sep
G-factor										
Below average	7,824	0.44	0.02	-0.03	0.07	21-Jul	0.07	-0.01	0.14	2-Apr
Above average	8,880	-0.39	0.02	-0.02	0.05	30-Apr	0.03	-0.02	0.09	20-Oct
MMSE										
≥26	13,720	-0.09	0.02	-0.01	0.06	31-May	0.03	-0.02	0.07	13-Oct
<26	2,933	0.49	0.08	-0.01	0.18	25-Sep	0.14	0.00	0.29	24-Apr
Dementia diagnosis < 5 years										
Excluded	16,351	-0.01	0.02	-0.01	0.04	9-Jun	0.04	0.00	0.08	12-Oct
Working status										
Occupied	2,806	-0.23	0.05	-0.04	0.14	26-Apr	0.10	-0.04	0.23	29-Jun
Not occupied	8,750	0.14	0.05	0.00	0.10	14-Jun	0.02	-0.04	0.09	29-Jun
std-log-Stroop Color naming										
All	16,706	0.01	0.04	0.01	0.07	15-Feb	0.04	0.00	0.08	22-Apr
Age-group										
Middle-aged	7,123	-0.18	0.03	-0.01	0.07	19-Sep	0.05	-0.01	0.11	31-Mar
Young-elderly	5,310	-0.04	0.03	-0.02	0.08	29-Jul	0.01	-0.07	0.09	3-May
Old elderly	4,273	0.37	0.05	-0.02	0.12	5-Sep	0.02	-0.04	0.08	23-Apr
Sex										
Men	7,156	0.11	0.04	0.00	0.08	22-Jul	0.08	0.02	0.13	31-Mar
Women	9,550	-0.05	0.04	0.01	0.08	3-Sep	0.02	-0.03	0.08	30-Apr
CES-D scale										
<16	14,356	-0.01	0.04	0.01	0.07	21-Aug	0.04	0.00	0.08	19-Apr
≥16	2,155	0.11	0.08	-0.01	0.17	11-Jan	0.15	0.01	0.29	16-Oct
Education										
Low	1,411	0.53	0.12	0.02	0.22	30-Sep	0.12	-0.03	0.27	30-Apr
Low/intermediate	6,788	0.02	0.06	0.01	0.10	18-Aug	0.03	-0.04	0.09	31-Mar
High general	4,966	-0.01	0.04	0.00	0.09	4-Aug	0.01	-0.05	0.08	31-Mar
University	3,346	-0.18	0.01	-0.04	0.07	29-Apr	0.07	-0.02	0.15	27-Sep
G-factor										
Below average	7,825	0.47	0.04	-0.01	0.09	16-Sep	0.06	-0.01	0.13	29-Apr
Above average	8,881	-0.41	0.03	0.00	0.07	27-Aug	0.05	0.00	0.10	11-Apr

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
MMSE										
≥26	13,722	-0.09	0.04	0.01	0.07	3-Aug	0.03	-0.02	0.07	20-Apr
<26	2,933	0.49	0.10	0.02	0.19	25-Sep	0.17	0.04	0.31	25-Apr
Dementia diagnosis < 5 years										
Excluded	16,353	-0.01	0.04	0.01	0.07	20-Aug	0.04	0.00	0.08	21-Apr
Working status										
Occupied	2,806	-0.21	0.03	-0.06	0.12	24-Sep	0.08	-0.05	0.21	26-Dec
Not occupied	8,750	0.14	0.03	-0.02	0.07	21-Apr	0.03	-0.03	0.09	29-Jun
std-log-Stroop Interference										
All	16,707	0.01	0.04	0.02	0.07	5-Mar	0.05	0.01	0.09	6-Apr
All										
Age-group	7,123	-0.28	0.00	-0.03	0.04	14-Jul	0.04	-0.02	0.09	27-Oct
Middle-aged	5,310	-0.06	0.04	-0.01	0.10	17-Oct	0.02	-0.06	0.11	24-Jun
Young-elderly	4,274	0.58	0.03	-0.04	0.11	24-Aug	0.07	0.01	0.13	26-Feb
Old elderly										
Sex	7,156	0.02	0.04	0.00	0.08	27-Sep	0.07	0.01	0.13	31-Mar
Men	9,551	0.00	0.05	0.01	0.09	22-Aug	0.07	0.01	0.12	31-Jan
Women										
CES-D scale	14,357	-0.02	0.04	0.01	0.06	29-Aug	0.04	0.00	0.08	5-Apr
<16	2,155	0.15	0.08	-0.02	0.18	30-Sep	0.09	-0.05	0.23	14-Apr
≥16										
Education	1,412	0.67	0.05	-0.08	0.17	17-Mar	0.03	-0.15	0.21	28-Aug
Low	6,788	0.09	0.05	0.01	0.09	18-Aug	0.05	-0.01	0.12	1-Mar
Low/intermediate	4,966	-0.04	0.08	0.03	0.13	13-Sep	0.08	0.01	0.15	31-Mar
High general	3,346	-0.36	0.02	-0.03	0.07	5-Nov	0.06	-0.01	0.13	31-Mar
University										
G-factor	7,826	0.64	0.01	-0.04	0.06	12-Sep	0.04	-0.04	0.11	4-Feb
Below average	8,881	-0.55	0.05	0.03	0.08	15-Sep	0.06	0.03	0.10	15-Apr
Above average										
MMSE	13,723	-0.12	0.05	0.02	0.07	4-Sep	0.04	0.00	0.08	19-Apr
≥26	2,933	0.63	0.03	-0.06	0.13	29-Jul	0.03	-0.11	0.17	27-Mar
<26										
Dementia diagnosis < 5 years	16,354	-0.02	0.04	0.01	0.07	6-Sep	0.05	0.01	0.09	3-Apr
Excluded										
Working status	2,806	-0.34	0.04	-0.04	0.12	2-Mar	0.06	-0.05	0.17	31-Jan

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Occupied	8,751	0.22	0.02	-0.02	0.07	16-Apr	0.04	-0.03	0.11	30-Apr
std-VFT										
All	16,707	0.00	0.04	0.01	0.07	28-Jan	0.05	0.00	0.09	16-Mar
Age-group										
Middle-aged	7,123	0.19	0.07	0.02	0.12	4-Nov	0.04	-0.03	0.12	27-Oct
Young-elderly	5,310	0.03	0.07	0.01	0.13	13-Feb	0.06	-0.03	0.16	6-Jan
Old elderly	4,274	-0.39	0.06	-0.01	0.13	31-Mar	0.07	0.01	0.13	21-Feb
Sex										
Men	7,156	0.03	0.05	0.00	0.10	7-Mar	0.06	-0.01	0.13	31-Mar
Women	9,551	-0.03	0.05	0.01	0.09	29-Dec	0.05	-0.01	0.12	31-Jan
CES-D scale										
<16	14,357	0.01	0.05	0.02	0.09	10-Jan	0.05	0.00	0.10	10-Mar
≥16	2,155	-0.08	0.11	0.02	0.21	22-May	0.17	0.02	0.31	18-May
Education										
Low	1,412	-0.48	0.09	-0.02	0.20	16-Jun	0.11	-0.06	0.28	29-Jul
Low/intermediate	6,788	-0.13	0.08	0.03	0.13	31-Jan	0.08	0.01	0.16	1-Mar
High general	4,966	0.04	0.05	-0.01	0.10	15-Mar	0.08	-0.01	0.16	31-Jan
University	3,346	0.40	0.07	0.00	0.14	17-Nov	0.05	-0.05	0.15	27-Oct
G-factor										
Below average	7,826	-0.60	0.05	0.01	0.10	10-Mar	0.09	0.03	0.16	20-Mar
Above average	8,881	0.52	0.03	-0.01	0.07	22-Nov	0.02	-0.05	0.08	13-Oct
MMSE										
≥26	13,723	0.11	0.04	0.00	0.07	1-Feb	0.04	-0.01	0.09	25-Mar
<26	2,933	-0.53	0.07	-0.02	0.15	24-Feb	0.08	-0.04	0.20	15-Feb
Dementia diagnosis < 5 years										
Excluded	16,354	0.02	0.04	0.01	0.07	2-Feb	0.05	0.01	0.10	16-Mar
Working status										
Occupied	2,806	0.22	0.10	0.00	0.21	29-Oct	0.12	-0.02	0.26	27-Oct
Not occupied	8,751	-0.15	0.06	0.02	0.11	13-Feb	0.05	-0.02	0.12	31-Mar
std-PPB both hands										
All	16,707	-0.01	0.08	0.05	0.11	0-Jan	0.02	-0.03	0.07	6-Apr
Age-group										
Middle-aged	7,123	0.29	0.11	0.06	0.16	10-Nov	0.08	0.01	0.15	27-Oct
Young-elderly	5,310	0.03	0.11	0.05	0.18	24-Jan	0.08	-0.01	0.18	27-Dec

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Old elderly	4,274	-0.54	0.05	-0.02	0.12	12-Jan	0.06	0.00	0.13	4-Mar
Sex										
Men	7,156	-0.23	0.06	0.01	0.11	17-Jan	0.03	-0.04	0.11	31-Mar
Women	9,551	0.16	0.09	0.05	0.13	19-Dec	0.05	-0.01	0.12	26-Nov
CES-D scale										
<16	14,357	0.00	0.06	0.03	0.10	4-Jan	0.02	-0.03	0.07	10-Apr
≥16	2,155	-0.07	0.10	0.00	0.20	12-Dec	0.02	-0.12	0.15	24-Oct
Education										
Low	1,412	-0.34	0.14	0.03	0.26	30-Nov	0.01	-0.16	0.18	27-Sep
Low/intermediate	6,788	0.00	0.09	0.04	0.14	7-Dec	0.06	-0.02	0.13	27-Oct
High general	4,966	-0.02	0.06	0.01	0.12	24-Jan	0.03	-0.06	0.11	31-Mar
University	3,346	0.14	0.08	0.01	0.14	18-Jan	0.01	-0.09	0.12	31-Mar
G-factor										
Below average	7,826	-0.54	0.06	0.01	0.10	22-Nov	0.03	-0.04	0.09	1-Nov
Above average	8,881	0.47	0.08	0.04	0.12	9-Jan	0.04	-0.02	0.10	4-Mar
MMSE										
≥26	13,723	0.06	0.08	0.04	0.11	7-Jan	0.03	-0.02	0.08	3-Apr
<26	2,933	-0.32	0.09	0.01	0.18	3-Dec	0.09	-0.03	0.22	20-Nov
Dementia diagnosis < 5 years										
Excluded	16,354	0.01	0.08	0.04	0.11	1-Jan	0.02	-0.03	0.07	6-Apr
Working status										
Occupied	2,806	0.20	0.07	-0.03	0.16	11-Jan	0.04	-0.09	0.17	27-Oct
Not occupied	8,751	-0.17	0.07	0.02	0.12	14-Dec	0.08	0.01	0.15	26-Nov
g-factor + delWLT										
All	13,833	-0.04	0.04	0.02	0.06	26-Oct	0.04	0.00	0.07	18-Oct
Age-group										
Middle-aged	5,385	0.34	0.06	0.03	0.10	15-Oct	0.04	-0.02	0.10	27-Sep
Young-elderly	4,667	0.08	0.03	-0.02	0.07	17-Oct	0.07	0.01	0.14	27-Nov
Old elderly	3,781	-0.64	0.02	-0.04	0.08	22-Dec	0.02	-0.03	0.06	4-Nov
Sex										
Men	5,898	-0.14	0.03	0.00	0.07	19-Oct	0.03	-0.02	0.09	27-Sep
Women	7,935	0.04	0.05	0.02	0.08	28-Oct	0.04	-0.01	0.09	27-Sep
CES-D scale										
<16	11,852	-0.01	0.05	0.03	0.08	18-Oct	0.04	0.00	0.08	21-Oct
≥16	1,823	-0.14	0.03	-0.05	0.11	31-May	0.17	0.04	0.30	17-Oct

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Education										
Low	1,163	-0.83	0.07	-0.03	0.16	28-Jul	0.16	0.01	0.31	28-Aug
Low/intermediate	5,528	-0.16	0.05	0.02	0.09	7-Nov	0.01	-0.04	0.07	27-Sep
High general	4,146	0.02	0.02	-0.02	0.07	20-Jan	0.05	-0.02	0.12	27-Oct
University	2,835	0.44	0.09	0.04	0.14	3-Oct	0.08	0.01	0.15	27-Sep
G-factor										
Below average	6,121	-0.86	0.01	-0.03	0.05	7-Oct	0.03	-0.03	0.08	29-Oct
Above average	7,712	0.65	0.02	0.00	0.05	8-Nov	0.01	-0.03	0.05	2-Oct
MMSE										
≥26	11,447	0.12	0.04	0.02	0.07	3-Nov	0.03	0.00	0.07	22-Oct
<26	2,337	-0.77	0.01	-0.07	0.10	13-Feb	0.04	-0.09	0.17	21-Apr
Dementia diagnosis < 5 years										
Excluded	13,550	0.00	0.04	0.02	0.06	26-Oct	0.03	-0.01	0.07	16-Oct
Working status										
Occupied	2,248	0.25	0.06	-0.04	0.15	9-Sep	0.11	-0.02	0.24	27-Oct
Not occupied	6,812	-0.25	0.02	-0.03	0.06	21-Oct	0.04	-0.02	0.10	27-Oct
std-Delayed WLT										
All	13,833	-0.02	0.02	-0.01	0.06	19-Dec	0.01	-0.04	0.06	24-Mar
Age-group										
Middle-aged	5,385	0.20	0.01	-0.05	0.07	16-Sep	0.02	-0.07	0.11	29-Jul
Young-elderly	4,667	0.02	0.07	0.00	0.13	3-Jan	0.06	-0.03	0.15	1-Dec
Old elderly	3,781	-0.34	0.03	-0.04	0.11	24-Feb	0.03	-0.04	0.09	7-Apr
Sex										
Men	5,898	-0.20	0.05	0.00	0.10	13-Dec	0.06	-0.01	0.14	26-Nov
Women	7,935	0.11	0.01	-0.04	0.05	26-Jan	0.05	-0.02	0.12	1-Mar
CES-D scale										
<16	11,852	-0.01	0.03	0.00	0.07	16-Nov	0.02	-0.03	0.08	3-Nov
≥16	1,823	-0.04	0.08	-0.03	0.18	5-May	0.19	0.03	0.35	13-May
Education										
Low	1,163	-0.50	0.02	-0.10	0.14	6-Nov	0.13	-0.05	0.31	27-Sep
Low/intermediate	5,528	-0.09	0.03	-0.02	0.08	11-Jan	0.05	-0.03	0.14	26-Dec
High general	4,146	0.00	0.06	0.00	0.12	10-Jan	0.01	-0.08	0.10	27-Oct
University	2,835	0.27	0.06	-0.01	0.14	18-Aug	0.03	-0.09	0.14	28-Aug
G-factor										
Below average	6,121	-0.42	0.04	-0.01	0.10	17-Dec	0.06	-0.02	0.14	2-Jan

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Above average	7,712	0.30	0.01	-0.04	0.05	22-Jun	0.05	-0.02	0.12	20-Sep
MMSE										
≥26	11,447	0.09	0.02	-0.02	0.05	1-Jan	0.02	-0.04	0.08	5-Nov
<26	2,337	-0.56	0.03	-0.06	0.12	8-Feb	0.07	-0.07	0.21	10-Feb
Dementia diagnosis < 5 years										
Excluded	13,550	0.01	0.02	-0.01	0.06	0-Jan	0.02	-0.03	0.07	16-Mar
Working status										
Occupied	2,248	0.12	0.13	0.02	0.24	2-Aug	0.12	-0.03	0.27	29-Jul
Not occupied	6,812	-0.14	0.06	0.00	0.12	21-Dec	0.07	-0.01	0.15	26-Nov
std-immediate WLT										
All	13,837	-0.02	0.04	0.01	0.08	13-Jan	0.05	0.00	0.11	18-Mar
Age-group										
Middle-aged	5,386	0.21	0.05	0.00	0.11	11-Dec	0.03	-0.06	0.12	1-Mar
Young-elderly	4,668	0.03	0.08	0.02	0.15	2-Jan	0.08	-0.02	0.18	17-Jan
Old elderly	3,783	-0.37	0.08	0.01	0.15	18-Mar	0.09	0.02	0.15	16-Mar
Sex										
Men	5,901	-0.18	0.08	0.03	0.13	8-Dec	0.06	-0.02	0.14	31-Jan
Women	7,936	0.10	0.04	0.00	0.09	6-Mar	0.08	0.01	0.15	1-Mar
CES-D scale										
<16	11,856	-0.01	0.04	0.00	0.07	21-Dec	0.05	-0.01	0.10	11-Mar
≥16	1,823	-0.04	0.10	-0.01	0.21	14-Mar	0.06	-0.10	0.23	13-May
Education										
Low	1,163	-0.55	0.08	-0.04	0.21	27-Mar	0.15	-0.03	0.34	31-Mar
Low/intermediate	5,530	-0.10	0.06	0.00	0.11	7-Feb	0.10	0.02	0.18	1-Mar
High general	4,147	0.03	0.08	0.01	0.14	30-Jan	0.04	-0.05	0.14	31-Mar
University	2,836	0.28	0.09	0.02	0.17	14-Oct	0.02	-0.09	0.12	27-Sep
G-factor										
Below average	6,124	-0.44	0.04	-0.02	0.10	28-Feb	0.06	-0.02	0.14	18-Mar
Above average	7,713	0.33	0.04	-0.01	0.08	18-Jan	0.07	0.01	0.14	16-Mar
MMSE										
≥26	11,451	0.09	0.04	0.00	0.08	11-Jan	0.03	-0.02	0.09	1-Apr
<26	2,337	-0.58	0.07	-0.03	0.16	18-Mar	0.10	-0.04	0.23	28-Feb
Dementia diagnosis < 5 years										
Excluded	13,554	0.00	0.04	0.01	0.08	13-Jan	0.06	0.01	0.11	14-Mar
Working status										

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Occupied	2,248	0.14	0.02	-0.09	0.13	2-Mar	0.06	-0.09	0.21	1-Mar
Not occupied	6,813	-0.16	0.07	0.01	0.13	2-Feb	0.06	-0.02	0.15	31-Jan
std-recognition WLT										
All	13,930	-0.01	0.03	-0.01	0.07	29-Jan	0.02	-0.04	0.07	13-Apr
Age-group										
Middle-aged	5,416	0.11	0.04	-0.02	0.10	16-Dec	0.03	-0.05	0.12	27-Oct
Young-elderly	4,717	0.01	0.07	0.00	0.14	19-Feb	0.06	-0.04	0.17	4-Feb
Old elderly	3,797	-0.21	0.05	-0.04	0.15	26-Feb	0.06	-0.01	0.14	17-Mar
Sex										
Men	5,932	-0.14	0.10	0.04	0.16	24-Dec	0.11	0.02	0.20	31-Jan
Women	7,998	0.08	0.04	-0.01	0.09	6-May	0.06	-0.01	0.14	30-Apr
CES-D scale										
<16	11,931	-0.01	0.03	-0.01	0.07	20-Jan	0.01	-0.05	0.07	21-Apr
≥16	1,838	-0.05	0.04	-0.08	0.16	13-Apr	0.06	-0.12	0.24	5-Jul
Education										
Low	1,176	-0.37	0.17	0.00	0.34	5-Jun	0.33	0.07	0.59	28-Aug
Low/intermediate	5,575	-0.05	0.07	0.01	0.13	22-Feb	0.10	0.00	0.19	1-Mar
High general	4,164	0.01	0.06	0.00	0.13	9-Jan	0.06	-0.04	0.16	26-Nov
University	2,851	0.17	0.07	-0.01	0.14	22-Oct	0.07	-0.04	0.17	27-Oct
G-factor										
Below average	6,185	-0.26	0.08	0.01	0.15	16-Mar	0.10	0.00	0.20	15-Apr
Above average	7,745	0.20	0.04	0.00	0.09	20-Nov	0.06	0.00	0.13	21-Dec
MMSE										
≥26	11,522	0.08	0.04	0.00	0.08	11-Jan	0.01	-0.05	0.07	22-Nov
<26	2,360	-0.44	0.13	-0.01	0.26	31-Mar	0.14	-0.05	0.34	13-Mar
Dementia diagnosis < 5 years										
Excluded	13,644	0.01	0.03	0.00	0.07	28-Jan	0.03	-0.03	0.08	27-Mar
Working status										
Occupied	2,254	0.09	0.01	-0.10	0.11	3-Apr	0.04	-0.12	0.20	30-Apr
Not occupied	6,865	-0.09	0.05	-0.02	0.12	1-Feb	0.04	-0.05	0.14	26-Dec
g-factor of participants with WLT data										
All	13,833	0.03	0.04	0.02	0.07	20-Oct	0.04	0.01	0.08	21-Oct
Age-group										
Middle-aged	5,385	0.46	0.07	0.03	0.11	18-Oct	0.06	0.00	0.11	27-Oct

Strata	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Young-elderly	4,667	0.12	0.03	-0.01	0.08	12-Sep	0.07	0.00	0.14	23-Nov
Old elderly	3,781	-0.62	0.02	-0.03	0.08	13-Nov	0.03	-0.02	0.08	30-Oct
Sex										
Men	5,898	-0.04	0.03	0.00	0.07	27-Sep	0.05	-0.01	0.10	27-Sep
Women	7,935	0.08	0.05	0.02	0.08	27-Oct	0.05	0.01	0.10	27-Oct
CES-D scale										
<16	11,852	0.06	0.05	0.03	0.08	15-Oct	0.05	0.01	0.09	24-Oct
≥16	1,823	-0.10	0.02	-0.06	0.10	25-Jun	0.14	0.02	0.27	13-Oct
Education										
Low	1,163	-0.73	0.07	-0.02	0.17	29-Jul	0.13	-0.02	0.28	28-Aug
Low/intermediate	5,528	-0.09	0.06	0.02	0.09	3-Nov	0.02	-0.04	0.07	27-Sep
High general	4,146	0.08	0.01	-0.04	0.05	26-Jan	0.05	-0.01	0.12	27-Oct
University	2,835	0.50	0.09	0.04	0.14	9-Oct	0.10	0.03	0.16	27-Oct
G-factor										
Below average	6,121	-0.80	0.02	-0.02	0.05	18-Sep	0.03	-0.03	0.09	20-Oct
Above average	7,712	0.72	0.03	0.00	0.05	10-Nov	0.01	-0.03	0.04	12-Oct
MMSE										
≥26	11,447	0.18	0.05	0.02	0.07	30-Oct	0.04	0.00	0.08	24-Oct
<26	2,337	-0.66	0.01	-0.07	0.09	27-Feb	0.05	-0.08	0.17	29-Apr
Dementia diagnosis < 5 years										
Excluded	13,550	0.06	0.04	0.02	0.06	18-Oct	0.04	0.00	0.07	19-Oct
Working status										
Occupied	2,248	0.43	0.05	-0.04	0.14	16-Oct	0.09	-0.04	0.22	27-Oct
Not occupied	6,812	-0.19	0.02	-0.02	0.06	10-Sep	0.05	-0.01	0.11	27-Oct

LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

Appendix 33. Selection of variation according to AIC and BIC

Cognitive test	AIC		BIC	
	Annual variation	Annual + harmonic variation	Annual variation	Annual + harmonic variation
g-factor	40731.25	40732.40	40777.59	40794.18
LDST	104392.90	104396.53	104439.24	104458.32
Stroop Reading	-13446.32	-13448.48	-13399.98	-13386.69
Stroop Color naming	-13744.78	-13748.14	-13698.44	-13686.35
Stroop Interference	5287.89	5291.31	5334.23	5353.10
VFT	101222.17	101225.17	101268.51	101286.96
PPB both hands	65166.18	65164.06	65212.53	65225.85
g-factor + delayed WLT	34334.57	34324.82	34379.78	34385.10
Delayed WLT	66046.18	66049.79	66091.39	66110.07
Immediate WLT	90101.48	90098.95	90146.69	90159.23
Recognition WLT	57522.05	57526.03	57567.30	57586.36

Bold values are lowest AIC or BIC, respectively. LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

Appendix 34. Seasonality of g-factor recalculated after excluding one component at a time (n=16,307)

Strata	Average	Annual seasonal variation				Annual + harmonics seasonal variation			
		Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
Excluding LDST	-0.01	0.06	0.04	0.09	25-Jan	0.05	0.01	0.09	28-Mar
Excluding VFT	-0.01	0.04	0.02	0.06	3-Feb	0.03	0.00	0.07	9-Apr
Excluding PPT both hands	-0.01	0.03	0.01	0.06	29-Aug	0.04	0.01	0.08	18-Nov
Excluding Stroop interference	-0.01	0.05	0.02	0.07	19-Jul	0.03	-0.01	0.07	22-Jul

LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task

Appendix 35. Seasonality of g-factor and cognitive tests – Unimputed dataset

Cognitive test	Observations	Average g-factor	Annual seasonal variation				Annual + harmonics seasonal variation			
			Seasonal variation	95%	CI	Nadir	Seasonal variation	95%	CI	Nadir
g-factor	14,378	0.04	0.05	0.03	0.08	11-Feb	0.05	0.02	0.09	5-Apr
LDST	14,378	0.03	0.03	0.00	0.06	20-Apr	0.04	0.00	0.08	3-May
log-Stroop Reading	14,375	-0.02	0.02	-0.01	0.05	18-Jan	0.05	0.01	0.10	16-Apr
log-Stroop Color naming	14,378	-0.02	0.04	0.01	0.07	23-Feb	0.06	0.01	0.10	24-Apr
log-Stroop Interference	14,378	-0.04	0.05	0.02	0.08	14-Mar	0.07	0.02	0.11	14-Apr
VFT	14,378	0.02	0.05	0.02	0.09	28-Jan	0.06	0.01	0.11	15-Mar
PPT both hands	14,378	0.02	0.08	0.05	0.12	10-Jan	0.04	-0.01	0.09	3-Apr
g-factor + delWLT	12,534	-0.03	0.04	0.01	0.06	17-Oct	0.04	0.00	0.08	17-Oct
Delayed WLT	12,534	-0.02	0.01	-0.03	0.04	12-Dec	0.01	-0.04	0.07	21-Mar
Immediate WLT	12,537	-0.02	0.03	0.00	0.07	25-Jan	0.05	0.00	0.11	17-Mar
Recognition WLT	12,605	-0.02	0.03	-0.01	0.07	31-Jan	0.02	-0.05	0.08	23-Apr
g-factor of participants with WLT data	12,534	0.04	0.04	0.02	0.07	17-Oct	0.04	0.01	0.08	22-Oct

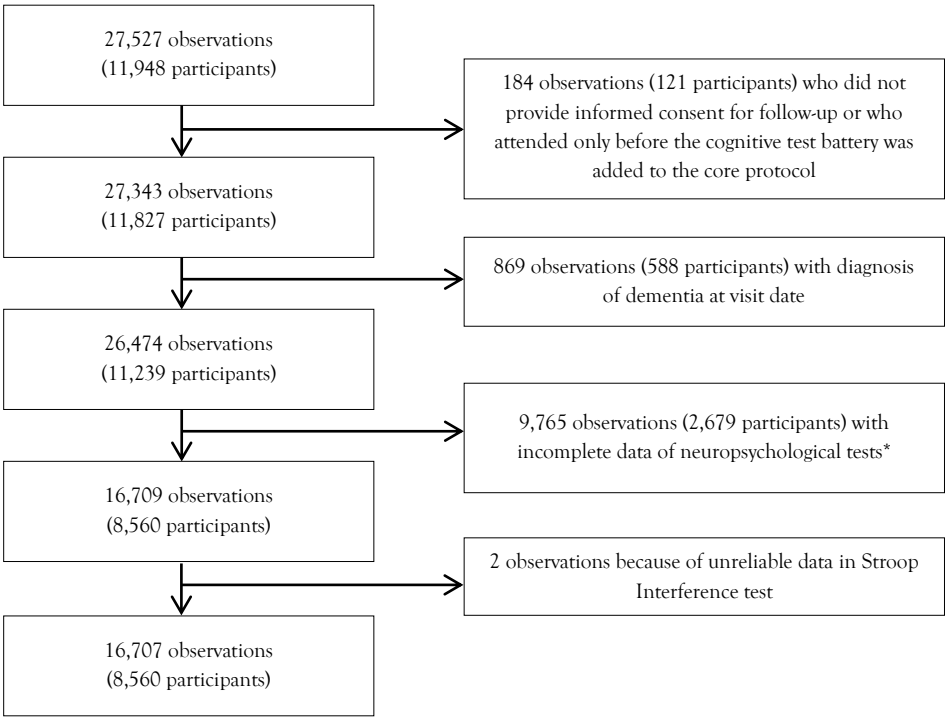
LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

Appendix 36. Seasonality of g-factor and cognitive tests – Sensitivity analysis 3: Only in years with large variation in monthly average temperature (difference equal or above the average difference: 15.9°C

Strata	Observations	Average	Annual seasonal variation			Annual + harmonics seasonal variation		
			Seasonal variation	95% CI	Nadir	Seasonal variation	95% CI	Nadir
g-factor	4,720	-0.11	0.06	(0.01, 0.11)	14-Feb	0.05	(-0.02, 0.12)	31-Mar
std-LDST	4,720	-0.10	0.06	(0, 0.12)	23-Apr	0.08	(0, 0.16)	30-Apr
std-log-Stroop Reading	4,720	0.06	0.05	(-0.02, 0.11)	1-May	0.07	(-0.03, 0.17)	29-Jun
std-log-Stroop Color naming	4,719	0.07	0.02	(-0.04, 0.09)	12-Sep	0.09	(0, 0.19)	29-Jul
std-log-Stroop Interference	4,720	0.07	0.05	(-0.02, 0.11)	8-Aug	0.07	(-0.02, 0.16)	27-Oct
std-VFT	4,720	-0.06	0.04	(-0.03, 0.11)	11-Feb	0.04	(-0.06, 0.14)	31-Mar
std-PPB both hands	4,720	-0.09	0.09	(0.02, 0.15)	3-Jan	0.05	(-0.04, 0.14)	27-Oct
g-factor + delWLT	4,468	-0.15	0.08	(0.02, 0.13)	29-Feb	0.08	(0.01, 0.15)	31-Mar
std-Delayed WLT	4,468	-0.08	0.09	(0.02, 0.16)	12-Feb	0.06	(-0.03, 0.15)	31-Mar
std-immediate WLT	4,468	-0.08	0.17	(0.09, 0.24)	27-Feb	0.18	(0.08, 0.28)	31-Mar
std-recognition WLT	4,517	-0.04	0.14	(0.06, 0.22)	12-Feb	0.11	(0, 0.22)	31-Mar
g-factor of participants with WLT data	4,468	-0.08	0.06	(0, 0.11)	28-Feb	0.05	(-0.02, 0.13)	31-Mar

*All cognitive tests, except g-factor, are standardized. LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

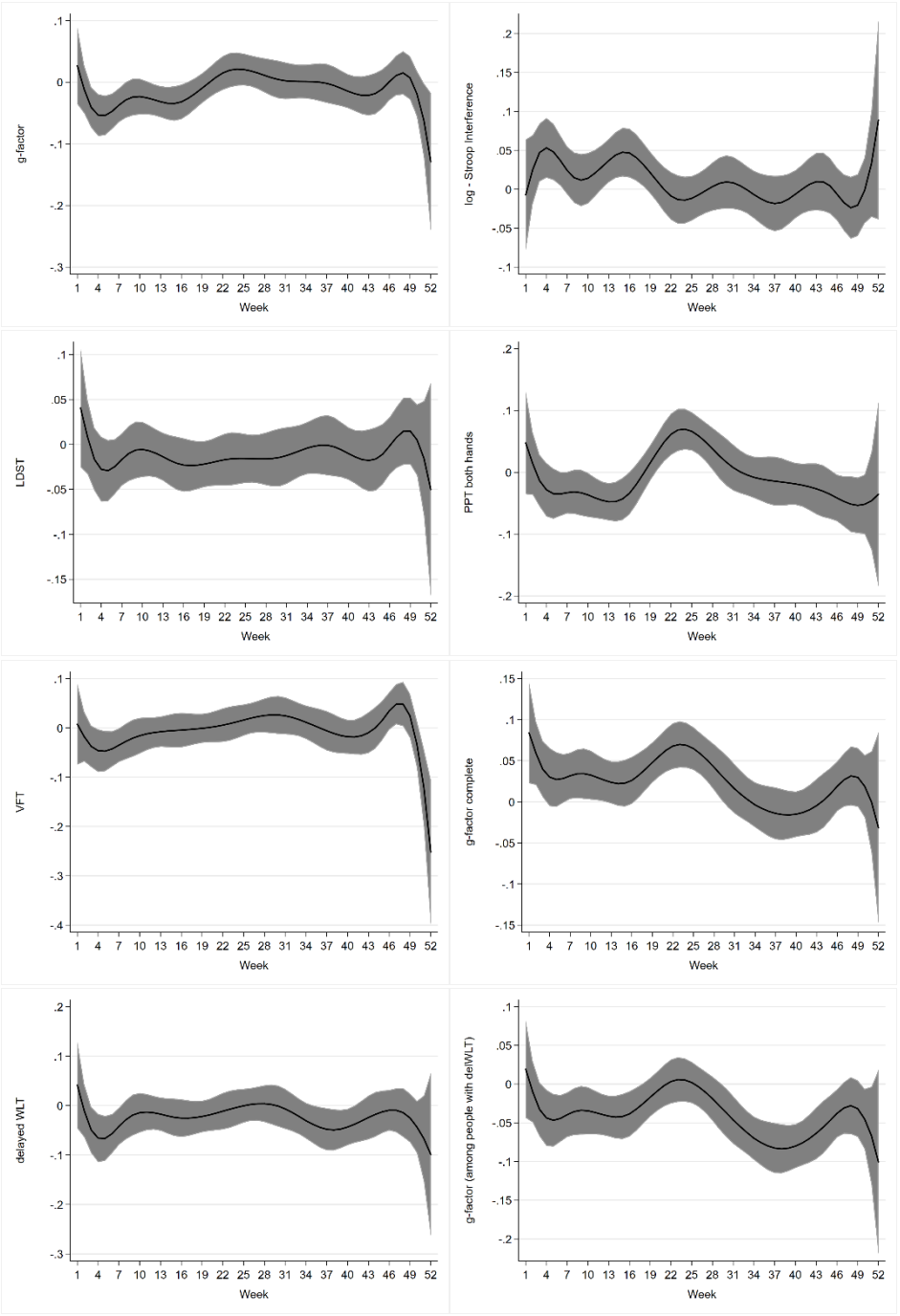
Appendix 37. Flowchart of data selection

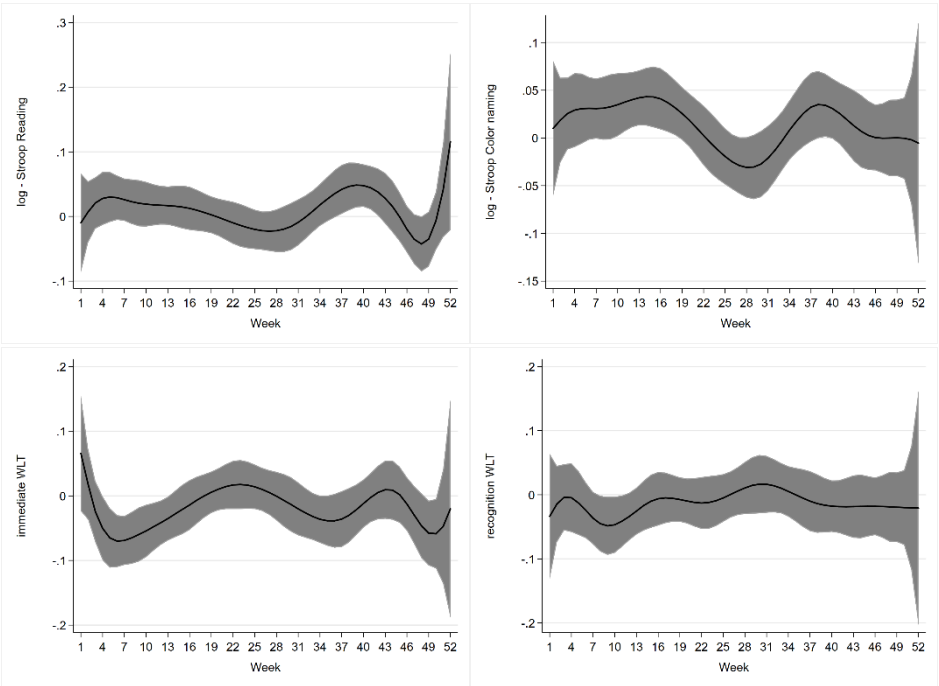


*Missing LDST = 3,847 observations; missing Stroop 3= 4,555 observations; missing VFT = 3,844 observations; missing PPB-both hands = 8,586 observations.

Imputation procedures: Sequential multiple imputation using chained equations was performed to impute missing values of covariates. This approach has been shown to work well in the context of complex longitudinal data, as in the Rotterdam Study.[178] The predictors used to impute the covariates were previous CVD, sex, age, cohort, visit, month, Stroop interference, VFT, LDST, date, date squared, systolic blood pressure, total-cholesterol, HDL-cholesterol, glucose, intake of antihypertensive, antidiabetic or statin medication, CES-D scale, body mass index (categories), smoking behavior, alcohol intake, physical activity (z-score of MET-hours/week), education, and housing status. To impute dichotomous variables (intake of antihypertensive, diabetic, or statin medication, and housing status) we used a logit function. To impute ordered categorical variables (education and body mass index (categories)) we used an ordered logit function. To impute categorical non-ordered variables (smoking behavior) we used a multinomial logit function. To impute continuous variables (physical activity and alcohol intake) we used a linear function. To ensure reproducibility, we used a random seed (2005). We created five imputed datasets, which were used in all the analysis. Covariates with missing values were: systolic blood pressure: 167 missing, HDL-cholesterol: 389 missing, total cholesterol: 382 missing, glucose: 383 missing, CES-D scale: 195 missing, physical activity: 4,402 missing, alcohol intake: 1,599 missing, intake of antihypertensive: 89 missing, antidiabetic intake: 85 missing, statin intake: 88 missing, housing status: 100 missing, education: 195 missing, body mass index: 130 missing, smoking behavior: 110 missing. Imputations were performed using the *mi impute* command of Stata software.

Appendix 38. Seasonality of cognitive tests – using flexible spines





LDST: Letter-Digit Substitution Task; VFT: Verbal Fluency Test; PPT: Purdue Pegboard Task; WLT: 15-Word Learning Test

2.2.4 Seasonality of antimicrobial resistance in critically important bacteria that pose a great threat to public health: A systematic review and meta-analysis

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ABSTRACT

Introduction: There is a perception that antimicrobial resistance (AMR) may display seasonal variation due to an increase use of antibiotics because seasonal outbreaks of infectious diseases. We aim to assess seasonality of AMR in five bacteria that imposed higher risk to public health.

Methods: Six online databases (Embase.com, Medline Ovid, Cochrane CENTRAL, Web of Science Core Collection, Biosis Ovid, and Google Scholar) were searched until September 2017. We selected observational studies describing resistant rates of *Campylobacter spp., Salmonella spp., Escherichia coli*, *Streptococcus pneumoniae*, and *Haemophilus influenza* at least in two different seasons, independently of sample source at any setting (e.g. humans and animals) and geographical region. Two authors appraised studies independently. Primary outcome measures were pooled odds ratios (OR) of seasonal AMR rates and 95% confidence intervals per bacteria type, antibiotic class, geographical region, and sample source. Pooled OR were calculated by using a random-effects meta-analysis.

Results: We included 32 references, of which 28 were meta-analyzed. AMR rates of *S. pneumoniae* and *H. influenzae* against penicillins and cephalosporines were lower in other seasons than in winter (OR = 0.72, 95% CI=0.66-0.78). AMR rates of *E. coli* were lower in other seasons than in summer, but these were heterogeneous according to sample source, geographical regions, and antibiotic classes (OR=0.70, 95%CI= 0.54-0.79). Multidrug-resistant rates of *S. pneumonia*e and *E. coli* were higher in winter and spring. No significant seasonal AMR rates were observed for *Salmonella spp.* and *Campylobacter spp.*

Conclusion: AMR rates of *S. pneumoniae*, *H. influenzae*, and *Escherichia coli* exhibit a seasonal variation, explained by seasonality of infectious diseases and to antibiotic use. There is need to increase awareness on rational antibiotic use and to strengthen stewardship to efficiently detect and address increasing AMR rates.

INTRODUCTION

The World Health Organization (WHO) published a list of critically important resistant bacteria that impose a high burden for public health. [228] This list includes bacteria such as *Streptococcus pneumoniae, Haemophilus influenzae, Escherichia coli, Campylobacter spp.,* and *Salmonella spp.* [228] Globally, these bacteria are responsible for 550 million respiratory and foodborne infections among humans, and 230.000 deaths every year. [229-231] In addition, almost 50% of all women will experience at least one episode of urinary tract infections (UTI's) caused by *E. coli* during their lifetime, leading to increased hospital stay and higher health care costs. [232,233]

As antimicrobial resistance (AMR) is increasing globally, the morbidity and mortality related to resistant infections are also expected to increase. Indeed, it has been estimated that AMR will be responsible for an extra 10 million of deaths in 2050.[234] The increase of AMR rates has been linked to antimicrobial use in human and in veterinary medicine.[41,44,234-236] Nevertheless, the use of antibiotics differs according to geographical region, mostly due to local practices and regulations regarding the use of antibiotics and husbandry practices in food-producing animals.[39,44,236-241] Moreover, such differences appear to also be determined by environmental factors, which in light of the increasing burden imposed by climate change, may influence the occurrence of diseases and higher prevalence of infections with resistant bacteria.[242]

Emerging evidence suggests that AMR rates vary with seasons because of the seasonal incidence of infectious diseases and the accompanying antimicrobial use. [39-45] In Europe and the USA, the use of antibiotics such as penicillins and cephalosporins increase in winter, along with the increase of respiratory infections caused by *S. pneumoniae* and *H. influenza.* [39-41,45,243] In humans, higher resistance rates of *Campylobacter spp.* to fluoroquinolones and macrolides have been reported in winter.[244,245] In contrast, high resistance rates of urinary *E. coli* to nitrofurantoin and sulphamides have been reported in summer. [246] In animals and carcasses, foodborne bacteria (e.g. *Salmonella spp.*, *Campylobacter spp*., and *E. coli*) were shown to have peak resistance rates to fluoroquinolones, macrolides, and tetracyclines in summer and autumn, when the incidence of enteric diseases is also high.[247-249]

There is a large variability in the seasonal patterns of AMR across the literature. Such variability can be attributed to differences in methodological approaches across studies, antimicrobial prescription settings in human and veterinary medicine, geographical regions and the incidence of infectious diseases. In this era of increasing AMR, it is pivotal to understand all phenomena contributing to the selection of antimicrobial resistant bacteria. Nevertheless, to the best of our knowledge, no previous effort has been made to systematically review evidence that addresses seasonal AMR and to assess the heterogeneity across the literature. Here, we systematically reviewed relevant published studies to assess seasonal AMR variation in the most important foodborne bacteria and respiratory bacteria, both in humans and in animals.

METHODS

Search strategy

We performed a systematic review of all published studies describing the seasonal variation in AMR of *Campylobacter spp., Salmonella spp., Escherichia coli*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. The search of relevant studies was performed until September 27th, 2017.

Embase.com, Medline Ovid, Cochrane CENTRAL, Web of Science Core Collection, Biosis Ovid, and Google Scholar databases were used to identify published observational studies.

There was no publication date or language restriction. The search was performed by using combined terms related to antibiotic resistance (e.g. "penicillin resistance") and bacteria (e.g. "'Campylobacter OR campylobacteriosis") with seasonality terms (e.g. "winter OR cold"). Additional studies were searched by reviewing the references list from the full-text retrieved studies and by contacting authors via e-mail. The full search strategy is available (Appendix 39, page 163).

Selection criteria and quality assessment

Studies were included if they presented resistance rates at least in two different seasons of *Campylobacter spp., Salmonella spp., Escherichia coli*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, from human and animal sources. Studies evaluating vaccination programs, literature reviews, abstracts, reports, thesis, and protocols were excluded. By working independently, all studies were reviewed by title and abstracts by four independent authors. Titles selected were full-text retrieved and read independently by two authors to identify those that fulfilled the selection criteria (Appendix 39, page 163), with a third investigator available in case of disagreement.

A modified version of the Newcastle-Ottawa Scale (NOS) was used to assess the quality of included studies (Appendix 39, page 163). We used as main quality criteria the representativeness of sample source, the length of follow-up, comparability of the population within the study period, reliability measure of antibiotic sensitivity and clear description of the outcome.

Data extraction and statistical analysis

From each study, relevant data were extracted and registered on a purposely designed form. We extracted general information (e.g. year, study design), bacteria type, antibiotic class, and season definition (Appendix 39, page 163). Furthermore, we extracted the frequency and the percentage of resistant and (if available) intermediate resistant bacteria per antibiotic class, and the total number of bacteria described. Frequency and percentage of multidrug-resistant (MDR) bacteria (i.e. bacteria resistant to two or more antibiotics) was also extracted.

Seasons were defined as winter (December to February), summer (June to August), autumn (September to October), spring (March to April). Depending on the geographical region of the study, and information provided by the authors, we also used the season definition as cold (November to April), warm (May to October), wet (Jun to September), and dry (October to May). Country of the studies were categorized into regions according to the WHO Member States regions to Eastern Mediterranean, Europe, America, South East Asia, and Western Pacific. Antibiotic drugs were categorized into classes as aminoglycosides, penicillins, cephalosporins, phenicols, fluoroquinolone, macrolides, nitrofurans, sulphamides, trimethoprim, and tetracyclines ((Appendix 39, page 163).

We performed several analyses to compare seasonal AMR per bacteria type, antibiotic class, sample source and geographical region. First, for each study, we calculated the resistance rates per season, stratified according to bacteria type, antibiotic class, and sample source, using as numerator the number of resistant isolates and as denominator the total number of isolates

tested per season. If the denominator (total number of isolates per season) was not available, we assumed that the sampling was equally distributed across the full length of the study. Thus, we calculated the denominator by dividing the overall number of isolates tested in the study by the number of seasons included in the study (i.e. dividing by four if the study presented resistance rates for four seasons). In addition, for studies in which monthly resistance rates were provided, resistance rates were pooled according to season category within each bacteria type and antibiotic class. Finally, for studies in which monthly resistance rates were provided per month and per year, we first pooled the monthly resistance rates per year into season category, and then seasonal resistance rates were calculated by dividing the sum of resistant isolates per month of the four months of the corresponding season by the sum of the isolates examined per month of the four months of the corresponding season. These rates were calculated per antibiotic class and sample source.

Second, using the rates calculated for each study in the previous step, we calculated the ratio of resistance rates between any season vs. winter, which was the reference category. The ratios were calculated stratified by bacteria type, antibiotic class, and sample source. For those studies that only compared spring and autumn, spring was the reference group. For the studies in which seasons were defined as cold and warm, or wet and dry, cold and wet were the reference groups, respectively. In addition, for the studies in which resistance rates were presented comparing June – August vs. other nine months of the year, June-August was defined as summer and was the reference group.

Finally, we carried out random-effects meta-analyses to calculate pooled odd ratios (OR) for each comparison (any season vs. winter), stratified according to bacteria type, antibiotic class, bacteria, and sample source. As a subgroup analysis, we recalculated the meta-analyses according to study region. We calculated 95% confidence intervals for each pooled odds ratio. Heterogeneity was measured using the I-squared test. We examined publication bias for each comparison by examining asymmetry funnel plots and using Egger's test. All tests were two-tailed and p-values ≤ 0.05 were significant. All analysis was carried out using the statistical software Stata MP 14 and R Studio.

RESULTS

Study selection and main characteristics

A total of 3,261 studies were identified through database searching, of which 211 studies were selected for full-text assessment. After the final appraised, 32 studies were included for the qualitative synthesis, of which 28 studies were quantitative analyzed (see Figure 8 (page 159) for reasons of exclusion). No additional studies were identified by either manual research or contact to authors. 18 of 32 studies reported a random recruitment of data across seasons (at least two seasons), and 13 of 32 adjusted prevalence resistant rates by season (Appendix 40, page 168).

For the calculation of seasonal AMR, in 8 studies, [238,245,246,249-254] the denominator was imputed by dividing the overall number of isolates by the number of seasons. In other 8 studies, [41,44,45,244,255-258] for the denominator was necessary to pooled monthly resistance rates by season first, and the seasonal AMR were calculated. In 3 studies, [239,251,259] monthly resistance rates per

year were firstly categorized by season, and then seasonal AMR were calculated as previously described.

In addition, for the meta-analysis winter was mainly the reference group, however, cold season was the reference group in 3 studies, [250,256,260] wet season in other 3 studies, [243,256,261] and summer (June-August) was the reference group in two studies. [258,262]

Most of the studies were cross-sectional (n=23) and performed in human samples (n=23). Studies were mainly performed in Europe (n=14) and reported resistance rates of *E. coli* (n=13). In humans, *E. coli* was isolated in patients with UTI´s (n=6), whereas in animals, *E. coli* originated from the gut (n=4) (Table 16, page 155). In humans, the highest resistance rates were found against penicillins, cephalosporins and macrolides in both respiratory bacteria (*S. pneumoniae* and *H. influenzae*). The highest resistance rate for urinary *E. coli* was against nitrofurantoins, aminoglycosides and cephalosporins. In animals, antibiotic resistance in foodborne bacteria (*E. coli, Salmonella spp, and Campylobacter spp.*) was most commonly against fluoroquinolones, macrolides and tetracyclines (Table 16, page 155). Additional characteristic of included studies is available (see Appendix 41, page 169).

Seasonal variation in antimicrobial resistance of S. pneumoniae and H. influenzae

Included studies mainly described resistance rates of *S. pneumoniae* and *H. influenzae* causing respiratory infections in children. The AMR rates in *S. pneumoniae* were lower in other seasons than in winter (pooled OR = 0.72, 95% CI=0.66-0.78, I^2=17.9%, Egger's P=0.11) (Figure 9, page 160). In addition, AMR rates were lower in dry and warm months and in autumn (see Appendix 42, page 172).

Lower resistance rates in other seasons than in winter were observed for penicillins (OR= 0.69, 95% CI=0.62-0.77) cephalosporins (OR= 0.78, 95% CI=0.66-0.92), and MDR isolates (OR= 0.75, 95% CI=0.50-1.13) (Figure 9, page 160). Only one study examined the seasonality of AMR rates in *H. influenzae* to penicillins and it was not significant (Table 17, page 157). The seasonal AMR rates both in *H. influenzae* and *S. pneumoniae* were similar after stratifying by geographical region (Table 17, page 157). We identified relative relatively low heterogeneity (I^2<60 %) (Figure 9, page 160), and no evidence of publication bias according to Egger´s test and by visual inspection of funnel plots (Table 17, page 157 and Appendix 43, page 173).

Seasonal variation in antimicrobial resistance of E. coli

The AMR rates in urinary *E. coli* were lower in other seasons than in winter (pooled OR= 0.96, 95%CI=0.89-1.03, I^2=56.0%, Egger's P= 0.23) (Figure 10, page 161). Based on two studies, [258,262] resistance rates were lower in the other nine months of the year compared to summer (pooled OR=0.70, 95%CI= 0.54-0.79, I^2=68%, Egger's P=0.12) (Appendix 44, page 174).

Higher AMR rates were observed in summer than in winter to nitrofurans (OR= 1.41, 95% CI=1.06-1.88) and tetracyclines (OR= 2.02, 95% CI=1.30-3.13) (Figure 10, page 161). Also, resistance to sulphamides, tetracyclines and multidrug-resistance occurred at higher rates in spring and winter (Figure 10, page 161).

Additionally, seasonal AMR rates varied according to geographical region. In Europe, resistance rates were lower in winter, whereas in Western Pacific and American countries these

were higher in summer (Table 18, page 158). We identified medium heterogeneity (I^2 >50%) and no evidence of publication bias for these meta-analyses (Table 18, page 158 and Appendix 43, page 173).

In animals, included studies mainly described resistance rates of commensal *E. coli* from healthy food-producing animals and carcasses. The seasonal antimicrobial resistance rate was higher in other seasons than in winter (pooled OR=1.14, 95% CI=1.02-1.28, I^2=75.3%, Egger's P= 0.30) (Figure 11, page 162). Resistance to phenicols and macrolides occurred at higher rates in summer than in winter, and resistant to sulphonamides occurred at higher rates in winter and spring (Figure 11, page 162). Furthermore, seasonal AMR rates varied by geographical region. In Europe, resistant rates were lower in other seasons than in winter, whereas in Eastern Mediterranean and American countries these were higher in summer (Table 18, page 158). We identified high heterogeneity (I^2 >80%), and evidence of publication bias in some meta-analyses (Table 18, page 158 and Appendix 43, page 173).

Seasonal variation in antimicrobial resistance of Salmonella spp. and Campylobacter spp.

No significant seasonality was observed in AMR of *Salmonella spp.* and *Campylobacter spp*. from humans with foodborne diseases or from healthy food-producing animals and carcasses. In one study performed in humans, [245] the resistance rates of *Campylobacter* spp. to fluoroquinolones and macrolides were lower in summer than in winter (OR= 0.86, and 95% CI=0.73-1.01). In one study performed in humans, [261] the occurrence of multidrug- resistant *Salmonella spp.* was similar in dry vs. wet seasons (OR= 1.01, and 95% CI=0.51-1.98).

In animals, resistance rates of *Campybolacter spp.* were higher in autumn than in spring, although the difference was not statistically significant (pooled OR =1.52, 95%CI=0.97-2.39, I^2=76.6%, Egger's P= 0.48). Similar findings were observed for AMR to fluoroquinolones, macrolides, and tetracyclines (Appendix 45, page 175). One study performed with chicken carcasses [247] showed higher rates of AMR of *Salmonella spp*. in summer than in winter, although the difference was not statistically significant (OR= 2.48, and 95% CI=0.55-11.11, I^2=26.6%, Egger's P= 0.01) (Appendix 45, page 175).

DISCUSSION

Our systematic review and meta-analysis suggests there is seasonality in the AMR rates of *S. pneumoniae*, *H. influenzae* and *E. coli*. For *Salmonella spp.* and *Campylobacter spp*. there is no evidence of seasonal variation in AMR rates. AMR rates of *S. pneumoniae* and *H. influenzae* were higher in winter. AMR rates of *E. coli* in patients with urinary tract infections were higher in summer, as well as in *E. coli* isolates from food-producing animals.

Based on the available evidence, two main determinants may be dynamically contributing to the seasonality of AMR rates. First, the seasonality of antibiotic use secondary to the seasonal variation of the incidence of infectious diseases may exert a selective pressure that results in a seasonal variation of AMR rates.[41,43-45,235] Indeed, under the continued selective pressure exerted by antibiotic use, bacteria can develop different co-selective mechanisms such as co-resistance (i.e. various resistant determinants in the same genetic element such as plasmid) and cross-resistance (i.e. same genetic determinant responsible for resistance to all antibiotics from the

same class), which may furthermore lead to a resistance to a broad spectrum of antibiotics (i.e. multidrug-resistance).[263] Second, the seasonal variation of the incidence of infectious diseases, per se, may contribute to the increment of circulating resistant strains, what not only contributes to the seasonality of the AMR rates, but also to the increasing need for antibiotics of second and third line treatment options.[42,238,243,250,252] These factors should be closely analyzed in future studies to fully understand its role in the dynamics of the seasonality of AMR rates.

Nevertheless, our results need to be interpreted with caution as large heterogeneity was observed in the seasonal variation of AMR rates, especially in *E. coli* isolates (I^2>80%). Part of the heterogeneity is attributable to the specific characteristics of the study population (e.g. age, sex, presence of comorbidities). However, a large part of the heterogeneity may also be explained by the geographical regions where the studies were conducted underscoring the influence of local factors modifying the patterns of antibiotic use and, consequently, of AMR occurrence. For example, it has been observed that differences in drug regulations and the structure of the pharmaceutical market across countries may lead to differences in the seasonality of AMR rates.[235] This includes also the regulation on antimicrobial prescription at various levels of the healthcare system (e.g. primary and secondary care).[44,45,249] Furthermore, within the same country, socio-cultural determinants and educational level can influence the patient demands and the proclivity of medical doctors for prescribing antibiotics.[242,264] Besides, climate variability according to regions has been linked to health consequence such as outbreaks of food-borne diseases and air pollution, and therefore changes in antibiotic use and further resistance.[242] Finally, emerging evidence suggests that region differences in animal-farming practices,[246,253] including the antimicrobial use, may also influence the seasonality of AMR rates. These findings indicate the importance of developing national surveillance and monitoring programs of antimicrobial use and resistance to fully understand local determinants of use and, therefore, the underlying selective pressure of AMR occurrence.

Seasonality of AMR rates in humans

The AMR rates of *S. pneumoniae* and *H. influenzae* showed a marked winter-peak seasonal variation. This pattern is expected as respiratory infections exhibit a clear seasonal pattern with peak in cold-months, thus leading to an increase in antibiotic use. [41,45,235,243,250,254] The antibiotic use was between 24% and 38% higher in winter than in summer, [39,40] most of which corresponded to the increase of penicillins and cephalosporins, which are broadly used for respiratory infections. [235,238,256,265,266] Other studies have reported a winter peak in other antibiotics used for respiratory infections, such as amoxicillin, amoxicillin-clavulanic acid, macrolides, and quinolones. [246,267] These overlapping patterns would correspond to periods of multidrug-resistant respiratory bacteria around winter. [41] One potential explanation is that under the continued pressure of antibiotic use during the winter, the multidrug-resistant strains become more prevalent in the community.

AMR rates of *E. coli* tended to be higher in summer than in other seasons, as has been described in other studies. [42,268] Nevertheless, this trend was highly heterogeneous according to geographical region and antibiotic classes. On one hand, resistance rates from Western Pacific (e.g. Australia) and American countries (e.g. USA) were rather high in winter unlike those from

Europe (e.g. Norway and England), where the rates were higher in summer. It is possible that this geographical cluster can be attributed to region-specific patterns of antimicrobial prescription. [44,251,268,269] On the other hand, we found that resistance rates to sulphamides and tetracyclines were higher in spring and winter, respectively. This pattern might be attributable to previous use of these antibiotic classes to treat diseases such as skin infections, respiratory infections and UTI's. [44] Additionally, previous studies have shown a 1 to 2 month-lag between the increase of use of a particular antibiotic class such as quinolone and β-lactams and the increase of AMR of *E. coli* to these antibiotics. [44,45] It might be argued that this phenomenon occurs also with other antibiotic classes.

Seasonality of AMR rates in animals

We did not find consistent seasonality of AMR rates for samples obtained from animals, probably due to the large heterogeneity in the seasonal patterns observed across studies. The studies reporting a seasonal variation in AMR rates in animal samples suggested as potential explanation the different husbandry practices of food-producing animals. [236,239,240] On one hand, resistance strains of *Campylobacter spp., E. coli and Salmonella spp.* has been described by keeping animals outdoors in summer, probably because this practice increases the risk of contact with other AMR bacteria sources, such as farm pets, [270] wild animals, [271] and contaminated farm water and soil. [272] On the other hand, the intensive housing, commonly used in colder months, may increase the effective transmission of resistant and non-resistant bacteria due to crowding. [240] Such mechanism was explained for the spring-peak of AMR rates of *E. coli* to β-lactams, sulphamides, tetracyclines, and trimethoprim. [240,253,259] Similarly, the summer-peak of respiratory and enteric infections with *E. coli* and *Campylobacter spp.* in pigs and poultry has been related to a summer increase of resistance to fluoroquinolones and macrolides. [236,249] Previous studies suggested a decrease of resistant rates in *E. coli* strains from food-producing animals after the reduction of antimicrobial use, together with good farming practices. [273]

The seasonality of resistant bacteria among food-producing animal poses special treat to public health, highlighting the potential role of animal sources in the transmission and spread of AMR bacteria to humans through the food-chain. [245,274-276] For example, resistant *E. coli* has been described as a reservoir of resistance genes for potentially pathogens bacteria that can be transmitted to humans. [259] A higher consumption of chicken meat in winter may be the underlying mechanism of the winter-peak of human Campylobacteriosis resistant to fluoroquinolones and macrolides in the winter. [244,245] Additionally, higher rates of multidrug-resistant *Salmonella spp.* strains have been isolated in summer from chicken carcasses. [247,261]

Strengths and limitations

To our knowledge, this is the first comprehensive systematic review and meta-analysis addressing seasonal variation in AMR in humans and animals of critically important bacteria for the public health. We have addressed, across the evidence, the multiples sources of heterogeneity emerging from differences factors underlying the seasonal patterns according to antibiotic type, bacteria, geographical region and sample source. However, some limitations need to be acknowledged. First, we could not systematically examine the role of potential explicative factors of the observed

seasonal patterns, such as antimicrobial prescriptions and infections incidence, because it was not systematically examined in the studies included in this review and because it fell beyond the scope of our study. However, our results coincide with the well-known seasonality of infections caused by *S. pneumonia* [238,243,250,252] and urinary *E. coli.* [251] Consequently, these patterns appear as relevant determinants contributing to the observed seasonal variation in AMR rates.

Second, because of incomplete reporting of the findings in eight studies included in our review, we assumed that the sampling of the isolates for resistance determination was at random throughout the year. Nevertheless, if the sample procedure is not at random throughout the year, a spurious seasonality can occur if, for example, more isolates are taken in peak-periods of infection in a population who are more likely to have resistant infections and higher exposure to antibiotics. [41] Finally, although we found evidence of publication bias in only a few comparisons, we cannot rule out its influence on our results. Indeed, because most of the studies included in our review were performed in European and North American countries, the potential variation that could occur in countries with different seasonal incidence of infections and antibiotic use could be underrepresented in our meta-analyses. The same holds true for seasonal variation in AMR of *Campylobacter spp.* and *Salmonella spp.*

Implications

Our study shows that the seasonality of AMR is more consistent for respiratory infections than for infections caused by food-borne bacteria. This finding has multiple implications. First, the fact that respiratory infections have a more consistent seasonality, thus leading to a more consistent seasonal variation of AMR of these bacteria could be attributed both to the increase of resistant strains, and to the newly-generated resistance as a consequence of increased use of antibiotics in winter. In clinical settings, and from a public health perspective, these findings highlight the need for a proactive preparation for the seasonal increase of bacterial infections and it subsequent antibiotic use, which may mostly affect susceptible population, such as elderly and children. Additionally, active surveillance and reaction protocols are required in order to rapidly identify and address outbreaks of AMR rates. These are required as the burden imposed by climate change through extreme climatic conditions may favor outbreak of infections, and therefore the need to increase antibiotic use. Additionally, the international collaboration may be fundamental in reducing the heterogeneity in the practices of antibiotic prescription through enhancing stewardship programs in hospitals, what may contribute to reduce practices of unnecessary antibiotic use and further spread of MDR strains.

Second, we found a large heterogeneity in the seasonality of AMR rates in food-producing animals, which can be largely attributed to the fact that all studies were performed to screen resistance in commensal *E. coli*. In spite of this, the patterns identified within the studies consistently identified the husbandry practices, including antibiotic use, as the main determinant of seasonal AMR variation. Because of the risk that poses for public health, it remains a priority to handle the practices of food-producing animals and to implement antimicrobial stewardship on farms promoting rational antibiotic use, and therefore helping to reduce risk of transmission of resistant bacteria to humans through the food-chain.

Finally, studies included in our systematic review suggested that periods of MDR can occur due to the accumulation of resistance strains of respiratory bacteria and potentially, of infections caused by food-borne bacteria. This may induce complicated infections accompanied with higher risk of mortality, higher cost of treatment and longer length of stay in hospitals, [233,277] especially in developing countries, where certainly the impact of AMR is much higher. [278] This longer occupancy in bed might be taken into consideration in the planning of availability of beds in hospitals. Furthermore, governments should consider implementing strong politics for surveillance and increased the response against AMR and the factors that underlay its seasonality, which may be worsen the upcoming challenges of climate change. [241]

Conclusion

In this comprehensive systematic review, seasonality of AMR was more consistent in respiratory infections caused by *S. pneumoniae* and *H. influenzae,* whereas seasonality of AMR in *E. coli* was rather heterogeneous according to sample source, geographical region and antibiotic class. Due to few available studies in humans, we could not find significant seasonal variation of AMR in *Salmonella spp.* and *Campylobacter spp*, however, seasonal fluctuations of these bacteria can occur in relation with husbandry practices in animals. Based on the available evidence, the seasonal variation in AMR among bacteria depends on the seasonal fluctuation of antibiotic use due to the incidence of infectious disease. Since emerging evidence associated the occurrence of diseases with environmental changes and its impact to the public health, future studies are required to better understand the factors underlying the seasonality of AMR. Resources are required to global standardization of stewardship programs for antibiotics in hospitals and farms.

Table 16. Main characteristics of selected studies (n=32)

Study (Ref)	Study design	Country	Region	Sample source	Isolates source	AMS testing interpretation	AMR pattern	Season category
					Streptococcus pneumoniae			
Albanese et al. (2002) [250]	Cross-sectional	USA	AM	H	Patients with respiratory infections	CSLI	PEN	3
Baquero et al. (1996) [238]	Prospective	Spain	EU	H	Patients with respiratory infections	CSLI	PEN	1
Boken et al. (1995) [265]	Cross-sectional	USA	AM	H	Children aged 2 to 24 months with respiratory infections	CSLI	PEN	2
Dagan et al. the (2008)† [41]	Prospective	Israel	EU	H	Children aged > years months with respiratory infections	CSLI	PEN, CEP, MC	N/A
Marco et al., (2000) [279]	Cross-sectional	Spain	EU	H	Patients with respiratory infections	CSLI	PEN, CEP, MC	1
Guevara et al. (2008) [243]	Cross-sectional	Costa Rica	AM	H	Children until 2 years with otitis media	CSLI	PEN, MC	4
Hoberman et al. (2005) [255]	Cross-sectional	USA	AM	H	Children 2 months to 7 years with respiratory infection	CSLI	PEN, MC, TM/SUL	1
Marchisio et al. (2001) [237]	Longitudinal	Italy	EU	H	Healthy children aged 1 to 7 years.	CSLI	PEN, MC	2
Siripongpreeda et al. (2010) [256]	Retrospective	Thailand	SEA	H	Patients aged <18 with respiratory infection	CSLI	PEN	3/4
Stacevičiene et al. (2016) [267]	Prospective	Lithuania	EU	H	Children aged <6 years with respiratory infection	EUCAST	MDR	1
Tam et al. (2015) [254]	Cross-sectional	USA	AM	H	Children aged < 5 years with respiratory infection	N/A	PEN	1
Vardhan & Allen (2003) [257]	Prospective	England	EU	H	Children with respiratory infection	CSLI	PEN	1
					Escherichia coli			
Abatih et al. (2009) [239]	Cross-sectional	Denmark	EU	A	Fecal pig samples and pig carcasses	CSLI	AMG, PEN, SUL	1
Gencay (2014)[260]	Cross-sectional	Turkey	EU	A	Sheep carcasses	CSLI	CEP	3
Gow et al. (2008) [240]	Cross-sectional	Canada	AM	A	Fecal samples from healthy cattle and calves	CSLI	AMG, PEN, CEP, FEN, FQ, MDR, SUL, TET, TM/SUL	2
Talebiyan et al. (2014) [253]	Cross-sectional	Iran	EM	A	Fecal samples from broiler flocks	CSLI	AMG, FEN, FQ, MC, SUL, TET, TM/SUL	1
Alali et al. (2008) [259]	Longitudinal	USA	AM	A/H	Fecal samples from swine and sewage water	CSLI	PEN, MDR, MC	1
Meumann et al. (2015)† [44]	Retrospective	Australia	WP	H	General patient population with urinary tract infections	EUCAST	PEN, AMG, CEP, FQ	N/A
Fasugba et al. (2016) [251]	Cross-sectional	Australia	WP	H	Inpatients in hospital with urinary tract infection	CSLI	AMG, PEN, CEP, FQ, NIF, TM, TM/SUL	1

Sun et al. (2012)† [45]	Cross-sectional	USA	AM	H	Inpatients and outpatients isolates	CSLI	PEN, AMG	N/A
Usui et al. (1973) [262]	Cross-sectional	Japan	WP	H	Patients with urinary tract infections	N/A	AMG, CEP, FEN, TET	7
Vorland et al. (1985) [246]	Cross-sectional	Norway	EU	H	Outpatients with urinary tract infections	CSLI	NIF, SUL	1
Wormald, P. J (1971) [258]	Retrospective	England	EU	H	Outpatients with and without urinary tract infections	CSLI	PEN, FQ, NIF, SUL, TET	7
Campylobacter sp./E.coli								
Taylor et al. (2009) [249]	Cross-sectional	England	AM	A	Fecal samples from pig	CSLI	FQ, MC, TET	1
Rao et al. (2010) [236]	Cross-sectional	Canada	AM	A	Fecal samples in cattle	CSLI	FQ, MC, TET, AMG, PEN, CEP, FEN, MDR, SUL, TM/SUL	2
Campylobacter spp.								
Andrzejewski et al. the (2013) [270]	Cross-sectional	Poland	EU	A	Fecal samples from farm cats and dogs	CSLI	FQ	6
Sulonen et al. (2007) [248]	Cross-sectional	Finland	EU	A	Fecal samples from laying hens	CSLI	FQ	2
Talsma et al. (1994)† [244]	Cross-sectional	Netherlands	EU	H	General patient population	CSLI	N/A	N/A
Van Hees et al. (2007) [245]	Cross-sectional	Netherlands	EU	H	General patient population	N/A	MC, FQ	1
Salmonella spp.								
Dar et al. (1992) [261]	Cross-sectional	India	SEA	H	Patients with suspected typhoid fever	N/A	MDR	4
Lee et al. (2016) [247]	Cross-sectional	Korea	WP	A	Chicken carcasses	CSLI	AMG, MDR	5
Haemophilus influenzae								
Hashida et al. (2008) [280]	Cross-sectional	Japan	WP	H	Healthy children aged 1 to 6 years with	CSLI	PEN	5

*AMS = Antibiotic susceptibility, † = excluded studies for meta-analysis, A = animal, H = Human, 1= Spring, Summer, Autumn and Winter, 2 = Autumn and Spring, 3 = Warm and Cold, 4 = Dry and Wet, 5 = Summer/Winter, 6 = Autumn/Winter, 7= Summer/ Other months, CSLI = Clinical Laboratory Standards Institute, EUCAST = European Committee on Antibiotic Susceptibility Testing, N/A = not available. EU= Europe, SEA = South-East Asia, WP = Western Pacific, AM = Americas, EM = Eastern Mediterranean. AMG =

Table 17. Comparison of antimicrobial resistance rates in respiratory bacteria between seasons according to geographical regions (n=28). Winter and spring were reference groups

Geographical region	Antibiotic Class	n studies	OR	95% CI	I^2 (%)	Egger's p-value
			All seasons vs Winter			
Streptococcus pneumoniae						
Europe	All classes	4	0.72	0.65 - 0.79*	29.6	0.10
	Penicillins	3	0.69	0.60 - 0.78*	37.8	
	Cephalosporines	1	0.78	0.66 - 0.92*	23.2	
	Multidrug-resistant	1	0.75	0.50 - 1.13	10.4	
America	Penicillins	2	0.66	0.44 - 1.00	0.0	0.85
			Autumn vs Spring			
Streptococcus pneumoniae						
Europe	All classes	5	0.92	0.80 – 1.06	0.0	0.10
	Penicillins	4	0.93	0.77 – 1.11	0.0	
	Cephalosporines	1	0.96	0.65 – 1.42	48.7	
America	Penicillins	3	0.52	0.08 – 3.47	89.3	0.85
Haemophilus influenzae						
Europe	Penicillins	1	0.55	0.27 – 1.11	-	0.67

Table 18. Comparison of antimicrobial resistance rates of urinary *E. coli* (isolates from humans) and commensal *E. coli* (isolates from animals) between seasons according to geographical regions (n=28). Winter was reference group.

Geographical region	Antibiotic Class	Studies (n)	OR	95% CI	I^2 (%)	Egger's p-value
				All seasons vs Winter		
Escherichia coli from patients with urinary tract infections						
Europe	All classes	2	1.05	0.85 - 1.29	72.1	0.80
	Penicillins	1	0.61	0.37 - 1.01	78.2	
	Fluoroquinolones	1	1.23	0.77 - 2.12	48.0	
	Nitrofurans	2	1.44	1.12 - 1.88*	0.0	
	Sulphamides	2	0.98	0.77 - 1.23	65.0	
	Tetracycline	1	1.41	0.85 - 2.34	72.0	
Western Pacific	All classes	1	0.97	0.92 - 1.03	0.0	0.73
	Aminoglicosides	1	1.11	0.86 - 1.45	60.7	
	Penicillins	1	1.13	0.94 - 1.35	0.0	
	Cephalosporins	1	0.98	0.87 - 1.11	0.0	
	Fluoroquinolones	1	0.81	0.67 - 0.98	0.0	
	Nitrofurans	1	1.13	0.89 - 1.44	0.0	
	Tri/sulpha	1	0.92	0.79 - 1.06	0.0	
	Trimethoprim	1	0.91	0.81 - 1.01	0.0	
America	Cephalosporins	1	0.84	0.72 - 0.97	59.9	0.12
Escherichia coli from food-producing animals and carcasses						
Eastern Mediterranean	All classes	1	1.21	0.92 - 1.59	71.5	0.44
	Aminoglicosides	1	0.71	0.25 - 2.00	39.6	
	Fenicoles	1	2.79	1.71 - 4.56*	45.3	
	Fluoroquinolones	1	1.02	0.82 - 1.28	0.0	
	Macrolides	1	1.14	0.28 - 4.61	84.3	
	Tetracycline	1	1.54	0.88 - 2.70	71.8	
	Tri/sulpha	1	0.47	0.24 - 0.94*	69.1	
Europe	All classes	2	0.45	0.83 - 1.09	66.0	0.00
	Aminoglicosides	1	0.91	0.76 - 1.09	40.4	
	Penicillins	1	0.89	0.74 - 1.08	0.0	
	Fluoroquinolones	1	5.41	0.85 - 34.47	78.1	
	Tetracycline	1	1.1	0.89 - 1.35	47.1	
	Sulphamides	1	0.78	0.65 - 0.93*	34.0	
America	Multidrug-resistant	1	1.26	1.06 - 1.50*	82.0	0.00

Figure 8. Study selection process flow-diagram (PRISMA flow chart).

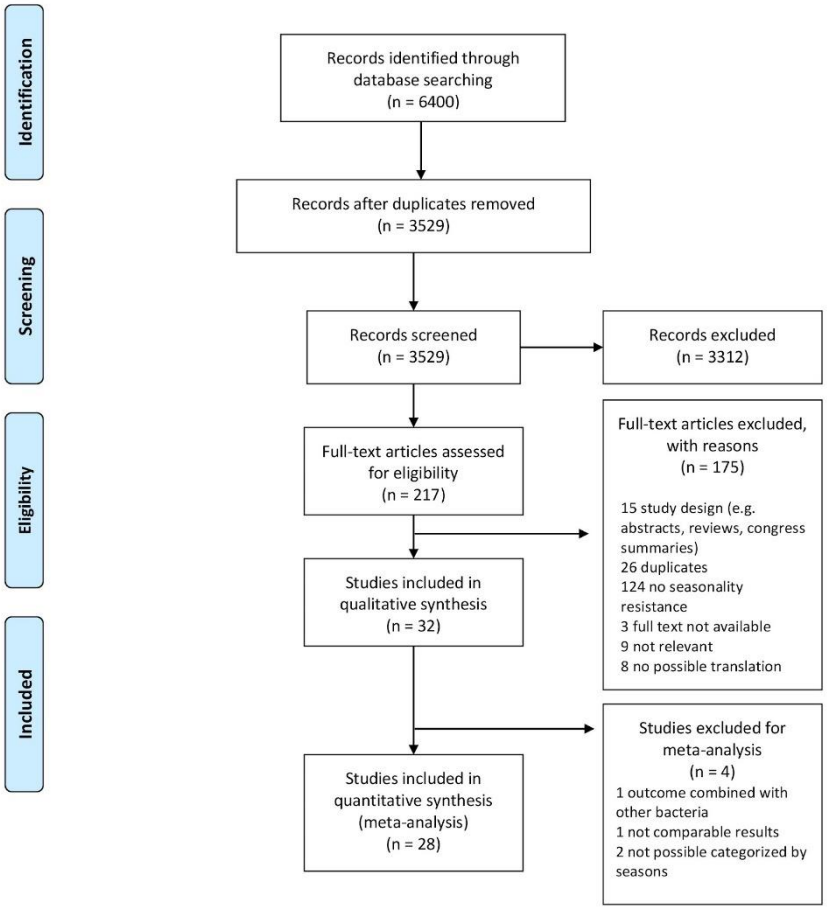
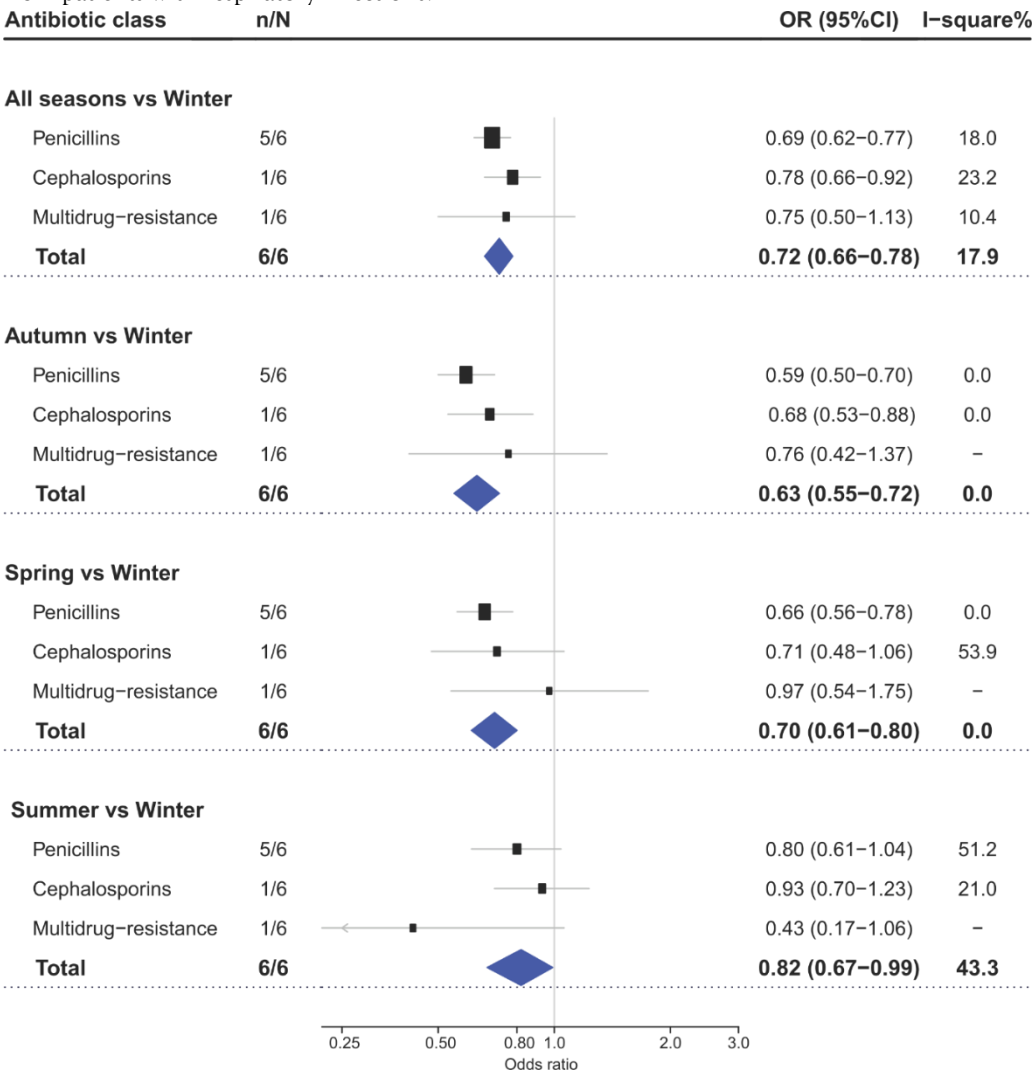
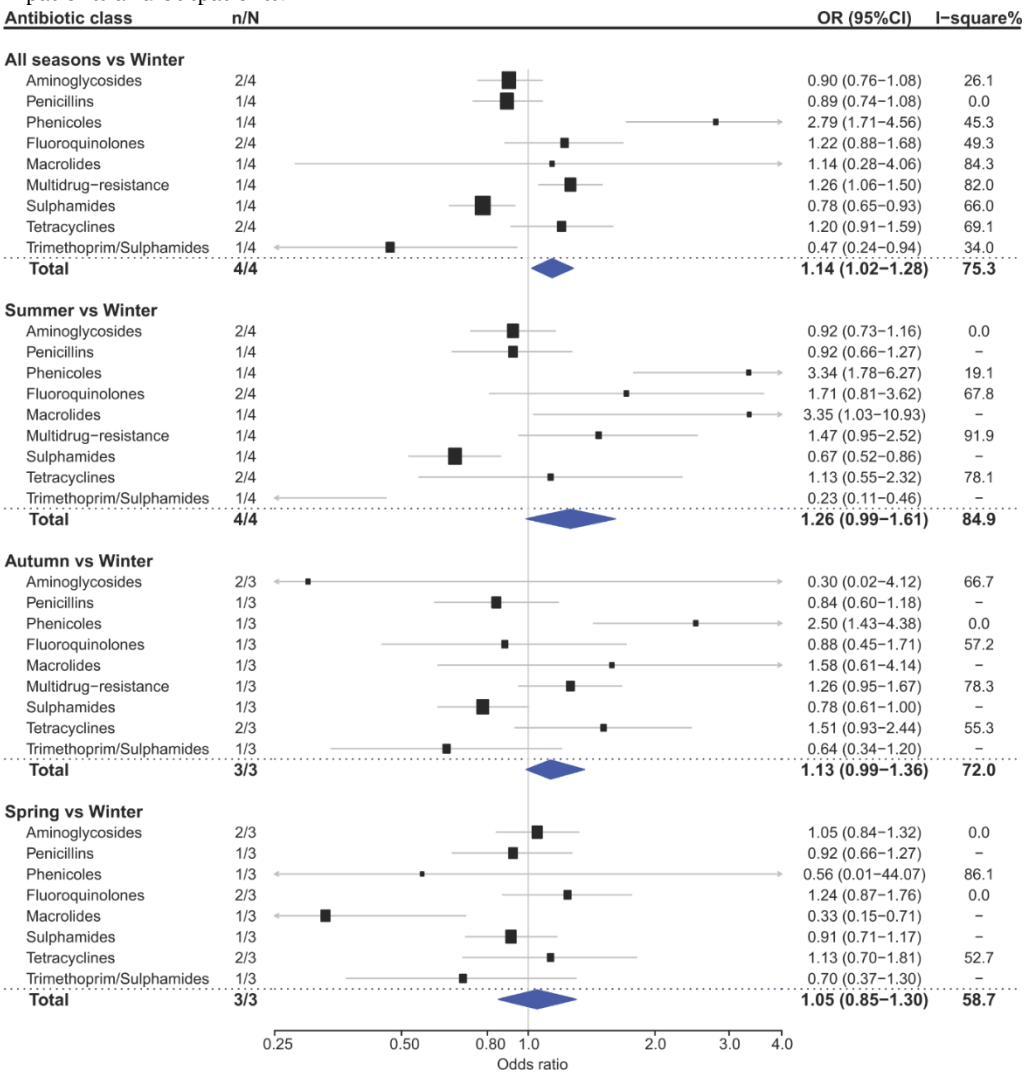


Figure 9. Forest plot of overall seasonality of antimicrobial resistance in Streptococcus *pneumoniae* from patients with respiratory infections.



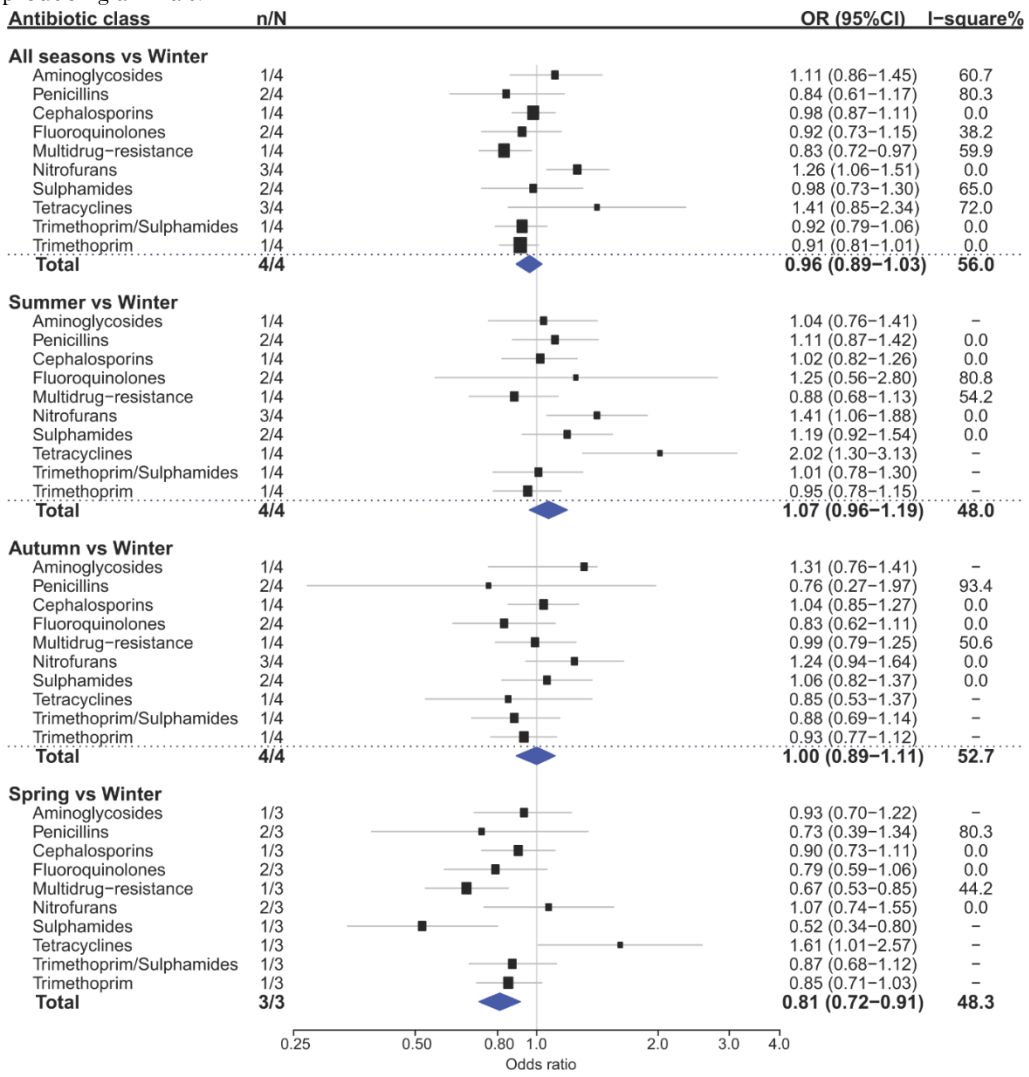
Solid line, refers to no difference in seasonal AMR between the two groups. Winter season was the reference group = 1. n/N = number of included studies in that comparison/total number of included studies. OR, pooled odds ratio of seasonal AMR with 95% Confidence intervals.

Figure 10. Forest plot of overall seasonality of antimicrobial resistance of urinary *E. coli* from inpatients and outpatients.



Solid line, refers to no difference in seasonal AMR between the two groups. Winter season was the reference group = 1. n/N = number of included studies in that comparison/total number of included studies. OR: pooled odds ratio of seasonal AMR with 95% Confidence intervals.

Figure 11. Forest plot of overall seasonal variation in antimicrobial resistance of *E. coli* from food-producing animals.



Solid line, refers to no difference in seasonal AMR between the two groups. Winter season was the reference group = 1. n/N = number of included studies in that comparison/total number of included studies. OR: pooled odds ratio of seasonal AMR with 95% Confidence intervals.

SUPPLEMENTARY MATERIAL

Appendix 39. Review protocol

1) Databases search strategy and terms

Embase.com: ('antibiotic sensitivity'/exp OR 'antibiotic agent'/exp/dd_ad OR 'penicillin resistance'/de OR (('drug resistance'/de OR 'drug sensitivity'/de) AND 'antiinfective agent'/exp) OR 'multidrug resistance'/exp OR (((antibiotic* OR antibacter* OR anti-biotic* OR antiinfect* OR anti-infect* OR anti-bacter* OR antimicrob* OR anti-microb* OR penicillin* OR aminoglycosid* OR lactam* OR cephalosporin* OR fenicole* OR fluorochinono* OR macrolide* OR trimethoprim* OR tetracyclin* OR sulphamide* OR abyssomicin* OR acetomycin* OR actinorhodine* OR aditoprim* OR agglomerin* OR alafosfalin* OR aldecalmycin* OR alisamycin* OR allicin* OR ambruticin* OR ansamitocin* OR ansamycin* OR aplasmomycin* OR aristeromycin* OR asukamycin* OR atpenin* OR auricularum* OR aurograb* OR avilamycin* OR bafilomycin* OR baliz* OR baquiloprim* OR beroline* OR betafectin* OR betamipron* OR boromycin* OR borrelidin* OR brilacidin* OR butalactin* OR cadazolid* OR calcimycin* OR carbadox* OR carbazomycin* OR chloramphenicol* OR ciadox* OR cinoquidox* OR citrinin* OR concanamycin* OR coumamycin* OR cryptosporin* OR cycloheximide* OR dalfopristin* OR dealanylalahopcin* OR dioxidine* OR echinomycin* OR edeine* OR efepristin* OR emimycin* OR endusamycin* OR eperezolid* OR epetraborole* OR epiderstatin* OR epiroprim* OR ethylhydrocupreine* OR evernimicin* OR everninomicin* OR flopristin* OR fosmidomycin* OR furaquinocin* OR furazidin* OR furazolium* OR fusafungine* OR fusidate-sodium* OR fusidic-acid* OR gepotidacin* OR grisein* OR hatomamicin* OR hedamycin* OR heliomycin* OR hidamicin* OR hymeglusin* OR iclaprim* OR ikarugamycin* OR inostamycin* OR kalafungin* OR kelfiprim* OR kidamycin* OR kinamycin* OR kinamycin* OR lactacystin* OR lactivicin* OR laidlomycin* OR lanopepden* OR lasalocid* OR lavanducyanin* OR lenoremycin* OR linezolid* OR linopristin* OR lonomycin* OR lotilibcin* OR lydicamycin* OR lysocellin* OR macrolide* OR malyngolide* OR manumycin* OR methylenomycin* OR mikamycin* OR monensin* OR monensin* OR mureidomycin* OR mycolog* OR myxothiazol* OR narasin* OR negamycin* OR nybomycin* OR olaquindox* OR paldimycin* OR patulin* OR pentalenolactone* OR platensimycin* OR pluramycin* OR polyactin* OR polyfungin* OR posizolid* OR pristinamycin* OR prothracarcin* OR pseudomonic-acid* OR pyrroxamycin* OR quinomycin* OR quinupristin* OR radezolid* OR radicicol* OR ranbezolid* OR simaomicin* OR simocyclinone* OR spectinomycin* OR squalamine* OR streptogramin* OR streptovitacin* OR tedizolid* OR terdecamycin* OR tetracycline* OR tetronasin* OR tetronomycin* OR tetroxoprim* OR thiolactomycin* OR tibezonium-iodide* OR tizoxanide* OR toyocamycin* OR toyocamycin* OR trichostatic-acid* OR trichostatin* OR trimethoprim* OR trimethoprim* OR triostin* OR trospectomycin* OR tuftsin* OR tuftsin* OR tutofusin* OR urdamycin* OR validamycin* OR vernamycin* OR virginiae-butanolide* OR virginiamycin* OR volpristin* OR zibrofusidic-acid* OR zorbamycin* OR fluoroquinolon* OR quinolon* OR multidrug* OR multi-drug* OR methicillin* OR ticarcillin* OR ampicillin* OR ciprofloxacin* OR cefprozil* OR cefaclor* OR amoxicillin* OR streptomycin*) NEAR/10 (sensitiv* OR susceptib*)) OR resistan*):ab,ti) AND ('Campylobacter'/exp OR 'campylobacteriosis'/exp OR 'Salmonella'/exp OR 'salmonellosis'/de OR 'Escherichia coli'/exp OR 'Escherichia coli infection'/exp OR 'Streptococcus pneumoniae'/exp OR 'pneumococcal infection'/exp OR 'Haemophilus influenzae'/exp OR 'Haemophilus infection'/exp OR 'bacterial meningitis'/exp OR 'bacterial pneumonia'/exp OR 'otitis media'/de OR 'acute otitis media'/de OR (Campylobacter* OR Salmonell* OR 'Escherichia coli' OR e-coli OR (Streptococc* NEAR/3 pneumon*) OR (Haemoph* NEAR/3 (influen* OR meningit*)) OR ((respirator* OR food-born* OR foodborn*) NEAR/3 pathogen*) OR ((enteric* OR typhoid*) NEAR/3 fever*) OR pneumococc* OR (bacteri* NEAR/3 meningit*) OR (otitis NEAR/3 (media OR infect*)) OR ((Communit* OR bacter*) NEAR/3 pneumonia*)):ab,ti) AND ('temporal analysis'/exp OR 'meteorological phenomena'/exp OR ((time NEAR/3 series*) OR season* OR Autumn* OR spring OR summer* OR winter* OR meteorolog* OR weather* OR climate* OR ((temporal* OR month*) NEAR/6 (variat* OR higher* OR lower* OR compar* OR divers* OR risk OR fluctuat* OR peak OR pattern* OR dynamic* OR trend* OR monitor* OR decline* OR decrease* OR increase* OR incline* OR change* OR associat*)) OR (throughout NEAR/3 year)):ab,ti)

Medline Ovid: (exp "Drug Resistance, Microbial"/ OR (("Drug Resistance"/) AND exp "Anti-Infective Agents"/) OR "Drug Resistance, Multiple"/ OR (((antibiotic* OR antibacter* OR anti-biotic* OR antiinfect* OR anti-infect* OR anti-bacter* OR antimicrob* OR anti-microb* OR penicillin* OR aminoglycosid* OR lactam* OR cephalosporin* OR fenicole* OR fluorochinono* OR macrolide* OR trimethoprim* OR tetracyclin* OR sulphamide* OR abyssomicin* OR acetomycin* OR actinorhodine* OR aditoprim* OR agglomerin* OR alafosfalin* OR aldecalmycin* OR alisamycin* OR allicin* OR ambruticin* OR ansamitocin* OR ansamycin* OR aplasmomycin* OR aristeromycin* OR asukamycin* OR atpenin* OR auricularum* OR aurograb* OR avilamycin* OR bafilomycin* OR baliz* OR baquiloprim* OR beroline* OR betafectin* OR betamipron* OR boromycin* OR borrelidin* OR brilacidin* OR butalactin* OR cadazolid* OR calcimycin* OR carbadox* OR carbazomycin* OR chloramphenicol* OR ciadox* OR cinoquidox* OR citrinin* OR concanamycin* OR coumamycin* OR cryptosporin* OR cycloheximide* OR dalfopristin* OR dealanylalahopcin* OR dioxidine* OR echinomycin* OR edeine* OR efepristin* OR emimycin* OR endusamycin* OR eperezolid* OR epetraborole* OR epiderstatin* OR epiroprim* OR ethylhydrocupreine* OR evernimicin* OR everninomicin* OR flopristin* OR fosmidomycin* OR furaquinocin* OR furazidin* OR furazolium* OR fusafungine* OR fusidate-sodium* OR fusidic-acid* OR gepotidacin* OR grisein* OR hatomamicin* OR hedamycin* OR heliomycin* OR hidamicin* OR hymeglusin* OR iclaprim* OR ikarugamycin* OR inostamycin* OR kalafungin* OR kelfiprim* OR kidamycin* OR kinamycin* OR kinamycin* OR lactacystin* OR lactivicin* OR laidlomycin* OR lanopepden* OR lasalocid* OR lavanducyanin* OR lenoremycin* OR linezolid* OR linopristin* OR lonomycin* OR lotilibcin* OR lydicamycin* OR lysocellin* OR macrolide* OR malyngolide* OR manumycin* OR methylenomycin* OR mikamycin* OR monensin* OR monensin* OR mureidomycin* OR mycolog* OR myxothiazol* OR narasin* OR negamycin* OR nybomycin* OR olaquindox* OR paldimycin* OR patulin* OR pentalenolactone* OR platensimycin* OR pluramycin* OR polyactin* OR polyfungin* OR posizolid* OR pristinamycin* OR prothracarcin* OR pseudomonic-acid* OR pyrroxamycin* OR quinomycin* OR quinupristin* OR radezolid* OR radicicol* OR ranbezolid* OR simaomicin* OR simocyclinone* OR spectinomycin* OR squalamine* OR streptogramin* OR streptovitacin* OR tedizolid* OR terdecamycin* OR tetracycline* OR tetronasin* OR tetronomycin* OR tetroxoprim* OR thiolactomycin* OR tibezonium-iodide* OR tizoxanide* OR toyocamycin* OR toyocamycin* OR trichostatic-acid* OR trichostatin* OR trimethoprim* OR trimethoprim* OR triostin* OR trospectomycin* OR tuftsin* OR tuftsin* OR tutofusin* OR urdamycin* OR validamycin* OR vernamycin* OR virginiae-butanolide* OR virginiamycin* OR volpristin* OR zibrofusidic-acid* OR zorbamycin* OR fluoroquinolon* OR quinolon* OR multidrug* OR multi-drug* OR methicillin* OR ticarcillin* OR ampicillin* OR ciprofloxacin* OR cefprozil* OR cefaclor* OR amoxicillin* OR streptomycin*) ADJ10 (sensitiv* OR susceptib*)) OR resistan*).ab,ti.) AND (exp "Campylobacter"/ OR exp "Campylobacter Infections"/ OR exp "Salmonella"/ OR exp "Salmonella Infections"/ OR exp "Escherichia coli"/ OR exp "Escherichia coli Infections"/ OR exp "Streptococcus pneumoniae"/ OR exp "Pneumococcal Infections"/ OR exp "Haemophilus influenzae"/ OR exp "Haemophilus Infections"/ OR exp "Meningitis, Bacterial"/ OR exp "Pneumonia, Bacterial"/ OR "otitis media"/ OR (Campylobacter* OR Salmonell* OR "Escherichia coli" OR e-coli OR (Streptococc* ADJ3 pneumon*) OR (Haemoph* ADJ3 (influen* OR meningit*)) OR ((respirator* OR food-born* OR foodborn*) ADJ3 pathogen*) OR ((enteric* OR typhoid*) ADJ3 fever*) OR pneumococc* OR (bacteri* ADJ3 meningit*) OR (otitis ADJ3 (media OR infect*)) OR ((Communit* OR bacter*) ADJ3 pneumonia*)).ab,ti.) AND ("Climate"/ OR Seasons/ OR Weather/ OR ((time ADJ3 series*) OR season* OR Autumn* OR spring OR summer* OR winter* OR meteorolog* OR weather* OR climate* OR ((temporal* OR month*) ADJ6 (variat* OR higher* OR lower* OR compar* OR divers* OR risk OR fluctuat* OR peak OR pattern* OR dynamic* OR trend* OR monitor* OR decline* OR decrease* OR increase* OR incline* OR change* OR associat*)) OR (throughout ADJ3 year)).ab,ti.)

Cochrane: ((((antibiotic* OR antibacter* OR anti-biotic* OR antiinfect* OR anti-infect* OR anti-bacter* OR antimicrob* OR anti-microb* OR penicillin* OR aminoglycosid* OR lactam* OR cephalosporin* OR fenicole* OR fluorochinono* OR macrolide* OR trimethoprim* OR tetracyclin* OR sulphamide* OR abyssomicin* OR acetomycin* OR actinorhodine* OR aditoprim* OR agglomerin* OR alafosfalin* OR aldecalmycin* OR alisamycin* OR allicin* OR ambruticin* OR ansamitocin* OR ansamycin* OR aplasmomycin* OR aristeromycin* OR asukamycin* OR atpenin* OR auricularum* OR aurograb* OR avilamycin* OR bafilomycin* OR baliz* OR baquiloprim* OR beroline* OR betafectin* OR betamipron* OR boromycin* OR borrelidin* OR brilacidin* OR butalactin* OR cadazolid* OR calcimycin* OR carbadox* OR carbazomycin* OR chloramphenicol* OR ciadox* OR cinoquidox* OR citrinin* OR concanamycin* OR coumamycin* OR cryptosporin* OR cycloheximide* OR dalfopristin* OR dealanylalahopcin* OR dioxidine* OR echinomycin* OR edeine* OR efepristin* OR emimycin* OR endusamycin* OR eperezolid* OR epetraborole* OR epiderstatin* OR epiroprim* OR ethylhydrocupreine* OR evernimicin* OR everninomicin* OR flopristin* OR fosmidomycin* OR furaquinocin* OR furazidin* OR furazolium* OR fusafungine* OR fusidate-sodium* OR fusidic-acid* OR gepotidacin* OR grisein* OR hatomamicin* OR hedamycin* OR heliomycin* OR hidamicin* OR hymeglusin* OR iclaprim* OR ikarugamycin* OR inostamycin* OR kalafungin* OR kelfiprim* OR kidamycin* OR kinamycin* OR kinamycin* OR lactacystin* OR lactivicin* OR laidlomycin* OR lanopepden* OR lasalocid* OR lavanducyanin* OR lenoremycin* OR linezolid* OR linopristin* OR lonomycin* OR lotilibcin* OR lydicamycin* OR lysocellin* OR macrolide* OR malyngolide* OR manumycin* OR methylenomycin* OR mikamycin* OR monensin* OR monensin* OR mureidomycin* OR mycolog* OR myxothiazol* OR narasin* OR negamycin* OR nybomycin* OR olaquindox* OR paldimycin* OR patulin* OR pentalenolactone* OR platensimycin* OR pluramycin* OR polyactin* OR polyfungin* OR posizolid* OR pristinamycin* OR prothracarcin* OR pseudomonic-acid* OR pyrroxamycin* OR quinomycin* OR quinupristin* OR radezolid* OR radicicol* OR ranbezolid* OR simaomicin* OR simocyclinone* OR spectinomycin* OR squalamine* OR streptogramin* OR streptovitacin* OR tedizolid* OR terdecamycin* OR tetracycline* OR tetronasin* OR tetronomycin* OR tetroxoprim* OR thiolactomycin* OR tibezonium-iodide* OR tizoxanide* OR toyocamycin* OR toyocamycin* OR trichostatic-acid* OR trichostatin* OR trimethoprim* OR trimethoprim* OR triostin* OR trospectomycin* OR tuftsin* OR tuftsin* OR tutofusin* OR urdamycin* OR validamycin* OR vernamycin* OR virginiae-butanolide* OR virginiamycin* OR volpristin* OR zibrofusidic-acid* OR zorbamycin* OR fluoroquinolon* OR quinolon* OR multidrug* OR multi-drug* OR methicillin* OR ticarcillin* OR ampicillin* OR ciprofloxacin* OR cefprozil* OR cefaclor* OR amoxicillin* OR streptomycin*) NEAR/10 (sensitiv* OR susceptib*)) OR resistan*):ab,ti) AND ((Campylobacter* OR Salmonell* OR 'Escherichia coli' OR e-coli OR (Streptococ* NEAR/3 pneumon*) OR (Haemoph* NEAR/3 (influen* OR meningit*)) OR ((respirator* OR food-born* OR foodborn*) NEAR/3 pathogen*) OR ((enteric* OR typhoid*) NEAR/3 fever*) OR pneumococ* OR (bacteri* NEAR/3 meningit*) OR (otitis NEAR/3 (media OR infect*)) OR ((Communit* OR bacter*) NEAR/3 pneumonia*)):ab,ti) AND (((time NEAR/3 series*) OR season* OR Autumn* OR spring OR summer* OR winter* OR meteorolog* OR weather* OR climate* OR ((temporal* OR month*) NEAR/6 (variat* OR higher* OR lower* OR compar* OR divers* OR risk OR fluctuat* OR peak OR pattern* OR dynamic* OR trend* OR monitor* OR decline* OR decrease* OR increase* OR incline* OR change* OR associat*)) OR (throughout NEAR/3 year) OR 'periodicity'):ab,ti)

Web of science : TS=(((((antibiotic* OR antibacter* OR anti-biotic* OR antiinfect* OR anti-infect* OR anti-bacter* OR antimicrob* OR anti-microb* OR penicillin* OR aminoglycosid* OR lactam* OR cephalosporin* OR fenicole* OR fluorochinono* OR macrolide* OR trimethoprim* OR tetracyclin* OR sulphamide* OR abyssomicin* OR acetomycin* OR actinorhodine* OR aditoprim* OR agglomerin* OR alafosfalin* OR aldecalmycin* OR alisamycin* OR allicin* OR ambruticin* OR ansamitocin* OR ansamycin* OR aplasmomycin* OR aristeromycin* OR asukamycin* OR atpenin* OR auricularum* OR aurograb* OR avilamycin* OR bafilomycin* OR baliz* OR baquiloprim* OR beroline* OR betafectin* OR betamipron* OR boromycin* OR borrelidin* OR brilacidin* OR butalactin* OR cadazolid* OR calcimycin* OR carbadox* OR carbazomycin* OR chloramphenicol* OR ciadox* OR cinoquidox* OR citrinin* OR concanamycin* OR coumamycin* OR cryptosporin* OR cycloheximide* OR dalfopristin* OR dealanylalahopcin* OR dioxidine* OR echinomycin* OR edeine* OR efepristin* OR emimycin* OR endusamycin* OR eperezolid* OR epetraborole* OR epiderstatin* OR epiroprim* OR ethylhydrocupreine* OR evernimicin* OR everninomicin* OR flopristin* OR fosmidomycin* OR furaquinocin* OR furazidin* OR furazolium* OR fusafungine* OR fusidate-sodium* OR fusidic-acid* OR gepotidacin* OR grisein* OR hatomamicin* OR hedamycin* OR heliomycin* OR hidamicin* OR hymeglusin* OR iclaprim* OR ikarugamycin* OR inostamycin* OR kalafungin* OR kelfiprim* OR kidamycin* OR kinamycin* OR kinamycin* OR lactacystin* OR lactivicin* OR laidlomycin* OR lanopepden* OR lasalocid* OR lavanducyanin* OR lenoremycin* OR linezolid* OR linopristin* OR lonomycin* OR lotilibcin* OR lydicamycin* OR lysocellin* OR macrolide* OR malyngolide* OR manumycin* OR methylenomycin* OR mikamycin* OR monensin* OR monensin* OR mureidomycin* OR mycolog* OR myxothiazol* OR narasin* OR negamycin* OR nybomycin* OR olaquindox* OR paldimycin* OR patulin* OR pentalenolactone* OR platensimycin* OR pluramycin* OR polyactin* OR polyfungin* OR posizolid* OR pristinamycin* OR prothracarcin* OR pseudomonic-acid* OR pyrroxamycin* OR quinomycin* OR quinupristin* OR radezolid* OR radicicol* OR ranbezolid* OR simaomicin* OR simocyclinone* OR spectinomycin* OR squalamine* OR streptogramin* OR streptovitacin* OR tedizolid* OR terdecamycin* OR tetracycline* OR tetronasin* OR tetronomycin* OR tetroxoprim* OR thiolactomycin* OR tibezonium-iodide* OR tizoxanide* OR toyocamycin* OR toyocamycin* OR trichostatic-acid* OR trichostatin* OR trimethoprim* OR trimethoprim* OR triostin* OR trospectomycin* OR tuftsin* OR tuftsin* OR tutofusin* OR urdamycin* OR validamycin* OR vernamycin* OR virginiae-butanolide* OR virginiamycin* OR volpristin* OR zibrofusidic-acid* OR zorbamycin* OR fluoroquinolon* OR quinolon* OR multidrug* OR multi-drug* OR methicillin* OR ticarcillin* OR ampicillin* OR ciprofloxacin* OR cefprozil* OR cefaclor* OR amoxicillin* OR streptomycin*) NEAR/9 (sensitiv* OR susceptib*)) OR resistan*)) AND ((Campylobacter* OR Salmonell* OR "Escherichia coli" OR e-coli OR (Streptococ* NEAR/2 pneumon*) OR (Haemoph* NEAR/2 (influen* OR meningit*)) OR ((respirator* OR food-born* OR foodborn*) NEAR/2 pathogen*) OR ((enteric* OR typhoid*) NEAR/2 fever*) OR pneumococ* OR (bacteri* NEAR/2 meningit*) OR (otitis NEAR/2 (media OR infect*)) OR ((Communit* OR bacter*) NEAR/2 pneumonia*))) AND (((time NEAR/2 series*) OR season* OR Autumn* OR spring OR summer* OR winter* OR meteorolog* OR weather* OR climate* OR ((temporal* OR month*) NEAR/5 (variat* OR higher* OR lower* OR compar* OR divers* OR risk OR fluctuat* OR peak OR pattern* OR dynamic* OR trend* OR monitor* OR decline* OR decrease* OR increase* OR incline* OR change* OR associat*)) OR (throughout NEAR/2 year) OR "periodicity")))

Biosis Ovid: ((((antibiotic* OR antibacter* OR anti-biotic* OR antiinfect* OR anti-infect* OR anti-bacter* OR antimicrob* OR anti-microb* OR penicillin* OR aminoglycosid* OR lactam* OR cephalosporin* OR fenicole* OR fluorochinono* OR macrolide* OR trimethoprim* OR tetracyclin* OR sulphamide* OR abyssomicin* OR acetomycin* OR actinorhodine* OR aditoprim* OR agglomerin* OR alafosfalin* OR aldecalmycin* OR alisamycin* OR allicin* OR ambruticin* OR ansamitocin* OR ansamycin* OR aplasmomycin* OR aristeromycin* OR asukamycin* OR atpenin* OR auricularum* OR aurograb* OR avilamycin* OR bafilomycin* OR baliz* OR baquiloprim* OR beroline* OR betafectin* OR betamipron* OR boromycin* OR borrelidin* OR brilacidin* OR butalactin* OR cadazolid* OR calcimycin* OR carbadox* OR carbazomycin* OR chloramphenicol* OR ciadox* OR cinoquidox* OR citrinin* OR concanamycin* OR coumamycin* OR cryptosporin* OR cycloheximide* OR dalfopristin* OR dealanylalahopcin* OR dioxidine* OR echinomycin* OR edeine* OR efepristin* OR emimycin* OR endusamycin* OR eperezolid* OR epetraborole* OR epiderstatin* OR epiroprim* OR ethylhydrocupreine* OR evernimicin* OR everninomicin* OR flopristin* OR fosmidomycin* OR furaquinocin* OR furazidin* OR furazolium* OR fusafungine* OR fusidate-sodium* OR fusidic-acid* OR gepotidacin* OR grisein* OR hatomamicin* OR hedamycin* OR heliomycin* OR hidamicin* OR hymeglusin* OR iclaprim* OR ikarugamycin* OR inostamycin* OR kalafungin* OR kelfiprim* OR kidamycin* OR kinamycin* OR kinamycin* OR lactacystin* OR lactivicin* OR laidlomycin* OR lanopepden* OR lasalocid* OR lavanducyanin* OR lenoremycin* OR linezolid* OR linopristin* OR lonomycin* OR lotilibcin* OR lydicamycin* OR lysocellin* OR macrolide* OR malyngolide* OR manumycin* OR methylenomycin* OR mikamycin* OR monensin* OR monensin* OR mureidomycin* OR mycolog* OR myxothiazol* OR narasin* OR negamycin* OR nybomycin* OR olaquindox* OR paldimycin* OR patulin* OR pentalenolactone* OR platensimycin* OR

pluramycin* OR polyactin* OR polyfungin* OR posizolid* OR pristinamycin* OR prothracarcin* OR pseudomonic-acid* OR pyrroxamycin* OR quinomycin* OR quinupristin* OR radezolid* OR radicicol* OR ranbezolid* OR simaomicin* OR simocyclinone* OR spectinomycin* OR squalamine* OR streptogramin* OR streptovitacin* OR tedizolid* OR terdecamycin* OR tetracycline* OR tetronasin* OR tetronomycin* OR tetroxoprim* OR thiolactomycin* OR tibezonium-iodide* OR tizoxanide* OR toyocamycin* OR toyocamycin* OR trichostatic-acid* OR trichostatin* OR trimethoprim* OR trimethoprim* OR triostin* OR trospectomycin* OR tuftsin* OR tuftsin* OR tutofusin* OR urdamycin* OR validamycin* OR vernamycin* OR virginiae-butanolide* OR virginiamycin* OR volpristin* OR zibrofusidic-acid* OR zorbamycin* OR fluoroquinolon* OR quinolon* OR multidrug* OR multi-drug* OR methicillin* OR ticarcillin* OR ampicillin* OR ciprofloxacin* OR cefprozil* OR cefaclor* OR amoxicillin* OR streptomycin*) ADJ10 (sensitiv* OR susceptib*)) OR resistan*).ab,ti.) AND ((Campylobacter* OR Salmonell* OR "Escherichia coli" OR e-coli OR (Streptococ* ADJ3 pneumon*) OR (Haemoph* ADJ3 (influen* OR meningit*)) OR ((respirator* OR food-born* OR foodborn*) ADJ3 pathogen*) OR ((enteric* OR typhoid*) ADJ3 fever*) OR pneumococ* OR (bacteri* ADJ3 meningit*) OR (otitis ADJ3 (media OR infect*)) OR ((Communit* OR bacter*) ADJ3 pneumonia*)).ab,ti.) AND (" Climatology"/ OR ((time ADJ3 series*) OR season* OR Autumn* OR spring OR summer* OR winter* OR meteorolog* OR weather* OR climate* OR ((temporal* OR month*) ADJ6 (variat* OR higher* OR lower* OR compar* OR divers* OR risk OR fluctuat* OR peak OR pattern* OR dynamic* OR trend* OR monitor* OR decline* OR decrease* OR increase* OR incline* OR change* OR associat*)) OR (throughout ADJ3 year)).ab,ti.)

Google scholar: "antibiotic|antibacterial|biotic sensitivity|susceptibility|resistance" Campylobacter|Salmonella|"Escherichia|e coli"|Streptococca|Haemophilae|"respiratory|foodborne pathogens" "time series"|season|seasonal|weather|climate|"temporal variation|fluctuation"

Additional searching: Reference review off all included studies (pulled for possible inclusion). The search was done independently by both PM and MJ. Any study felt suitable by either of the authors was included for further examination. Contact of authors via e-mail. The search was done by PM, authors from included (good qualify) studies were contacted via e-mail looking for suggestions of suitable studies.

2) Selection criteria

The appraised by tittle of all studies found by search on online database was done by PM, MC, MJ, JS, MG authors. Final selection of included studies by reviewing tittle and abstract was done by PM and MJ, and disagreements were discussed with MC.

The detail inclusion/exclusion criteria considered for this study is below:

a. Inclusion criteria

*Design: Include all studies (cross-sectional, cohorts, case-control, time-series, surveillance) that evaluated seasonal incidence and/or prevalence of antibiotic resistance or antibiotic susceptibility in any of the following bacteria Campylobacter *spp. Salmonella spp. Escherichia coli*, *Streptococcus pneumoniae* and *Haemophilus influenza.* independently from the type of disease caused.

*Population: Include all studies that measured antibiotic resistant/susceptibility criteria in any of the following sample sources: soil, water, humans, animals or animal products.

*Outcome: Include studies that measured antibiotic resistance/susceptibility through objective determination on isolated bacteria strains, for example:

- Dilution method,
- Disk-diffusion method,
- E-test,
- Automated methods,
- Genotypic methods such as PCR and DNA hybridization methods,
- Minimal inhibitory concentration (MIC)

*Include studies that measured any type of resistance genes to any of the bacteria under study.

b. Exclusion criteria

*Exclude literature reviews, abstracts, congress information, reports, thesis, meta-analysis, and protocols.
*Exclude studies that obtained antibiotic resistant patter based on surveys.
*Exclude studies that has been conducted only in one season (e.g. only in winters).
*Exclude studies that has measured antibiotic-resistant patterns having as main objective evaluated vaccination programs.

3) Quality assessment

Quality assessment was based on a adapted version from the Newcastle-Ottawa Quality Assessment Scale for cross-sectional studies by [2811]. The three factors considered were:

Sample selection criteria (maximum 4 points):

1- Representativeness of the sample/sample size
   a. Truly representative of the average in the target population (random sampling) and justified sample size based on population characteristics (i.e. farms production, hospitals, slaughter houses, river length). **

b. Somewhat representative of the average in the target population (convenience sampling or other sampling method), justified sample size based on population characteristics. *
c. No description of the sampling strategy/sample size not justified.

2- Study time period
a. The time period was satisfactory (i.e. monthly observations) making possible the categorization of the four seasons (summer, winter, spring and autumn) **
b. The time period was partly satisfactory making possible the categorization of two seasons (including cold and warm seasons). *
c. The time period was unsatisfactory making difficult the categorization of seasons.

Comparability on the basis of the design or analysis (maximum 2 points):
3- Comparability of the population (control of confounders):
a. Population is comparable throughout the time period of study (have the same characteristics) and adjusted for at least an important factor (i.e. seasonal prevalence of resistant strains, population, year). **
b. Population is not comparable throughout the time period of study (different characteristics) but adjusted for at least an important factor. *
c. Population is not comparable and not adjusted.

Outcome (maximum 2 points):
4- Assessment of the exposure:
a. Medical records or laboratory assessment with a validated antimicrobial susceptibility testing and interpretation method based on standard minimal concentration break points*
b. Self-report (i.e. survey, questionnaire).
c. No description of antimicrobial susceptibility testing and interpretation method.

5- Data analysis and statistical test:
a. Total number of samples, frequencies and proportion of susceptible and non-susceptible strains are clearly described, as well as the statistical test used to analyze the data including p-values. *
b. The data described is incomplete, the statistical test is not appropriate or not well describe.

[1]Modesti, P. A., G. Reboldi, F. P. Cappuccio, C. Agyemang, G. Remuzzi, S. Rapi, E. Perruolo, G. Parati and E. S. H. W. G. o. C. R. i. L. R. Settings (2016). "Panethnic Differences in Blood Pressure in Europe: A Systematic Review and Meta-Analysis." PLOS ONE **11**(1): e0147601.

4) Data extraction and statistical analysis

Data extraction: PM extracted the data from included studies. MC did a review of the data extracted. Data was recorded in a proper excel sheet, including: author, published year, study design, country, follow-up time period, sample source (animal, human), sample origin (e.g. cattle, poultry, hospital, care center), number of total isolates tested in the study, sampling method, previous use of antibiotics, culture isolation method, biochemical testing, antimicrobial susceptibility testing method, bacteria type, bacteria serotype, season definition, antibiotic class tested in the study, antibiotic type, number of resistant isolates per antibiotic class, resistant rates (%) per bacteria and per antibiotic class, number of susceptible isolates per antibiotic class, susceptible rates (%) per bacteria and per antibiotic class, number of intermediate resistant isolates per antibiotic class, intermediate resistant rates (%) per bacteria and per antibiotic class

5) Statistical analysis

All statistical analysis was carry on by PM and supported by MC. For the statistical analysis was necessary to categorized variables as follows:

Geographical regions: All countries included in each category are listed below:
-Eastern Mediterranean: Iran.
-Europe: Czech Republic, Denmark, England, Finland, France, Israel, Italy, Lithuania, Norway, Poland, Spain, Netherlands and Turkey.
-America: Canada, Costa Rica and United States.

-*South East Asia:* Thailand and India.
-*Western Pacific:* Australia, China, Japan and Korea.

Antibiotic classes: All antibiotic types included in each category are listed below:
-*Aminoglycosides:* Amikacin, Gentamicin, Kanamycin and Streptomycin.
-*Penicillins:* Amoxycillin/clavulanic acid, Amoxycillin, Ampicillin, Penicillin, Carbenicillin, Piperacillin.
-*Cephalosporines:* Cefaclor, Cefalexin, Cefazolin, Cefepime, Cefixime, Cefotaxime, Cefoxitin, Ceftazidime, Ceftiofur, Ceftriaxone, Cefuroxime, Cefaloridine, Cefalothin, Cefaxolin.
-*Phenicols:* Chloramphenicol and Florfenicol.
-*Fluoroquinolones/Quinolones:* Ciprofloxacin, Danofloxacin, Difloxacin, Enrofloxacin, Levofloxacin, Norfloxacin, Ofloxacin, Nalidixic acid.
-*Macrolides:* Azithromycin, Carithromycin, Doxycycline, Erythromycin and Tylosin.
-*Nitrofurans:* Nitrofurantoin and Furazolidone.
-*Sulphamides*: Sulfadiazine, Sulfamethazine, Sulphamethoxazole and Sulphonamide.
-Trimethoprim: Trimethroprim.
-*Tetracyclines:* Tetracycline, Chlortetracycline, Doxycycline, Liquamycin, Oxytetracycline.

Sample source: The variables included in each category are listed below:
-*Animals:* cats, cattle, dog, poultry, sheep, and swine.
-*Human:* samples at population level, hospitals, and sewage water.

Appendix 40. Quality assessment of included studies (n=32).

A cut-off of NOS score of 7 or more to considered as "good quality" was used. This criterion was based on (see McPheeters et al. 2012*; see Appendix G page 103-104 in http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0049229/). Bold letter indicate author that were contacted for additional search studies.

No.	First autor	Study year	Sample Criteria		Comparability	Outcome		Total score	Quality assessment interpretation
			Representativeness of the sample	Study time period	Control of confounders	Assessment of the exposure	Data analysis and statistical testing		
1	Vorland	1985	*	*	**	*	*	6	Fair quality
2	Stacevičiene et al.	2016	*	**	**	*	*	7	Good quality
3	Rao et al.	2010	**	*	**	*	*	7	Good quality
4	Baquero et al.	1999	*	**	*	*	*	7	Good quality
5	Sulonen et al.	2007	**	*	-	*	-	4	Low quality
6	Usui et al.	1973	-	**	-	-	*	3	Low quality
7	Boken et al.	1995	*	*	*	*	*	5	Fair quality
8	Talebiyan et al.	2014	*	**	*	*	*	6	Fair quality
9	Vardhan et al.	2003	**	**	-	*	-	5	Fair quality
10	Taylor et al.	2009	*	*	**	*	*	6	Fair quality
11	Fasugba et al.	2016	*	**	**	*	*	7	Good quality
12	Siripongpreeda et al.	2010	*	**	*	*	*	6	Fair quality
13	Albanese et al.	2001	*	*	**	*	*	6	Fair quality
14	Alali et al.	2008	**	**	**	*	*	8	Good quality
15	Dar et al.	1992	*	**	-	*	-	4	Low quality
16	Hashida et al.	2008	*	*	*	*	*	5	Fair quality
17	Wormald et al.	1971	-	**	-	*	*	4	Low quality
18	Hoberman et al.	2005	*	**	**	*	*	7	Good quality
19	Gow et al.	2008	**	*	**	*	*	7	Good quality
20	Andrzejewska et al.	2013	*	*	-	*	-	3	Low quality
21	Lee et al.	2016	**	*	-	*	*	5	Fair quality
22	van Hees et al.	2006	**	**	-	-	*	5	Fair quality
23	Abatih et al.	2009	**	**	**	*	*	8	Good quality
24	Talsma et al.	1999	**	**	-	*	-	5	Fair quality
25	Guevara et al.	2008	**	*	*	*	*	6	Fair quality
26	Tam et al.	2015	*	**	*	-	*	5	Fair quality
27	Marchisio et al.	2001	**	*	*	*	*	6	Fair quality
28	Sun et al.	2012	*	**	**	-	*	6	Fair quality
29	Dagan et al.	2008	**	**	**	*	*	8	Good quality
30	Gencay	2014	**	*	*	*	*	6	Fair quality
31	Marco et al.	2000	*	*	*	*	*	5	Fair quality
32	Meumann et al.	2015	**	**	**	*	*	8	Good quality

*McPheeters, M.L., Kripalini, S., Peterson, N.B., Idowu, R.T. et al. (2012). Quality Improvement Interventions To Address Health Disparities. Evidence Report/ technology Assessment. Rockville (MD): Agency for Healthcare Research and Quality (US). http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0049222/pdf/TOC.pdf

Appendix 41. Detailed characteristic of included studies (n=32)

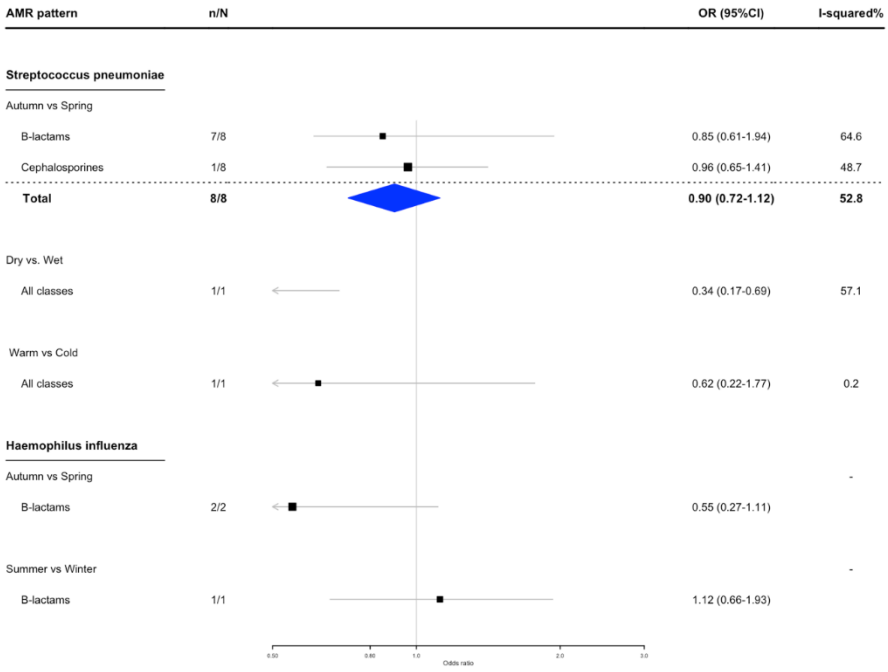
Study	Study design	Country	Region	Study period	Sample source	Sample source characteristic	AMCS* testing method	AMCS testing interpretation	AMR patterns	Season category	Outcome measure
					Escherichia coli						
Abatih et al (2009)	Cross-sectional	Denmark	EU	Between 1997 and 2005	A	Pig and isolates at slaughter houses	Sensititre	CSLI	AMG, PEN, SUP	1	Seasonal by year
Gencay (2014)	Cross-sectional	Tukey	EU	Between Jun 2012 and May 2013	A	Sheep at slaughter houses	E-test	CSLI	CEP	3	Seasonal
Gow et al (2008)	Cross-sectional	Canada	AM	Between Jan to May, 2002 and between Sep and Dec, 2002	A	Healthy cattle in production and calves	Dilution	CSLI	AMG, PEN, CEP, FEN, FQ, MDR, SUP, TET, TM/SUP	2	Seasonal
Talebiyan et al (2014)	Cross-sectional	Iran	EM	Apr, 2009 to Mar, 2012	A	Commercial broiler flocks	Disk-diffusion	CSLI	AMG, FEN, FQ, MC, SUP, TET, TM/SUP	1	Seasonal
Alali et al (2008)	Longitudinal	USA	AM	Feb 2004 to Jan 2007	A/H	Swine and sewage water	Dilution	CSLI	PEN, MDR, MC	1	Seasonal by year
Meumann et al (2015)†	Retrospective	Australia	WP	Between Jan, 2010 and Dec, 2012	H	Population-based	Disk-diffusion	EUCAST	PEN, AMG, CEP, FQ	N/A	Monthly
Fasugba et al (2016)	Cross-sectional	Australia	WP	From 2009 to 2013	H	Inpatients in hospital with urinary tract infection	Disk-diffusion	CSLI	AMG, PEN, CEP, FQ, NIF, TM, TM/SUP	1	Seasonal by year
Sun et al (2012)†	Cross-sectional	USA	AM	1997 to 2007	H	inpatients and outpatients isolates	N/A	CSLI	PEN, AMG	N/A	Monthly
Usui et al (1973)	Cross-sectional	Japan	WP	1969 to 1971	H	Patients with urinary tract infections	N/A	N/A	AMG, CEP, FEN, PMX, TET	7	Seasonal
Vorland et al (1985)	Cross-sectional	Norway	EU	1st Jun, 1979 through May, 1980	H	Outpatients with urinary tract infections	Disk-diffusion	CSLI	NIF, SUP	1	Seasonal
Wormald, P. J (1971)	Retrospective	England	EU	Ded, 1969 to Nov, 1970	H	Outpatients with and without urinary tract infections	Dilution	CSLI	PEN, FQ, PMX, NIF, SUP, TET	7	Monthly
					Streptococcus pneumonia						
Albanese et al (2002)	Cross-sectional	USA	AM	From 1995 through 1997	H	Patients from hospitals	N/A	CSLI	PEN	3	Seasonal

Study	Study design	Country	Region	Study period	Sample source	Sample source characteristic	AMCS* testing method	AMCS testing interpretation	AMR patterns	Season category	Outcome measure
Baquero et al (1996)	Prospective	Spain	EU	Between May 1996 and April 1997	H	Patients with respiratory community-acquire infections	Dilution	CSLI	PEN	1	Seasonal
Boken et al (1995)	Cross-sectional	USA	AM	April, 1994	H	Children age 2 to 24 months	Dilution	CSLI	PEN	2	Seasonal
Dagan et al (2008)†	Prospective	Israel	EU	From 1993 to 2003	H	Bedouin and Jewish children >5 years old	Disk-diffusion	CSLI	PEN	N/A	Monthly
Francesc et al (2000)	Cross-sectional	Spain	EU	Between May 1996 and April 1997	H	Patients with respiratory community-acquire infections	Dilution	CSLI	PEN, CEP	1	Seasonal
Guevara et al (2008)	Cross-sectional	Costa Rica	AM	Between 1999 to 2004	H	Outpatient children until 2 years with otitis media	Disk-diffusion	CSLI	PEN, MC	4	Seasonal
Hoberman et al (2005)	Cross-sectional	USA	AM	Between 1991 and 2003	H	Children age 2 months to 8 years with acute otitis media	Dilution	CSLI	PEN, MC, TM/SUP	1	Monthly
Marchisio et al (2001)	Longitudinal	Italy	EU	mid-Oct to mid-Nov and mid-April to mid-May, N/A year	H	Healthy children age 1 - 7 years	Sensititre	CSLI	PEN, MC	2	Seasonal
Siripongpreeda et al (2010)	Retrospective	Thailand	SEA	Jan, 1996 to Dec, 2007	H	Patients age < 18 year with Invasive pneumococci disease	Disk-diffusion	CSLI	PEN	3	Monthly
Stacevičiene et al (2016)	Prospective	Lithuania	EU	Feb, 2012 to Mar, 2013	H	Children age < 6 years with respiratory tract infection	Disk-diffusion	EUCAST	MDR	1	Seasonal
Tam et al (2015)	Cross-sectional	USA	AM	From 2007 to 2009	H	Children age < 5 years suffering invasive pneumococcal disease	N/A	N/A	PEN	1	Seasonal
Vardhan & Allen (2003)	Prospective	England	EU	Between Jan, 1987 and Dec, 2000	H	Penicillin resistance isolates from pediatric clinical specimens	Rotary Stokes	CSLI	PEN	1	Monthly
Taylor et al (2009)	Cross-sectional	England	AM	Dec, 2002 to Oct 2003	A	Campylobacter spp./E.coli Pig finishing from farms	Dilution	CSLI	FQ, MC, TET	1	Seasonal
Rao et al (2010)	Cross-sectional	Canada	AM	Mar though Dec, 2004	A	Cattle housed in pens	Dilution	CSLI	FQ, MC, TET, AMG, PEN, CEP, FEN, MDR, SUP, TM/SUP	2	Seasonal

Study	Study design	Country	Region	Study period	Sample source	Sample source characteristic	AMCS* testing method	AMCS testing interpretation	AMR patterns	Season category	Outcome measure
Rao et al (2010)	Cross-sectional	Canada	AM	Mar though Dec, 2004	A	Cattle housed in pens	Dilution	CSLI	FQ, MC, TET, AMG, PEN, CEP, FEN, MDR, SUP, TM/SUP	2	Seasonal
						Campylobacter spp.					
Andrzejewska et al (2013)	Cross-sectional	Poland	EU	Nov to Dec, N/A year	A	Cats and dog from farms and private house holds	E-test	CSLI	FQ	6	Seasonal
Sulonen et al (2007)	Cross-sectional	Finland	EU	late Aug to early Oct, 2003 and Mar to April, 2004	A	Laying hens at organic farm level	Disk-diffusion	CSLI	FQ	2	Seasonal
Talsma et al (1994)†	Cross-sectional	Netherlands	EU	1994 to 1997	H	General patient population	Disk-diffusion	CSLI	N/A	N/A	Monthly
Van Hees et al (2007)	Cross-sectional	Netherlands	EU	2000 to 2004	H	Reports of Campylobacter isolates at populations level	N/A	N/A	MC, FQ	1	Seasonal
						Salmonella spp.					
Dar et al (1992)	Cross-sectional	India	SEA	Jan to Dec, 1990	H	Patients with suspected typhoid fever	Disk-diffusion	N/A	MDR	4	Monthly
Lee et al (2016)	Cross-sectional	Korea	WP	Jun to Aug, 2014 and Dec to Feb, 2015	A	Chicken carcasses from poultry processing plants	Disk-diffusion	CSLI	AMG, MDR	5	Seasonal
						Haemophilus influenza					
Hashida et al (2008)	Cross-sectional	Japan	WP	Jul, 2004 and Feb, 2005	H	Non-vaccinated children age 1 to 6 years from care centers	Dilution	CSLI	PEN	5	Seasonal

*AMS = Antibiotic susceptibility, † = excluded studies for meta-analysis, A = animal, H = Human, 1= Spring, Summer, Autumn and Winter, 2 = Autumn and Spring, 3 = Warm and Cold, 4 = Dry and Wet, 5 = Summer/Winter, 6 = Autumn/Winter, 7= Summer/ Other months, CSLI = Clinical Laboratory Standards Institute, EUCAST = European Committee on Antibiotic Susceptibility Testing, N/A = not available. EU= Europe, SEA = South-East Asia, WP = Western Pacific, AM = Americas, EM = Eastern Mediterranean. AMG = Aminoglycosides, PEN = Penicillins, CEP = Cephalosporines, FEN = Phenicols, FQ = Fluoroquinolone, MC = Macrolides, NIF = Nitrofurans, SUL = Sulphamides, TM = Trimethoprim, TET = Tetracyclines, and MDR = Multidrug-resistant.

Appendix 42. Forest plot of seasonal resistance variation in *S. pneumoniae* and *H. influenza* isolates.

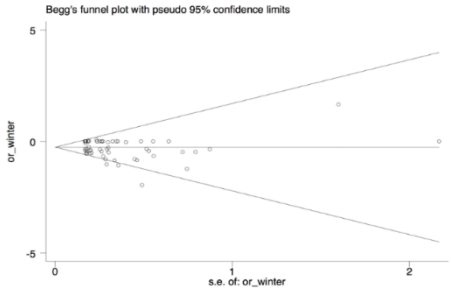


Solid line, refers to no difference in seasonal AMR between the two groups. Winter, wet and spring seasons were the reference groups = 1. n/N = number of included studies in that comparison/total number of included studies. OR, pooled odds ratio of seasonal AMR with 95% CIs.

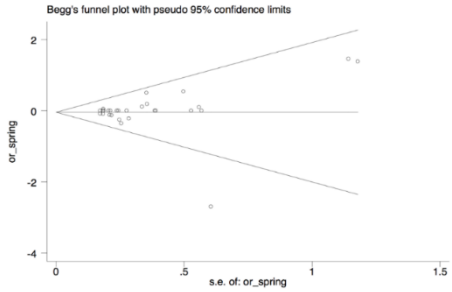
Appendix 43. Analysis of publication bias: Funnel plots

a) Funnel plots of *S. pneumonia* comparisons considering all antibiotic classes:

A1: All-seasons vs Winter

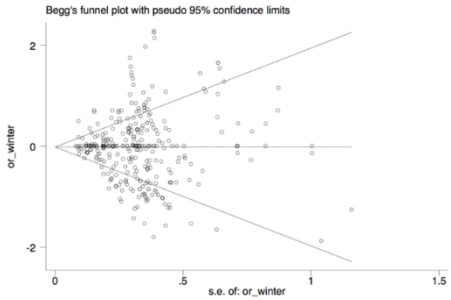


A2: Autumn vs Spring

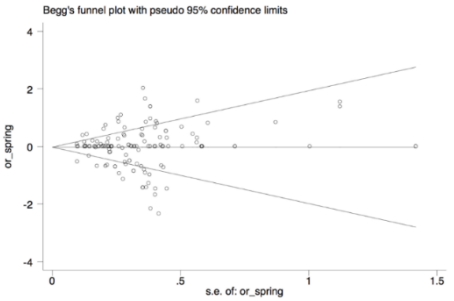


b) Funnel plots of urinary *E. coli* in humans comparisons considering all antibiotic classes:

B1: All-seasons vs Winter

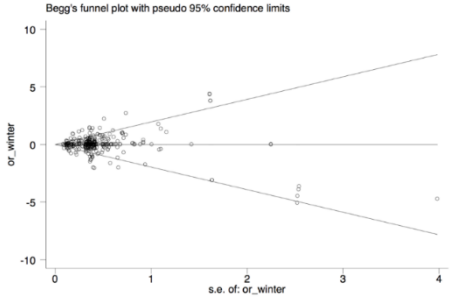


B2: Autumn vs Spring

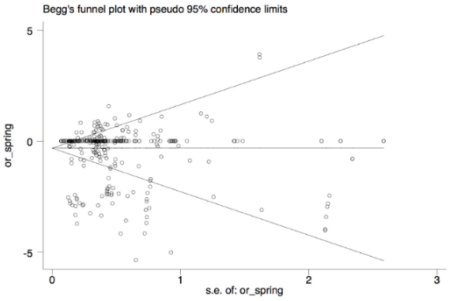


c) Funnel plots of *E. coli* from animals comparisons considering all antibiotic classes:

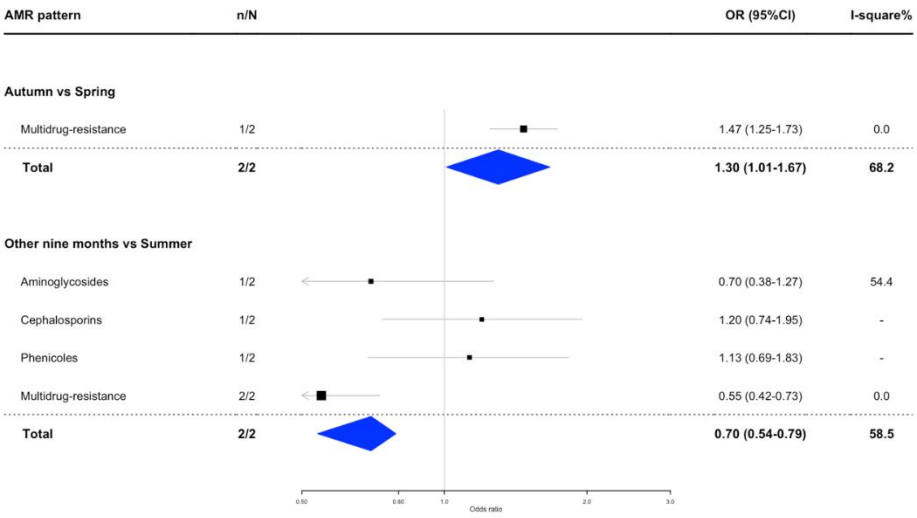
C1: All-seasons vs Winter



C2: Autumn vs Spring

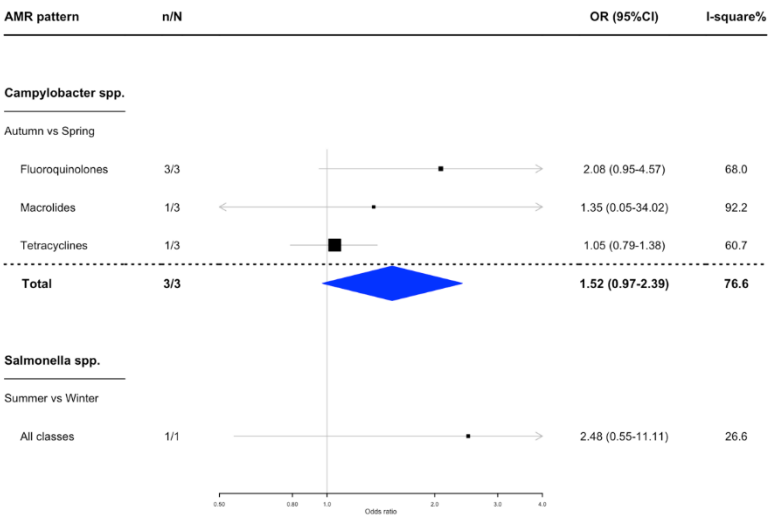


Appendix 44. Forest plot of seasonal resistance variation in urinary *E. coli*.



Solid line, refers to no difference in seasonal AMR between the two groups. Summer and spring seasons were the reference groups = 1. n/N = number of included studies in that comparison/total number of included studies. OR: pooled odds ratio of seasonal AMR with 95% CIs.

Appendix 45. Forest plot of seasonal antimicrobial resistance variation in *Campylobacter spp.* and *Salmonella spp.* isolates from healthy animals.



Solid line, refers to no difference in seasonal AMR between the two groups. Spring and winter seasons were the reference groups = 1. n/N = number of included studies in that comparison/total number of included studies. OR: pooled odds ratio of seasonal AMR with 95% CIs.

Chapter 3 Air pollution exposure and health effects in aging population

3.1 Exposure to air pollution among commuters according to mode of transport

3.1.1 Exposure to carbon monoxide, nitrogen dioxide, black carbon, fine and coarse particles according to mode of transport: systematic review and meta-analysis

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Adapted from The Lancet Public Health 2016; 2 (1), e23-e34

ABSTRACT

Introduction: Controversy exists about the differences in air pollution exposure and inhalation dose between mode of transport. We aimed to review air pollution exposure and inhaled dose according to mode of transport and pollutant and their effect in terms of years of life expectancy (YLE)

Methods: In this systematic review, we searched ten online databases from inception to April 13, 2016, without language or temporal restrictions, for cohort, cross-sectional, and experimental studies that compared exposure to carbon monoxide, black carbon, nitrogen dioxide, fine and coarse particles in active commuters (pedestrian or cyclist) and commuters using motorized transport (car, bus, massive motorized transport (MMT, i.e. train, subway or metro)) commuters. We excluded studies that measured air pollution exposure exclusively with biomarkers or on the basis of simulated data, reviews, comments, consensuses, editorials, guidelines, in-vitro studies, meta-analyses, ecological studies, and protocols. We extracted average exposure and commuting time per mode of transport and pollutant to calculate inhaled doses. We calculated exposure and inhaled dose ratios using active commuters as the reference and summarized them with medians and IQRs. We also calculated differences in YLE due to fi ne particle inhaled dose and physical activity

Results: We identified 4037 studies, of which 39 were included in the systematic review. Overall, car commuters had higher exposure to all pollutants than did active commuters in 30 (71%) of 42 comparisons (median ratio 1.22 [IQR 0.90–1.76]), followed by those who commuted by bus in 57 (52%) of 109 (1.0 [0.79–1.41]), by motorcycle in 16 (50%) of 32 (0.99 [0.86–1.38]), by a car with controlled ventilation settings in 39 (45%) of 86 (0.95 [0.66–1.54]), and by MMT in 21 (38%) of 55 (0.67 [0.49–1.13]). Overall, active commuters had higher inhalation doses than did commuters using motorized transport (median ratio car with controlled ventilation settings 0.16 [0.10–0.28]; car 0.22 [0.15–0.30]; motorcycle 0.38 [0.26–0.78]; MMT 0.49 [0.34–0.81]; bus 0.72 [IQR 0.50–0.99]). Commuters using motorized transport lost up to 1 year in YLE more than did cyclists.

Conclusion: Proximity to traffic and high air interchange increased the exposure to air pollution of commuters using motorized transport. Larger inhalation rates and commuting time increased inhaled dose among active commuters. Benefits of active commuting from physical activity are larger than the risk from an increased inhaled dose of fine particles.

INTRODUCTION

Worldwide, air pollution exposure is a public health issue associated with various health effects, including cardiovascular and respiratory disease, cancer, pregnancy complications, and adverse birth outcomes. [282] Air pollution exposure can be considered a function of the concentration of pollutants in a microenvironment and the time spent by individuals in that microenvironment. [283] Traffic emissions contribute the major part of air pollution in traffic-related microenvironments. [46] Commuters are exposed to the highest levels of pollutants,[47] which often do not meet air quality standards.

Findings from two previous systematic reviews [48,49] suggested that commuters using motorized transport (i.e., private or public car, train, metro, tram, bus, or subway) have a higher exposure to air pollution than do active commuters (i.e., pedestrian or cyclist). However, if the higher breathing parameters and trip time of an active commute than of a motorized commute are considered, inhaled and deposited doses of pollutants become higher among cyclists and pedestrians than among commuters using motorized transport.[284-287]. Authors of a systematic review [288] of health impact assessment studies concluded that consensus exists that despite the increased health risks associated with the higher inhaled dose of traffic-related pollutants among active commuters than among commuters using motorized transport, the benefits of physical activity from active commuting remain larger. Nevertheless, to our knowledge, no previous review of a modal comparison of air pollution exposure has systematically addressed the differences in inhaled dose of pollutant per mode of transport or the differential effect on years of life expectancy (YLE).

Therefore, we aimed to systematically review studies that compared air pollution exposure by mode of transport to examine differences in inhaled dose according to mode of transport and pollutant. Furthermore, we estimated the trade-off in YLE while taking into account the inhaled dose of fi ne particles and physical activity levels according to transportation

METHODS

Search strategy and selection criteria

In this systematic review, we searched ten databases (Embase, MEDLINE, Cinahl, the Cochrane Library, Web of Science, Scopus, PubMed, Google Scholar, ProQuest, and Scielo) in cooperation with a medical information specialist (WMB) to identify relevant studies that compared air pollution exposure between modes of transport among adult commuters from inception to April 13, 2016, with no language or temporal restrictions. We combined terms related to air pollution (e.g., “air pollution”) or specific air pollutants (e.g., “PM10”, “PM2.5”, or “CO”) with terms related to mode of transport (e.g., “traffic”, “subway”, “car”, “bicycle”, or “walk”). Full search strategies are provided in the
Appendix **46** (page 194).

We included all studies (cohort, cross-sectional, and experimental) that measured personal air pollution exposure while commuting by at least one active and one motorized mode of transport. We excluded studies that measured air pollution exposure exclusively with biomarkers or on the basis of simulated data, reviews, comments, consensuses, editorials, guidelines, in-vitro studies, meta-analyses, ecological studies, and protocols. We selected data only

for carbon monoxide (CO), black carbon (BC), nitrogen dioxide (NO_2), fine (particulate matter of ventilation settings (windows closed, air conditioning on or off, or air recirculation modes on or off) and those without controlled ventilation settings.

Working in pairs, three authors (MC, CMK, and KD) reviewed titles and abstracts of the entire list of studies identified by the search to select those that fulfilled the selection criteria. After initial appraisal, we retrieved full texts of selected titles. Full texts were appraised independently by two authors (MC and RF-P) to select those that fulfilled the selection criteria. Disagreements were solved through discussion and with consultation with a third independent author (OHF). We reviewed reference lists of the retrieved articles and previous systematic reviews for additional publications. We contacted experts in the field to identify additional references that should be considered. Selection criteria and study selection procedures, data extraction and quality assessment are described in detail in Appendix 47 (page 196) and Appendix 48 (page 197).

Statistical analysis

We registered extracted data from each article in a purposely designed form, including for study design, measurement period, modes of transport, monitoring device, commuting time, and number of measurements. We extracted summary and dispersion measurements of exposure according to mode of transport and pollutant. If available, we extracted summary measurements stratified by season, day, period of monitoring, type of route, and city. If more than one summary measurement was reported for the same stratum, we preferably extracted arithmetic means, then geometric means, and, finally, medians. We extracted summary measurements of inhalation and uptake dose (per h or trip), the model, and the parameters used for the estimation. We used the most complete report when multiple papers of the same study were available. We addressed quality of the studies in terms of the comparability of the exposure measured between mode of transport (i.e., time and route standards), external validity (i.e., background and meteorological conditions and commuting standards), measurement standardization, and data reporting. We used a modified version of the Newcastle-Ottawa Scale for assessing the quality of observational studies (Appendix 48, page 197).

To uniformly summarize the exposure data extracted, we standardized the units of concentrations by applying standard conversion factors.[289] We calculated the median and IQR of averages of exposure concentration per mode of transport and pollutant and the percentage of exposure averages above the European Union ambient air quality standards [290] (except for BC, because there is no standard defined). Within each study, we calculated the exposure ratio according to mode of transport using cyclists' exposure as the reference. We summarized exposure ratios as medians and IQRs per mode of transport and pollutant and calculated the percentages of ratios above 1. Also, we meta-analyzed exposure ratios using random-effects models.[291] We assessed heterogeneity with I^2 [292]. We assessed variability within studies by estimating the SE from the variance for ratios of the mean. [291] We visually inspected publication bias with funnel plots and used Egger's tests to assess asymmetry. All tests were two-tailed and we considered p values of 0.05 or less significant. For 13 studies that did not include cyclists, we used pedestrians' exposure as the reference (reported separately to the studies that included cyclists).

For two additional studies, we used pedestrians' exposure as the reference because for some comparisons in these studies only comparisons with pedestrians were possible.

We calculated inhaled doses of pollutants (inhaled amount per trip) as the average exposure concentration (reported by authors) multiplied by minute ventilation (m^3/h) multiplied by trip time (min; reported by authors) multiplied by a conversion factor, if applicable. We used minute ventilation as suggested by the US Environmental Protection Agency [293] for each mode of transport (Appendix 49, page 198). Then, we calculated the inhalation dose ratio between mode of transport using the inhaled dose of cyclists (or pedestrians, accordingly) as the reference. We summarized ratios as medians and IQRs.

Finally, we estimated the trade-off in YLE due to fine particle inhaled dose and physical activity, according to mode of transport. We used fine particles because it has the most consistent evidence for all-cause mortality risk.[294] We calculated the loss or gain of YLE due to fine particle inhaled dose and physical activity levels for a person commuting by a given mode of transport. We based calculations on fine particle exposure and a set of assumptions regarding weekly levels of physical activity per mode of transport (Appendix 49, page 198). We built the assumptions for a given scenario where one hypothetical person spends 7 days in four microenvironments: at work, at home, sleeping, and commuting by one of the modes of transport over a 7 km route twice a day. We did a sensitivity analysis for the person commuting over a 3.5 km route. We calculated the net gains or losses by comparing each mode of transport to a reference scenario (cyclists or pedestrians, accordingly) and summarizing them as medians and IQRs. A detailed description of the procedures is provided in the Appendix 50 (page 199). All analyses were performed in Stata (version 14.0).[72]

RESULTS

After screening 4,037 potentially relevant studies, we retrieved and assessed 228 full texts, of which 54 fulfilled the initial selection criteria and 39 reported on exposure to the pollutants of interests and were included in the systematic review (Table 19 (page 185) and Figure 12 (page 190)); we excluded three duplicate studies from the systematic review but included them in the table of study characteristics). The studies were done in European (n=24), west Pacific (n=11), American (n=3), and Southeast Asian (n=1) countries. Further characteristics are provided in the Appendix 51 (page 200).

Irrespective of pollutant, car commuters had higher air pollution exposure than did active commuters in 30 (71%) of 42 comparisons (median 1.22 [IQR 0.90–1.76]), followed by those who commuted by bus in 57 (52%) of 109 (1.0 [0.79–1.41]), by motorcycle in 16 (50%) of 32 (0.99 [0.86–1.38]), by a car with controlled ventilation settings in 39 (45%) of 86 (0.95 [0.66–1.54]), and by MMT in 21 (38%) of 55 (0.67 [0.49–1.13]). (Appendix 53-54, pages 215-216). We observed differences in exposure ratio per mode of transport and pollutant (Figure 13, page 191 and Appendix 55, page 217). We obtained similar estimations by meta-analyzing the exposure ratios, but we identified a large heterogeneity (higher than 90% in most comparisons; Appendix 56 (page 220)). We did not find evidence of publication bias (Appendix 60, page 232).

Inhalation or uptake pollutant dose was available in 12 of the studies included in the systematic review (Appendix 57, page 222). Uptake dose was estimated as the deposition of

pollutants in the airway. VE as breathing parameter was heterogeneous across studies. Cyclists followed by pedestrians had the highest uptake dose of pollutants. Minute ventilation as a breathing parameter was heterogeneous across studies. Five studies [284,286,287,295,296] used surrogates of activity intensity to derive minute ventilation, whereas the remaining studies [297-302] used published parameters. In Figure 14 (page 192), we compare the distribution of exposure and inhaled dose ratios on the basis of our calculation of inhalation dose. For all motorized modes of transport, the median of the inhaled dose ratio was lower than the exposure ratio. Active commuters had a higher inhalation dose of pollutants than did commuters who used motorized transport (median ratio car with controlled ventilation settings 0.16 [IQR 0.10–0.28]; car 0.22 [0.15–0.30]; motorcycle 0.38 [0.26–0.78]); MMT 0.49 [0.34–0.81]; bus 0.72 [0.50–0.99]) due to increased respiratory parameters. A ratio of inhaled dose lower than the ratio of exposure, with respect to the y axis, suggests that the relative inhaled dose of pollutant among cyclists, in the denominator, is higher than their relative exposure. We observed small differences between exposure and inhaled dose ratios for the comparison of pedestrians with cyclists.

Figure 15 (page 193) shows the difference in YLE due to fine particle exposure and physical activity per mode of transport. Median losses in YLE were up to 1 year larger among commuters using motorized transport than among cyclists because of less physical activity, despite the lower inhaled dose of fine particles (Appendix 58, page 228) using motorized transport compared with cyclists because of the longer commuting time of pedestrians than of cyclists. In a sensitivity analysis, we tested varying commuting times and consistently observed YLE gains in favor of active transport (Appendix 59, page 230), as the difference between life-years lost due to fine particle exposure and life-years gained due to physical activity remained roughly the same for a 3.5 km route as for a 7 km route with the relative risk of physical activity of 0.80[303] (age 20–30 years: median –1.50 years [IQR –1.69 to –1.08]; age 40–64 years: –1.26 [–1.44 to –0.77]; age ≥65 years: –0.59 [–0.82 to –0.26]).

Regarding quality of studies, comparability of exposure between mode of transport was high (at least three stars according to the Newcastle-Ottawa Scale) in 16 experimental studies (**Appendix 52**, page 214). We noted a very low comparability in 13 experimental studies (two or fewer stars). Ten studies were observational, which aimed to measure rather than compare exposure between modes of transport. Irrespective of pollutant, exposure levels to CO, NO_2, and fine and coarse particles were above ambient air quality standards [290] among cyclists in 50 (56%) of 89 exposure averages, among pedestrians in 22 (46%) of 48, among those who commuted by car in 22 (55%) of 40, among those who commuted by a car with controlled ventilation settings in 45 (52%) of 87, among those who commuted by MMT in 25 (48%) of 52, and among those who commuted by motorcycle in 24 (65%) of 37. The distribution of pollutant exposure level per mode of transport is shown in the appendix. Fine particles were more frequently above ambient air quality standards than were the other pollutants (155 [83%]) of 187 exposure averages). Detailed information about ascertainment of air pollution exposure was provided in 33 (85%) studies. Sample size or dispersion measurements were not reported in four (10%) studies. Complete reporting of background and meteorological conditions was found in 21 (54%) studies. Standardization of all modes of transport measured and reporting of it was found in 20 (51%) studies.

DISCUSSION

Car and bus commuters had the highest levels of air pollution exposure, followed by those commuting by a car with controlled ventilation settings, cyclists, and pedestrians, whereas the lowest was experienced by MMT commuters and motorcyclists. Cyclists, followed by pedestrians, had the highest inhalation and uptake dose of pollutants because of increased minute ventilation and trip time. Compared with people commuting by car, by a car with controlled ventilation settings, and by motorcycle, the negative effect on YLE of increased inhaled dose did not overcome the positive effect of physical activity when commuting actively. Commuter exposure can be reduced by increasing the distance from traffic emissions, reducing air exchange with use of ventilation settings in motorized mode of transport, and choice of routes with l ow emissions and high dispersion of pollutants (e.g., parks), as well as eff orts to reduce local and regional emissions. We observed a large heterogeneity across the evidence. Further research should consider inhaled and uptake dose while commuting to address air pollution effects on health.

In agreement with previous systematic reviews [48,49] the differences in air pollution exposure between mode of transport in this study can be explained mainly by the position of the commuter with respect to the gradient of pollutant concentration [285,295,299,301,304-307] and the commuter's microenvironment sensitivity to surrounding pollutant concentration. The gradient of pollutant concentration depends on the rate of emissions and the dispersion and decay of pollutants in the air,[282,308] which is influenced, among others, by meteorological[309,310] and route [295,301,310] attributes. The close contact of commuters using motorized transport to the traffic line explains their higher levels of air pollution exposure than those for active commuters.[48,49,302] Indeed, bus commuters and cyclists have lower exposure when they travel via separated bus lanes or cycle routes or travel close to kerb than when they do not.[48,283,295,302,306] Also, pedestrians, who usually travel on the pavement, have a lower exposure than do cyclists.[49,306] We observed the lowest exposure among MMT commuters, except for exposure to BC, most probably because they often travel on railways or through tunnels separated from ground traffic.[297] The main sources of exposure for MMT commuters involve walking stages, when approaching the stations, [304] and while waiting inside the stations.[48,301,311] Commuters using ground motorized transport (i.e., car and bus) on overcongested routes with high emission levels had high pollutant exposure because of high emissions, long trip time, and frequent idling.[48,49,300] Additionally, canyon-like street configuration reduces the dispersive and catalytic action of environmental and meteorological factors, thus trapping the pollutants.[285,301,312]

Commuters' microenvironment sensitivity to surrounding pollutants depends on the rate of air interchange of the microenvironment. Active commuters, and commuters using motorized transport with open windows, have a high rate of air interchange, increasing their exposure to high pollutant concentrations [301,311,313] and pollutants hotspots like intersections and traffic lights.[297,301,313-316] This leads to a pattern of concentration peaks in active commuters' exposure, whereas commuters using motorized transport have a constant concentration exposure. Physical barriers like controlled ventilation settings in cars help to extract and filter fine and coarse particles from the vehicle microenvironment.[311,313,317,318] Moreover, physical barriers make a large difference in highly contaminated environments,[300] where both commuters using motorized transport and active commuters have similar exposure levels to fi ne and coarse particles.[300,318,319]

Nevertheless, people commuting with a car with controlled ventilation settings had an increased exposure to CO,[287,300,302,306,317,320] attributed to self-pollution due to filtration of surrounding emissions and products from engine combustion.

Commuters' microenvironment sensitivity to traffic related air pollution is largely determined by built environment attributes that increase their proximity to traffic emissions, by an absence of physical barriers like ventilation settings, and by increased respiratory parameters leading to increased airway deposition of pollutants. Therefore, active commuters might benefit from air pollution forecasting and on-road advice to actively protect themselves from exposure—e.g., by choosing uncongested routes. Incentives to shift from private motorized to active and public transport should be accompanied by urban planning standards and policies, such as dedicated lanes, separated cycle routes and pavements, improved ventilation in vehicles and at stops and stations for public transport, a boosted transition to environmentally friendly vehicles, and other eff orts aimed to reduce both combustive and non-combustive traffic-related emissions.[46] Moreover, large societal benefits are obtained from an active commuter-friendly environment, which affects additional traffic-related risk factors, like noise, traffic injuries, quality of life, and social cohesion, among others.[321,322]

By contrast with overall exposure, the inhaled dose of pollutants was higher among active commuters than among commuters using motorized transport. This finding is mainly explained by the increased minute ventilation, leading to increased air volume and frequency of breathing, deeper inhalation, and larger inhalation of pollutants in active commuters than in commuters using motorized transport.[284] Active commuters, especially pedestrians, also have a longer trip time than do commuters using motorized transport and thereby have increased exposure time.[284,285,296,300,318]

In agreement with previous studies,[288] the large losses in YLE among commuters using motorized transport due to less physical activity than in active commuters were not offset by the modest gains due to lower inhaled fi ne particles. YLE losses of commuting by car, by a car with controlled ventilation settings, and by motorcycle were larger than were the losses observed among public transport commuters (bus and MMT). This finding can be explained by the contribution of physical activity during the active stages of the trip, like when approaching stations or stops, despite additional sources of air pollution inhalation.[286,295,296,323]

To our knowledge, this study is the first systematic review of air pollution exposure and inhaled dose according to mode of transport. Our findings are in agreement with the systematic review by Mueller and colleagues,[288] which included 30 studies that assessed the net health benefits of active transport through health impact assessment, 17 of which addressed the negative effect of air pollution exposure. Nevertheless, none of the studies included by Mueller and colleagues [288] were included in our study as they did not comply with our selection criteria and research question. Also, all but one study analyzed by Mueller and colleagues [288] were done with data from European countries, the USA, New Zealand, and Australia, with mostly indirect air pollution exposure levels, and with heterogeneous assumptions and modelling frameworks. By contrast, we used fine particle exposure levels purposely measured for modal comparison in 23 studies and applied standard assumptions for inhaled and physical activity doses. Also, because of our selection criteria, we included further settings, also adding Asian and west Pacific cities, with

higher ambient air pollution than in the USA and most European countries. Under very high air pollution concentrations, the trade-off between air pollution exposure risks and active transport benefits has been suggested to not benefit active transport anymore.[324] Yet, our findings are consistently in favor of active transport.

Limitations of our analyses deserve attention. First, the external validity of the studies included in this report was affected by the heterogeneity of settings and methodological approaches. Nevertheless, on the basis of the observed heterogeneity, this systematic review encompasses various environmental conditions and makes our findings generalizable. Second, despite our comprehensive search, only eight studies were done in countries other than European and North American countries (China,[300,301,311,314] India,[325] Taiwan,[326] or Vietnam,[317] and Chile[304]). Although we did not find evidence of publication bias, these regions are under-represented in our review. Third, we did not take into account the additional toxicity of other pollutants. However, fine particle levels are a strong marker of traffic related air pollution, and we found that fine particles were more frequently above ambient air quality standards than were the other pollutants. Fourth, we assumed a rather unlikely scenario of pedestrians commuting daily for longer than 2 h. Walking is an important source of physical activity, and a large proportion of active commuters are pedestrians.[327] With a sensitivity analysis, we tested varying commuting times and consistently observed YLE gains in favor of active transport. Fifth, we focused on the long-term mortality effect of physical activity and fine particle exposure. However, examination of other short-term and long-term health effects would be beneficial, as well as other exposures, like noise and traffic injuries. Findings from previous studies suggest that regardless of the expected increment of traffic injuries along the shift from motorized to active commuting, the reduction in motorized traffic volume and the increment of an active commuter friendly environment would contribute to a reduction of the burden of traffic incidents.[288] Finally, we assumed a total replacement of mode of transport at each scenario modelled and a linear association of fine particle exposure and physical activity with mortality, by contrast with previous findings.[288,303,321] However, our approximation is intended to build on previous efforts to summarize air pollution exposure according to mode of transport to examine the effect of commuting parameters on inhaled doses and potential population-level effects. Health benefits strongly depend on specific local attributes,[288,321] such as the offer of mode of transport, apportionment of emissions, and built environment attributes, besides local policies and normative behavior. Decision making based on health impact assessment should take into account such local attributes.

Table 19. General characteristics of the studies

Author, year	Pollutants	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
Adams, 2001 [307] Adams, 2002 [328]	$PM_{2.5}$	Gravimetric analysis	🚲	🚗 🚌 🚆	Three week measurements in July 1999 and in February 2000	London; UK
Boogaard, 2009 [305]	$PM_{2.5}$	Light scattering	🚲	🚗	Eleven days (except Fridays) in late August-October 2006	[Apeldoorn, Delft, Den Bosch, Eindhoven, Groningen, Haarlem, Maastricht, Nijmegen, The Hague, Utrecht, Zwolle]; Netherlands
Brauer 1999 [329]	Particles concentration [≥1.0-<0-5.0 µg; ≥5.0 µg]	Light scattering	🚲 🚶	🚗 🚌 [Bus and Seabus] 🚆	May-October 1999	Vancouver; Canada
Briggs, 2008 [318]	PM_{10}- $PM_{2.5}$ $PM_{2.5\text{-}1}$	Light scattering	🚶	🚗	Seven weekdays during May and June 2005	London; UK
Chertok, 2004 [330]	NO_2	NS	🚲 🚶	🚗 🚌 🚆	13-27 September 2002	Sydney; Australia
De Bruin, 2004 [331]	CO	Electrochemical sensor	🚶	🚗 🚆 🚲 [M][Motorbike]	1997/1998 (1-year period)	Milan; Italy
de Nazelle, 2012 [295]	$PM_{2.5}$ CO BC	$PM_{2.5}$: Light scattering and gravimetric analysis CO: Electrochemical monitor BC: Optical sensor (aethalometer)	🚲 🚶	🚗 🚌	Four weeks beginning May 28th 2009	Barcelona; Spain
Dirks, 2012 [297]	CO	Electrochemical monitor	🚲 🚶	🚗 🚌 🚆 🚲 [M][Motorbike]	November 8th-December 17th; 2010	Auckland; New Zealand
Dons 2011 [298] Dons 2012[299]	BC	Aethalometer	🚲 🚶	🚗 [Driver and passenger] 🚆 [Train, light rail, metro] 🚌	16 participants only during summer/2010, eight of them plus 38 new volunteers were	Mol, Belgium

Author, year	Pollutants	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
					measured during winter 2010-2011	
Dor, 1995 [320]	CO	Electrochemical monitor	🚶	🚗 🚌	October1991-September1992	Paris; France
Duci, 2003 [332]	CO	Electrochemical monitor	🚶	🚗 🚌 🚆 [Electric train and rail]	Summer 1998 and November1998-February1999	Athens; Greece
Farrar, 2001 [312]	NO_2	Adsorbance (Spectrophotometer)	🚲	🚗 🚌	August-September 2000	Perth; Australia
Gee, 1999 [333]	$PM_{4.0}$	Gravimetric analysis	🚲	🚌	NS	Manchester; UK
Georgoulis, 2002 [334]	CO	Electrochemical monitor	🚲 🚶	🚗 🚌 🏍 [M]	February1997-January1998 February1997-March1998 June1997-June1998 March1997-January1998 October1996-December1997	Basel; Switzerland Athens; Greece Prague; Czeck Republic Milan; Italy Helsinki; Finland
Goel 2015 [325]	$PM_{2.5}$	Light scattering	🚲 🚶	🚗 [Open and closed windows] 🚌 [Open and closed windows] 🚆 🏍 [M][Auto-rickshaw and motorized 2-wheeler]	41 days between January – May 2014	Delhi; India
Gulliver, 2004 [319]	PM_{10} $PM_{2.5}$	Light scattering	🚶	🚗	Pilot: July 1999. Route 1: November/1999, March 2000. Route 2: April 2000.	Northampton; UK
Gulliver, 2007 [313]	TMP- PM_{10} PM_{10}- $PM_{2.5}$; $PM_{2.5}$-PM_1	Light scattering	🚶	🚗	10 different days between January-March 2005	Leicester; UK
Huang, 2012 [300]	$PM_{2.5}$ CO	$PM_{2.5}$: Spectrometer and gravimetric analysis	🚲	🚗 🚌	December2010-February2011.	Beijing; China

Author, year	Pollutants	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
		CO: Electrochemical sensor				
IntPanis, 2010 [284]	PM_{10} $PM_{2.5}$	Light scattering	🚲	🚗	8 days, June 2009	[Brussels, LLN, Mol]; Belgium
Kaur, 2005 [306] Kaur, 2009 [310]	$PM_{2.5}$ CO	$PM_{2.5}$: Gravimetric analysis CO: Electrochemical monitor	🚲 🚶	🚗 🚌	Four-week field campaign 28April/23May 2003	London; UK
Kingham 2013 [335]	PM_{10} $PM_{2.5}$ CO	PM_{10} & $PM_{2.5}$: Spectrometer CO: Electrochemical monitor	🚲	🚗 🚌	Weekdays between February 26th and March 26th 2009	Christchurch, New Zealand
Li, 2015 [301]	BC	Optical sensor (aethalometer)	🚲 🚶	🚗 🚌 🚇	6 non-rainy working days - August 2014	Shangai; China
Liu, 2015 [326]	PM_{10} $PM_{2.5}$	Light scattering	🚶	🚗 🚌 🚇	January-March between 2012-2014	Taipei; Taiwan
McNabola, 2008 [285]	$PM_{2.5}$	Gravimetric analysis	🚲 🚶	🚗 🚌	January2005-June2006	Dublin; Ireland
Morabia, 2009 [336]	$PM_{2.5}$	Light scattering	🚶	🚗 🚇	October2007-February2008	New York; USA
Moreno 2015 [337]	BC $PM_{2.5}$ CO	$PM_{2.5}$: Gravimetric analysis and light scattering BC: Optical sensor (aethalometer) CO: Electrochemical monitor	🚶	🚇[Tram] [Subway] 🚌	39 weekdays between October-November 2014	Barcelona; Spain
Nyhan, 2014 [286]	PM_{10} $PM_{2.5}$	Light scattering	🚲 🚶	🚌 🚇	NS	Dublin; Ireland
Onat, 2013 [316]	$PM_{2.5}$	Light scattering	🚶	🚌 🚇 🚗 [A/C on/off]	October 8th, November 16th 2008	Istambul; Turkey

Author, year	Pollutants	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
Ramos 2016 [302]	PM_{10}, PM_4, $PM_{2.5}$, PM_1 CO	PM: Light scattering CO: Electrochemical monitor	🚲	🚗 🚌 Metro-bus 🚲 [M]	December 2013 to March 2014; 15 non-rainy days	Lisbon; Portugal
Rank, 2001 [338]	Particles	Gravimetric analysis	🚲	🚗	18 June, 3 August 1998	Copenhagen; Denmark
Saksena, 2008 [317]	PM_{10} CO	PM_{10}: Nephelometer (Light scattering) CO: Electrochemical monitor	🚶	🚗 🚌 🚲 [M][Mobibike]	October 2006	Hanoi; Vietnam
Suárez, 2014 [304]	$PM_{2.5}$	Light scattering	🚲	🚗 🚌	Winter-spring 2011, summer-autumn 2012	Santiago de Chile; Chile
Van Wijnen, 1995 [287]	CO NO_2	CO: Gas chromatography NO_2: Adsorbance (Spectrophotometer)	🚲 🚶	🚗	January, May, August 1990	Amsterdam; The Netherlands
Vellopolou, 1998 [339]	CO	Electrochemical sensor	🚶	🚗 🚌 🚲 [M][Motorbike]	Five-months period beginning December1992	Athens; Greece
Vouitsis, 2014 [340]	PM_{10} $PM_{2.5}$ BC	PM: Light scattering BC: Optical sensor (aethalometer)	🚲	🚌 🚗 [Windows open and closed]	April 2011	Thessaloniki; Greece
Williams 2016 [341]	BC	Aethalometer	🚲 🚶	🚗[Windows open and closed] 🚌 🚆	April to October 2016	Brisbane/Eden; Australia
Wu, 2013 [311]	$PM_{2.5}$	Light scattering	🚲 🚶	🚗 🚌 🚆 🚲 [V]	5-10 March, 28March-3April, 5-11July 2011	Foshan; China
Yan, 2015 [314]	$PM_{2.5}$	PMC: Light scattering $PM_{2.5}$: Gravimetric analysis	🚶	🚌 🚆 [A/C on and off]	10-23 December 2011	Beijing; China

Author, year	Pollutants	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
Zuurbier, 2010 [296]	PM_{10} $PM_{2.5}$	PM_{10}: Gravimetric analysis $PM_{2.5}$: Light scattering	🚲 [Low and high traffic route]	🚌🚎[Diesel and electric] 🚗 [Diesel and gasoline]	June 2007-June 2008	Arnhem; Netherlands

🚲 Cyclists 🚶 Pedestrians 🚗 Car 🚌 Bus 🚎 Massive motorized transport 🚲 [M] Motorcycle. CO: Carbon monoxide, BC: Black carbon, NO_2: Nitrogen dioxide. PM: Particulate matter. A/C: Air conditioned. NS: Not specified.

Figure 12. Study selection

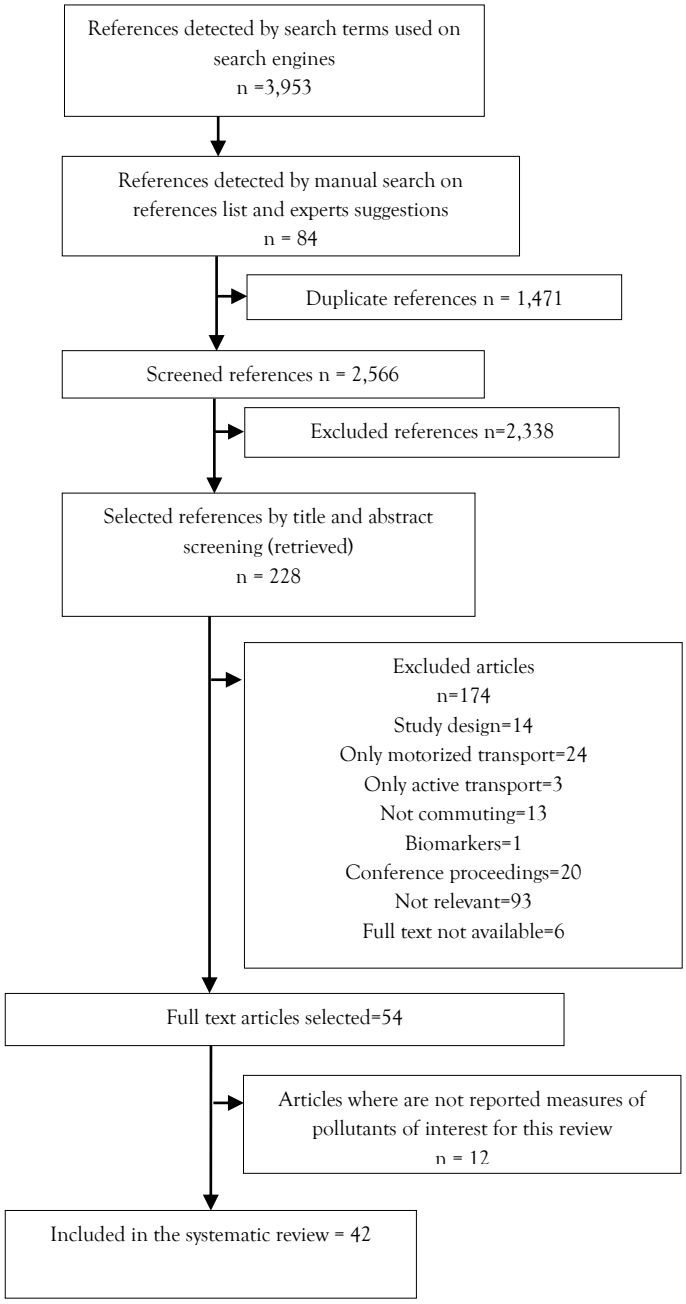
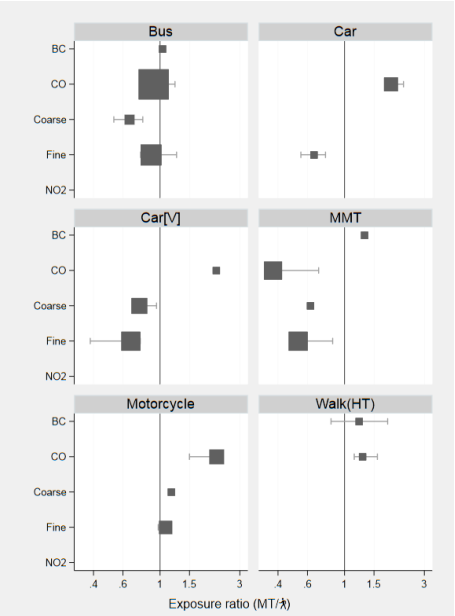
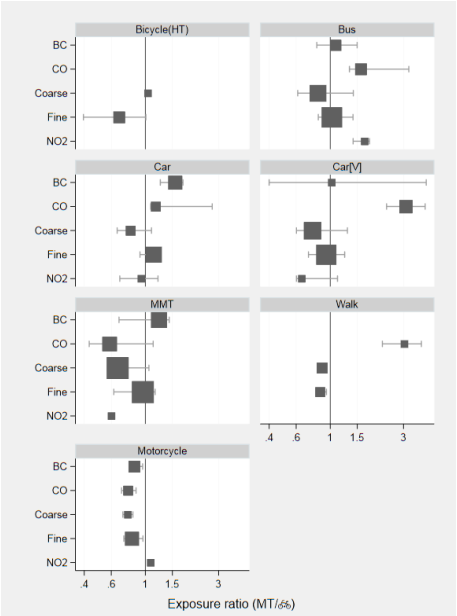


Figure 13. Distribution of ratio of pollutants exposure level among modes of transport, compared to (A) cyclists or (B) pedestrians' exposure

(A) Reference: Cyclists

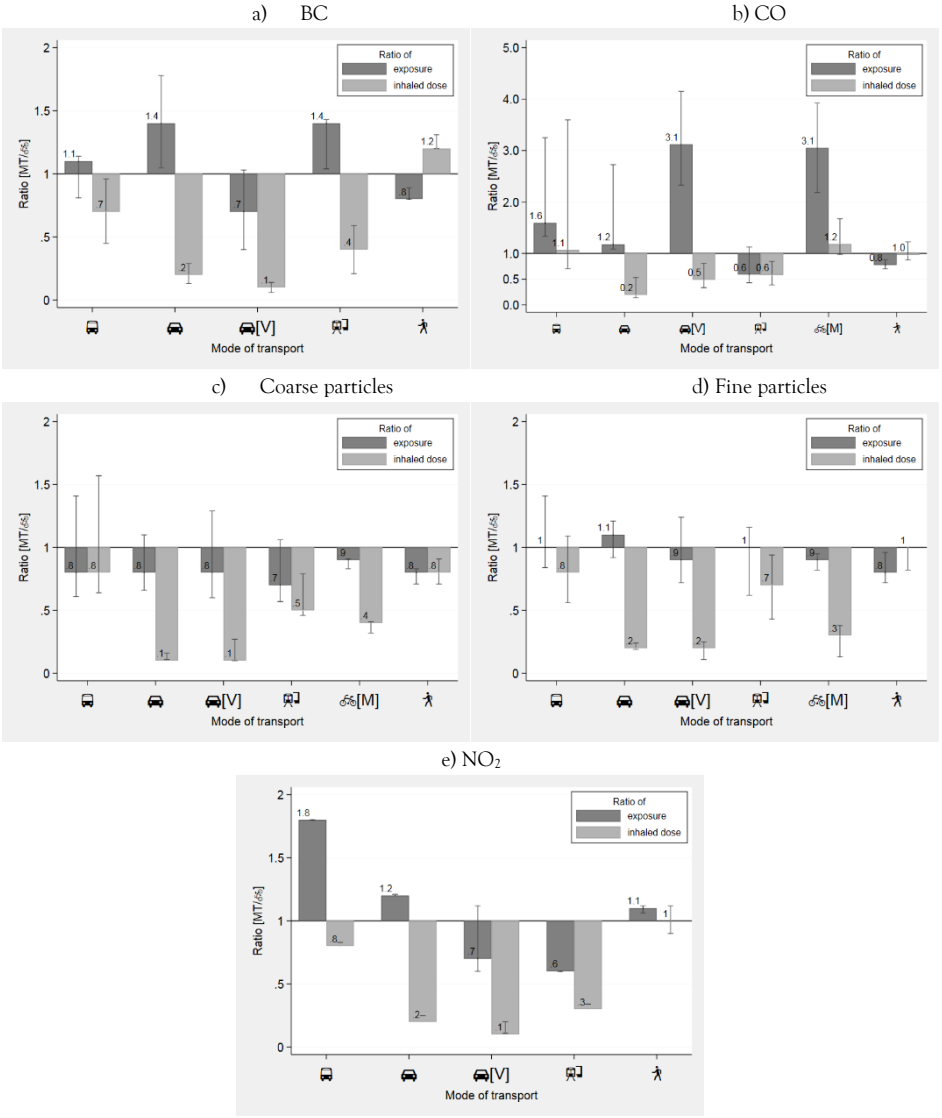


(B) Reference: Pedestrians



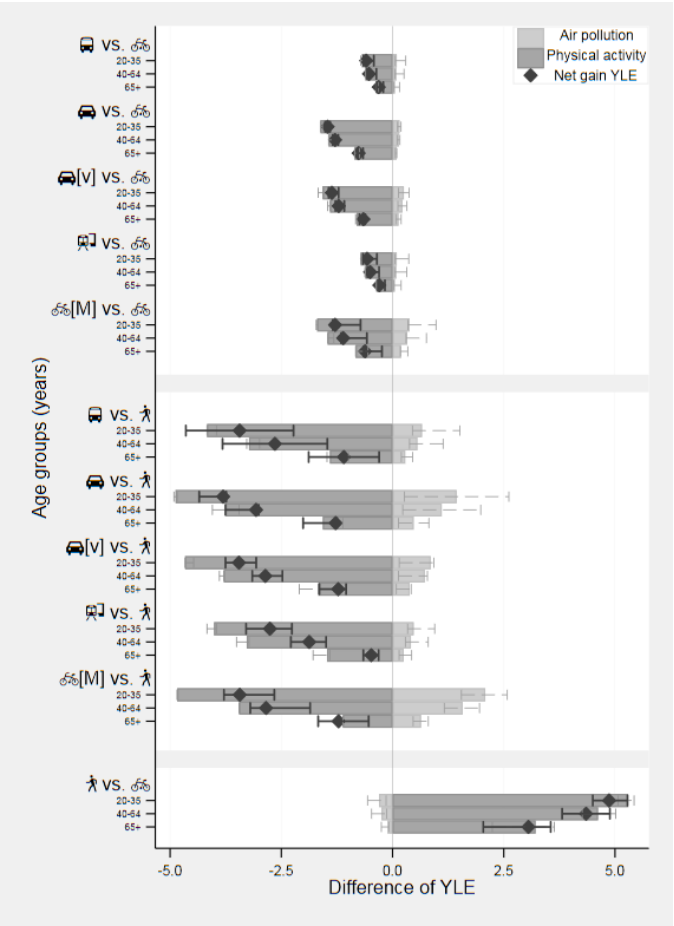
The squares size is weighted according to the number of comparisons used to calculate the median. The exact values of median and interquartile range are provided in Appendix 53 (page 215). 🚲 Cyclists 🚶 Pedestrians Car: Car driven without controlled ventilation settings Car[V] Car driven with controlled ventilation settings MMT: Massive motorized transport CO: Carbon monoxide, BC: Black carbon, Coarse: Coarse particles. Fine: Fine particles. NO_2: Nitrogen dioxide. Bicycle/Walk (HT) Comparison is cyclists/pedestrians in high traffic route (HT) vs. cyclists/pedestrians in low traffic route.

Figure 14. Comparison of ratio of exposure vs. ratio of inhaled dose to pollutants according to MT and pollutant (reference is cyclists)



Numbers in top of bars correspond to median of exposure/inhaled dose ratios. Cyclists Pedestrians Car driven without controlled ventilation settings [V] Car driven with controlled ventilation settings Bus Massive motorized transport [M] Motorcycle.

Figure 15. Gains of years of life expectancy per age-group due to air pollution exposure and physical inactivity, compared between any MT vs. bicycle (A) or walk (B) commuting of a 7km route per week*



YLE: Years of life expectancy. Cyclists Pedestrians Car driven without controlled ventilation settings [V] Car driven with controlled ventilation settings Bus Massive motorized transport [M] Motorcycle. Values of medians (bars) and interquartile ranges are provided in Appendix 58 (page 228).

SUPPLEMENTARY MATERIAL

Appendix 46. Search terms per search engine

Ten databases were searched in cooperation with a medical information specialist to identify relevant studies (Embase.com, Medline (Ovid), Cinahl (EBSCOhost), the Cochrane Library, Web of Science, Scopus, PubMed, Google Scholar, ProQuest and Scielo)

Embase.com: ('air pollution'/de OR 'air pollutant'/exp OR 'air pollution indicator'/de OR 'environmental exposure'/de OR 'exhaust gas'/de OR acetylene/de OR benzene/de OR '1, 3 butadiene'/de OR 'carbon monoxide'/de OR dust/de OR ethane/de OR ethylbenzene/de OR ethylene/de OR 'airborne particle'/de OR 'nitrogen dioxide'/de OR 'particulate matter'/de OR toluene/de OR xylene/de OR 'polycyclic aromatic hydrocarbon'/exp OR combustion/de OR 'black carbon'/de OR 'volatile organic compound'/de OR ((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEXT/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEXT/1 dioxide*) OR pm1 OR 'pm2 5' OR pm10 OR 'pm 1' OR 'pm 2 5' OR 'pm 10' OR soot OR toluene* OR xylene* OR ufp* OR 'black carbon' OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex):ab,ti) AND ('traffic and transport'/de OR 'motor vehicle'/exp OR 'railway'/de OR traffic/de OR bicycle/de OR 'car driving'/exp OR 'motorized transport'/de OR walking/de OR travel/de OR pedestrian/de OR (traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) NEAR/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*):ab,ti) AND (commut* OR telecommut* OR ((travel) NEAR/3 (work*)))

Medline (OvidSP): ("air pollution"/ OR exp "Air Pollutants"/ OR "environmental exposure"/ OR "Vehicle Emissions"/ OR acetylene/ OR benzene/ OR "Benzene Derivatives"/ OR "butadienes"/ OR "carbon monoxide"/ OR dust/ OR ethane/ OR ethylenes/ OR "nitrogen dioxide"/ OR "particulate matter"/ OR toluene/ OR "Polycyclic Hydrocarbons, Aromatic"/ OR ((air ADJ3 (clean*)) OR ((environment* OR personal) ADJ3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon ADJ monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* ADJ3 matter*) OR (nitro* ADJ dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic ADJ3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile ADJ3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex).ab,ti.) AND ("Transportation"/ OR exp "Motor Vehicles"/ OR "railroads"/ OR Bicycling/ OR walking/ OR travel/ OR (traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) ADJ3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*).ab,ti.) AND (commut* OR telecommut* OR ((travel) ADJ3 (work*)))

Cinahl (ebsco): (MH "air pollution+" OR MH "Air Pollutants+" OR MH "environmental exposure+" OR MH "Benzene Derivatives+" OR MH "carbon monoxide+" OR MH dust+ OR MH ethylenes+ OR MH "particulate matter+" OR MH toluene+ OR MH "Polycyclic Hydrocarbons, Aromatic+" OR ((air N3 (clean*)) OR ((environment* OR personal) N3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon N1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* N3 matter*) OR (nitro* N1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic N3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile N3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND (MH "Transportation+" OR MH "Motor Vehicles+" OR MH "railroads+" OR MH cycling+ OR walking+ OR travel+ OR (traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) N3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*)) AND (commut* OR telecommut* OR ((travel) N3 (work*)))

Cochrane: (((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEXT/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEXT/1 dioxide*) OR pm1 OR 'pm2 5' OR pm10 OR 'pm 1' OR 'pm 2 5' OR 'pm 10' OR soot OR toluene* OR xylene* OR ufp* OR 'black carbon' OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex):ab,ti) AND ((traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) NEAR/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*):ab,ti) AND (commut* OR telecommut* OR ((travel) NEAR/3 (work*)))

Web-of-science: TS=((((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEAR/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEAR/1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND ((traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) NEAR/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*)) AND (commut* OR telecommut* OR ((travel) NEAR/3 (work*))))

Scopus: TITLE-ABS-KEY((((air W/3 (clean*)) OR ((environment* OR personal) W/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon W/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* W/3 matter*) OR (nitro* W/1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic W/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile W/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND ((traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR

underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) W/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*)) AND (commut* OR telecommut* OR ((travel) W/3 (work*))))

PubMed: ("air pollution"[mh] OR "Air Pollutants"[mh] OR "environmental exposure"[mh] OR "Vehicle Emissions"[mh] OR acetylene[mh] OR benzene[mh] OR "Benzene Derivatives"[mh] OR "butadienes"[mh] OR "carbon monoxide"[mh] OR dust[mh] OR ethane[mh] OR ethylenes[mh] OR "nitrogen dioxide"[mh] OR "particulate matter"[mh] OR toluene[mh] OR "Polycyclic Hydrocarbons, Aromatic"[mh] OR ((air AND (clean*[tiab])) OR ((environment*[tiab] OR personal) AND expos*[tiab]) OR pollut*[tiab] OR microenvironment*[tiab] OR exhaust*[tiab] OR emission*[tiab] OR acetylene*[tiab] OR benzene*[tiab] OR butadiene*[tiab] OR (carbon ADJ monoxide*[tiab]) OR co OR carbonmonoxide*[tiab] OR Coarse OR dust OR ethane OR ethylbenzene*[tiab] OR ethylene*[tiab] OR ethene*[tiab] OR particle*[tiab] OR (particul*[tiab] AND matter*[tiab]) OR (nitro*[tiab] ADJ dioxide*[tiab]) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene*[tiab] OR xylene*[tiab] OR ufp*[tiab] OR "black carbon" OR (polycyclic AND (hydrocarbon*[tiab] OR carbon*[tiab])) OR pah OR pahs OR combust*[tiab] OR (volatile AND compound*[tiab]) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND ("Transportation"[mh] OR "Motor Vehicles"[mh] OR "railroads"[mh] OR Bicycling[mh] OR walking[mh] OR travel[mh] OR (traffic*[tiab] OR subway*[tiab] OR tram OR tramway*[tiab] OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail*[tiab] OR automobile*[tiab] OR bicycle*[tiab] OR motorcycle*[tiab] OR cycling OR walk*[tiab] OR bus OR busses OR buses OR foot OR bike OR transport*[tiab] OR vehicle*[tiab] OR ((commut*[tiab]) AND (mode OR type OR way OR public OR strateg*[tiab])) OR travel*[tiab] OR pedestrian*[tiab] OR passenger*[tiab] OR driver*[tiab])) AND (commut*[tiab] OR telecommut*[tiab] OR ((travel) AND (work*[tiab]))) AND publisher[sb]

Google Scholar: Pollution | pollutant | pollutants | exhaust | "particulate matter | pah | pahs | combustion | "black carbon" | vacs traffic | vehicle | railway | bicycle | car | driving | motorized | walking | pedestrian | pedestrians | subway | metro | underground | train | cycling commuters | commuting | commuter

Proquest: (ti(Pollution OR pollutant OR exhaust OR "particulate matter" OR pah OR pahs OR combustion OR "black carbon" OR vac OR vacs OR carbonmonoxide OR Coarse OR dust OR btex) OR ab(Pollution OR pollutant OR exhaust OR "particulate matter" OR pah OR pahs OR combustion OR "black carbon" OR vac OR vacs OR carbonmonoxide OR Coarse OR dust OR btex)) AND (ti(traffic OR vehicle OR railway OR bicycle OR car OR driving OR motorized OR walking OR pedestrian OR pedestrians OR subway OR metro OR underground OR train OR cycling) OR ab(traffic OR vehicle OR railway OR bicycle OR car OR driving OR motorized OR walking OR pedestrian OR pedestrians OR subway OR metro OR underground OR train OR cycling)) AND (ti(commuter*) OR ab(commuter*))

Scielo: (Pollution OR pollutant OR exhaust OR "particulate matter" OR pah OR pahs OR combustion OR "black carbon" OR vac OR vacs OR carbonmonoxide OR Coarse OR dust OR btex) AND (traffic OR vehicle OR railway OR bicycle OR car OR driving OR motorized OR walking OR pedestrian OR pedestrians OR subway OR metro OR underground OR train OR cycling) AND (commuter*)

Appendix 47. Selection criteria and study selection procedures, data extraction and quality assessment

Study selection and eligibility criteria

We included studies only in human adults. We excluded studies that measured AP exposure exclusively by biomarkers or based on simulated data, reviews, comments, consensuses, editorials, guidelines, in vitro studies, meta-analyses, ecological studies and protocols. We did not consider language or temporal limits.

Working in pairs, three authors reviewed titles and abstracts of the entire list of references to select those that fulfilled the selection criteria. After initial appraisal, full texts of selected titles were retrieved. Full texts were appraised independently by two authors to select those that fulfilled the selection criteria. Disagreements were solved through discussion, and upon consultation with a third independent author. Reference lists of the retrieved articles and previous systematic reviews were reviewed for additional publications. Experts in the field were contacted to identify additional references that should be considered.

Data extraction and quality assessment of the evidence

Extracted data from each article were registered in a form purposely designed, including study design, measurement period, MT, monitoring device, commuting time and number of measurements performed. Summary and dispersion measurements of exposures were extracted, according to MT and pollutant. If available, we extracted summary measurements stratified by season, day, period of monitoring, type of route and city. If more than one summary measurements were reported for the same strata, we extracted preferably arithmetic means, then geometric means and finally, medians. We extracted summary measurements of inhalation and uptake dose (per hour and/or per trip), model and parameters used for the estimation. We used the most complete report when multiple papers of the same study were available.

Quality of the studies was addressed in terms of the comparability of the exposure measured between MT, and the information provided to assess comparability. We used a modified version of the Newcastle-Ottawa scale for assessing the quality of observational studies (Appendix 48 (page 197)).

Appendix 48. Adjusted Newcastle-Ottawa Quality Assessment Scale

Selection (Maximum, four stars)

-1) Modal comparison

-a. Experimental [E]

-b. Observational [O]

-2) Background concentration and meteorological conditions

-a. Background concentration of ultrafine particles and meteorological conditions were provided/used for measurements standardization **

-b. Either background concentration of pollutants or meteorological conditions are provided but not used for standardization*

-c. Not measured or not provided

-3) Sources of heterogeneity

-a. Sources of heterogeneity for all modes of transport were standardized during measurements [i.e. for motorized modes, ventilation standards, type of car and fuel type; and for active modes, position in the road or in the sideway] **

-b. Standards were used in some modes of transport *

-c. No description or not standardized

Comparability (Maximum, four stars)

-1) Commuting time standards (start time and iterations)

-a. All modes of transport were measured at the same time **

-b. Not all the modes were measured at the same time, but the sampling time was standardized *

-c. No description or not standardized

-2) Route(s) standardized (same route followed by all the modes)

-a. Fixed route(s) followed by all the modes of transport **

-b. The fixed route(s) were not followed by all the modes, but were standardized *

-c. No description or not standardized

Outcome (Maximum, three stars)

-1) Ascertainment of exposure

-a. Sampling, device operation and analysis of the samples were standardized for all modes of transport **

-b. Sampling, device operation and analysis of the samples were standardized only for some modes of transport *

-c. No description or not standardized

-2) Sample size and dispersion measurements

-a. Average or total trip time and/or number of trips and dispersion data for summary measurements reported for all modes of transport *

-b. Incomplete reporting of trip time or number of trips for all modes of transport

Appendix 49. Parameters for estimation of gains in life expectancy by commuting over a 7km route by any MT vs. bicycle or walking during a week

Issue	Parameter	Values	Hours per day	Hours per week
Time (hours)	Motorized transport, except public transport		0.63	4.43
	Walk		2.50	17.50
	Bicycle		1.00	7.00
	Public transport		1.33	9.33
	Indoor (Sleep)		8.00	56.00
	Indoor (Work)		8.00	56.00
	Indoor (Home if motorized scenario)		7.37	51.57
	Indoor (Home if walk scenario)		5.50	38.50
	Indoor (Home if pedestrian scenario)		7.00	49.00
	Indoor (Home if public transport scenario)		6.67	46.67
Air pollution	Background AP factor†	0.74		
	Minute ventilation (m^3/hour)			
	Sleep	0.30		
	Home/Work	0.74		
	Trip: Motorized	0.28		
	Trip: Public transport	0.94		
	Trip: Walk	1.59		
	Trip: Bicycle	1.59		
	RR all-cause mortality per 10μg/m^3 of increment in $PM_{2.5}$ concentration	1.06		
Physical activity	MET mode – Motorized	1.5		
	MET mode – Public transport‡	2.0		
	MET mode – Walk	4.5		
	MET mode – Bicycle	4.5		
	MET sleep	1.0		
	MET home	2.25		
	MET work	2.25		
	RR all-cause mortality of seven hours per week of moderate physical activity vs 0 hours per week of moderate physical activity	0.76		

†This factor was used to estimate the background concentration of $PM_{2.5}$ in those studies where it was not provided. The factor was estimated as the average of the ratio between background $PM_{2.5}$ measured in fixed monitoring sites vs cyclists/pedestrians (if cyclists not measured), as reported in 25 studies included in the systematic review. ‡ This MET is assumed, taking into account that most trips made by public transport include approach stages, usually made by walking; thus, we assumed that 20 out of the 40 minutes of each public transport trip were approach stages of the trip made by walking.

Appendix 50. Procedures for estimation of gains in life expectancy by commuting over a 7-km route by any MT vs. bicycle or walking during a week

1. Scenarios: Within each study (n=25), we constructed a scenario for one hypothetical person spending seven days on four microenvironments: at work, at home, sleeping and commuting by one of the MT measured over a 7km route, twice a day (e.g., if cyclists and car commuters were measured, we built one scenario where the person commutes only by bicycle and other where the person commutes only by car). For each scenario, we calculated the weekly doses of physical activity and of inhaled fine particles. We assumed that at work and sleeping time were equal in all scenarios. The commuting time for each MT was calculated for a 7km route. At home time varied depending on the scenario to complete 24 hours.

2. Air pollution dose estimation: First, we estimated the daily inhaled fine particles dose of each scenario by adding the inhaled dose at each of the four activities, which were calculated applying the formula [1]: Inhaled dose [inhaled amount/trip]= Average exposure concentration (reported by the authors) x VE (m^3/hr.) x activity time (min), using the reported average exposure and standard VE depending on the activity[293], and weighted by the daily time spent at each activity.

3. Physical activity dose estimation: Second, we estimated the daily physical activity dose in MET-hours/week of each scenario by adding the energy expenditure weighted by the daily time spent at each activity. Both daily inhaled fine particles dose and physical activity dose were multiplied by seven.

4. Scenarios comparison: We calculated a weighted ratio of weekly inhaled fine particles dose and of physical activity dose by dividing the weekly inhaled fine particles dose and the weekly physical activity dose of each scenario by the corresponding weekly doses of the cyclists (or pedestrians) scenario.

5. Risk estimation between scenarios: We used the weighted ratio to calculate an adjusted dose-related all-cause mortality risk ratio (RR), derived from the RR for all-cause mortality estimated in previous meta-analyses. For fine particles exposure (1.06 per 10μg/m^3 change in fine particles concentration) [294] and for physical activity (0.76 for seven hours/week of moderate physical activity, compared to 0 hours/week).[303] Details of the procedures are explained in detail by de Hartog et al.[342]

6. Life-expectancy impact: The weighted RR were used to calculate the all-cause mortality rate per age group (20-39, 40-64 and +65 years) of the alternative scenarios ${}_mD_{xA}$ at each study, by applying the formula [2] ${}_mD_{xA}={}_mD_{xR}$*RR, where ${}_mD_{xR}$ is the mortality rate of the reference scenario (cyclist or pedestrian). As ${}_mD_{xR}$ we used the all-cause mortality rates per age group provided by the Global Burden of Disease for 2013[343] for the corresponding country of the study. The mortality rates were used to calculate the life expectancy at each scenario, using standard life tables.7. Summary: We calculated the difference of years of life expectancy between the alternative and the reference scenarios at each study. The differences were summarized in medians and interquartile ranges. The Appendix 50 (page 199) shows the assumptions used for the estimations.

Appendix 51. Methodological details of the studies

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	✔	?	✔	Partially	✖	NA	✖	✔	✔	✔	✔
Adams, 2001 - Fixed route study[307]/Adams, 2002 [328]	Volunteers carried personal samplers along three selected routes during four daily campaigns to resemble AM and PM peak and off peak traffic times	AM/PM peak and off peak. During summer, only PM. Not clear whether all modes were simultaneous on time	Route 1: Canyon like and congested Route 2: Mid congested Route 3: Less congested Cyclists also followed a side street route	Bus: Route 1: diesel open back; Route 2: diesel closed-shell; Route 3: both types Tube: Route 1 and 2: underground; route 3: mostly above ground Car: 6-year old diesel fueled estate car, and an 8-year petrol fueled in half measurements each Bicycle: Not specified	For motorized modes, included in-vehicle only and door-to-door measurements		Not specified	Sampling, device operation and analysis were standardized	Geometric standard deviation	Samples collected	Bicycle: 96 samples, Bus: 68, Car: 54 Tube: 66
	✔	?	✖	✔	✖	✔	✔	✔	✔	✔	✔
Boogaard, 2009[305]	Volunteers carried monitoring devices over 12 predefined routes of approx. 10-20min duration in 11 cities	Sampling between 12:00 and 19:00PM Not specified if commuting started at the same time	Shortest route per mode between origin and destination, in a radius of 2.5km within city center	Car: petrol-fueled	Routes consisted in before-, during and after-transport		Windows closed, A/C off and fanned ventilation on moderate setting	Sampling, device operation and analysis were standardized	Standard deviation	1-min averages	Car: 126 routes Bicycle: 120 routes
	✔	-	-	-	?	✔	✖	✔	✔	✔	✔
Brauer, 1999[329]	Monitoring devices were carried during ten 6hrs of scripted activity patterns simulating daily activity, including indoors, in-transit and outdoors microenvironments	Not specified	Not specified	Not specified	The change of microenvironment was registered, but not specified whether vehicle trips included or not walking trips	Same monitoring device	Not specified	Sampling, device operation and analysis were standardized	Geometric standard deviation	2-min averages	Bicycle: 61; Skytrain: 27; Bus: 153; Car: 154; Seabus: 17
Briggs, 2008[318]	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
	Monitors were carried over 48 routes to simulate typical trips (between 601-1361m total length)	Each route was walked once (or twice for a very short route), and driven repeatedly until the walk was completed	Walking route followed the road as closely as possible. Retour route was made over	Car: Diesel-fueled; car was previously ventilated Walk: followed road as closely as possible, taking centerline of sidewalks	Monitoring data not related directly with experiments were excluded		Windows closed, A/C off and fanned ventilation system on a	Sampling, device operation and analysis were standardized.	Standard deviation	Trip average	46 pairs of samples (car and walking)

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Author (year) Reference
			the same side of the car All types of routes were chosen.				moderate setting				
	✕	Partially	✕	Partially	✔	✔	✕	✔	✕	✔	✔
Chertok, 2004[330]	44-non-smoker volunteers commuting between home and work during at least 30 minutes, while carrying the BTEX and NO_2 samplers by one MT during the time of the study	Actual commuting trips of participants. All measurements on the same days to control for background ambient air pollution.	Actual commuting routes of participants	Participants commuting over their usual routes, but filled a diary journey. Cars used were a range of petrol fueled sedan models (1997). Specified routes of public transport.	Participants instructed to activate the samplers only while in the selected mode, and to travel directly between home and work		Not standardized	Sampling, device operation and analysis were standardized.	Not provided	Participants were given a weekly sampler, each sampler represents the exposure of, on average, 10-half-hour or longer commuting trips	Car=9 participants; Bus=4; Cycle=7 Train=11, Walk=9
	✕	✕	✕	✕	?	NA	✕	Partially	✔	✔	✔
De Bruin, 2004[331]	50 volunteers (office workers) aged 25-55 years from diverse home and work buildings typology carried personal monitors during 48-h and filled a 15-min resolution time-activity diary (actual data provided by 46 volunteers)	Actual commuting trips of participants	Actual commuting routes of participants	Not standardized	The diary log considered five micro-environments of in-transit modes, including walking, but it is not clear whether walking to approach stations was or not considered as part of one specific trip		Not standardized	Sampling and data analysis were standardized. Information about device operation standards is not provided	Standard deviation	15-min averages (estimated from 1-min exposure data)	Walking: 241 15-min periods, Train/metro: 57, Bus/tram: 158, Motorbike: 14, Car/taxi: 207
de Nazelle, 2012[295]	✔	Partially	Partially	✔	✕	✔	✕	✔	✔	✔	✔
	Commuters carried the monitoring devices in pairs of two different modes of transport	Measurements at peak (8-10h, 13-15h, 17-20h) and off peak (10-13h, 15-17h) times	The two routes were approximately the same, with	Routes and commutes were standardized for all the modes	Full trip from origin to destination, including walk		Driver's windows open	Sampling and device operation and data analysis were standardized	Standard deviation	Trip averages from 10-s and 1-s	Walk=48 trips, Bike=54,

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	over two selected 'round trip' commuting routes	Measurements performed in pairs of two different modes	variations for car and bus commuters Included high traffic roads, street canyons	Bike lane location ranging from the middle to a side of the road Car: diesel-fueled car	to bus stop or car park					measurements	Bus=34; Car=36
Dirks, 2012[297]	✗ Volunteers, regular commuters by a specific MT, were asked to carry a personal sampler over the same journey by one out of three set routes during one week	✗ Volunteers were asked to plan each journey to arrive at work at 9am and to leave to home at 5pm Each route was monitored during one week	✗ The offer of modes differed between routes: Route 1: Bus, car, motorcycle, run. Route 2. Bus, car. Route 3: Bus, car, run, train, bicycle	✗ Not standardized	? Volunteers were asked to log the start and end times of their journeys, but not specified whether intermediate legs were included	NA	✗ Not standardized	✔ Sampling and device operation were standardized	✔ Standard deviation	✔ 1-min concentrations	✔ Bus: 25 commutes, Car: 24, Run: 15, Motorbike: 8, Bicycle: 4, Train: 10
Dons, 2011[298]/ Dons, 2012[299]	✗ 62 volunteers carried the devices for seven consecutive days, reported microenvironments in an electronic diary fitted with a GPS.	✗ Volunteers were asked to carry the devices during seven days on their regular activities	✗ Volunteers followed their regular activities	✗ Not standardized	?/Partially Commuting data is disaggregated by mode of transport, but it is not specified whether multimodal trips (e.g. MMT) were disaggregated	NA	✗ Not standardized	✔ Sampling and device operation and analyses were standardized	✔ Standard deviation	✔ 5-min observations	✔ Car driver: 3190 5-min observations, car passenger: 645, bike: 1167, pedestrian: 1161, train: 72, light rail/metro: 72, bus: 190
Dor, 1995[320]	✔ Samplers and monitoring devices were carried by volunteers and placed on public transport vehicles over multiple specified routes	✗ Specific times for measurement in every mode. Car measurement on the same day of the week, during the same time frame (AM) and repeated every two weeks	✗ Specific routes for every mode Car route included canyon-like and suburban sections.	Partially Car: gasoline fueled, Public transport options are specified (bus and subway), Pedestrian route, no information about timing or parameters of the trips	Partially Car: during trips; public transport: inside wagons and bus; pedestrians: route provided	Partially Not clearly specified for no-car commutes	✗ Car occupants were asked to not smoke, but were free to heat and ventilate the car as wished	Partially Devices operation and analysis were standardized	Partially Not available for pedestrians, bus and metro commuters	✔ 1-min average intervals from 3-sec readings.	✔ Car: 19+22+8; not provided for the other modes

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	✔	Partially	Partially	✔	Partially	NA	✖	✔	✔	✔	✔
Duci, 2003[332]	Trips carrying several personal monitors were performed over seven standard routes.	Measurements conducted during morning (7.30-9.30), midday (14.30-16.30) and evening (19-21) rush hours Not measurements obtained during evening for car commutes.	In two routes were measured bus, trolley and car trips, in three only bus, in one trolley and in one electric train Pedestrian route was measured on sidewalks of the routes.	Cars were checked for the presence of indoor sources. Pedestrians: on sidewalks in proximity to selected routes MMT and bus over specified routes	10-min measurements at stations waiting for any MT were included.		Not specified	Sampling, devices operation and analysis were standardized	Standard deviation	1-min average measurements from 15-s intervals	Private car: 34 trips, bus: 144, trolley: 87, rail: 18, pedestrian: 30
Farrar, 2001[312]	✖	✖	✖	Partially	✔	NA	✖	Partially	✔	✔	✔
	Samplers were provided to bus drivers, cycling couriers and commuters, and were installed in taxis. Sampling times and operation differed per mode.	Participants recorded the exposure times on a form supplied	Actual commute routes	Bus: Diesel fueled, two were gas powered Taxis: gas powered Not specified for cyclists..	Monitors were opened while in transit		Car driving conditions and smoking was reported by drivers	Not standardized for all modes: Bus: monitors provided to bus drivers who opened the monitor while driving and carried the monitors when changed the bus Taxi: the monitors was installed din the vehicle and was open during a working week Cycling couriers had a monitor attached for one working week, which opened only during the working day Cycling commuters received a	Standard deviation	24-hour average concentration	Cityclippers: 23 24-hour periods, suburban buses: 64, taxis: 7, bicycle commuters: 8, couriers: 15

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
								monitor that was opened while commuting to and from work during 15 days			
	Partially	✔	Partially	Partially	✔	NA	-	✔	✔	✔	✔
Gee, 1999[333]	Air pollution samples were obtained with samplers placed in the driver cabins of two typical bus routes during a 3-hour period, and worn by cyclists who made repeated journeys over the route of the buses during the same time period	Sampling performed between 7-10am on weekdays	Cyclist followed the same route of one of the bus routes included in the study, and not always were the same	Despite standard bus routes, not information of further bus driving standards Not information about cycling standards	Measurements during target trips		Not specified	Sampling, device operation and analysis were standardized	Standard deviation	Whole route average concentration (3-hour period)	Bus:17 per bus. Cycle: 8
	✕	✕	✕	✕	✔	NA	✕	Partially	✕	✔	✔
Georgoulis, 2002[334]	Volunteers from five European cities carried a personal sampler, and filled a 15-min resolution activity diary	Actual commuting time	Actual commuter routes	Not standardized	Researchers extracted information of time spent in transfer and in street-transfer		Not standardized	Sampling and data analysis were standardized. Information about device operation standards is not provided	Geometric standard deviation	15-min averages based on 1-min measurements.	Car-taxi (participants): 251; Motorcycle: 21, Bus-tram: 148; Metro-train: 62; Walk-bicycle: 258
Goel, 2015[325]	✔	✕	?/Partially	Partially	?	NA	✔	✔	✔	✔	✔
	One way journeys over a defined route were monitored, mostly during morning commutes	No simultaneous modes of transport Measurements performed mostly between 8AM-1PM	One route consistent in sections of three roads was followed, Metro line is parallel to the study route. The tours (two trips) were made by one MT, except in 12 out 75 tours. Walking mode	Metro: One line was followed. Car: Ventilation settings, no further information provided Bus: Ventilation settings, no further information provided A-R and 2W, no further information provided	Not specified		Measurements in cars with A/C were set to air recycled mode	Sampling, device operation and analysis were standardized	Standard deviation	Average per mode	Cycle: 24 trips, 2W: 21, OW car: 16 AC car: 20 AR : 27, OW bus: 15 AC bus: 18 metro: 9

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
			consisted in crossing roads and approaching stops measurements.								
	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Gulliver, 2004[319]	Two measurement campaigns were performed, over one different route each, with two iterations per mode. The pilot measurements were performed over the route 1	Both modes started at the same time. Both modes performed two iterations of the route between 8-9AM and between 3-4PM. The first route was followed twice per time per mode The second was circular, the car was allowed up to three iterations while the pedestrian completed one iteration	Two routes with mixed traffic load. Route 1 was mostly over a heavily trafficked area. Route 2 was a circular route over mostly suburban roads, less trafficked than the Route 1	Car: One car, gasoline fueled (assumed, 1995 Ford Fiesta) Pedestrian: followed closely the road route over footpaths	Measurements obtained while commuting only		Windows were closed and no A/C or climatic controls were used	Sampling, device operation and analysis were standardized	Standard deviation	Hour average (two hourly iterations combined)	73 pairs of simultaneous in-car and walk measurements analyzed
	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Gulliver, 2007[313]	Measurements were performed simultaneously in two modes of transport. Two separate circular routes were monitored.	Car trip and walk started at the same time. Car was driven repeatedly until the walk was completed.	Both modes followed the same direction and route in each experiment. Both routes had mixed heavily trafficked and minor roads.	Car: Petrol-engine. Previous to each trip, the car was fully ventilated. Pedestrian followed the road route as closely as possible along the centerline of each pavement.			Windows were closed and moderate level of air flow	Sampling, device operation and analysis were standardized	Standard deviation	Trip [hour] average	Car=33 hours, Walking=33
Huang, 2012[320]	✔	✖	✔	Partially	?/✔	✔	✔	✔	✔	✔	✔
	Two routes were monitored during 18 sampling days. One third of the return trips were performed by one	Each trip performed at traffic heavy times (7-9.00 and 17-19.00) and light times (12-14.00).	Two routes were followed by all the modes Route 1 was broader and congested,	Car: gasoline-fueled car; Euro III engine. Bus: diesel-fueled; Euro III engine. Ventilation settings beyond the control of passengers; windows	Not specified whether bus and taxi measurements included waiting time		A/C on and windows closed.	Sampling, device operation and analysis were standardized	Standard deviation	Trip averages from 1-min interval reading.	Taxi: 43 trips, Bus: 45, Bicycle: 43

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	of the three modes of transport, respectively.		whereas the second was both congested and canyon-like.	were closed and A/C mostly, due to cold weather. No further information regarding cyclists.							
Int Panis, 2010[284]	✔ 55 healthy non-smoker volunteers were driven as passengers over one selected route, and then asked to ride the same route by bike	Partially The bike commute followed the car route with a minimum difference (between 3-8 min). The Brussels road was cycled twice	✔ Three routes were followed in each city with mixed traffic load	Partially Same car for all the commutes, not specified fuel type. Bicycle: The specified for timing and route	✔ Measurements only while commuting	✔	✔ Windows closed, A/C off and fanned ventilation system in mode 1	✔ Sampling and devices operation and analysis were standardized.	✔ Standard deviation	✔ Averages based on 1-s readings	✔ 43 pairs of bike-car trips
Kaur 2005[306]/Kaur 2009 [310]	✔ Groups of four volunteers were randomly assigned to travel along two set routes by five modes of transport	✔ Three timings (morning [8.30am], lunch [12.00pm] and afternoon [3.15pm]). An additional early evening [5.15pm] measurement during the first week for $PM_{2.5}$	Partially Two routes were randomly travelled. Route 1 was heavily trafficked. Route 2 had a mixture of congested sections and backstreets with very little traffic; this route was completely followed only on foot or bicycle, due to circulation restrictions for car and taxi, and bus riding only over part of the route.	✔ Car: petrol car with three-way catalyst and diesel black cab. Bus: diesel buses Bicycle: followed bus lanes over the routes Walking: The specified for timing and route	- Not specified	Partially CO measures only during three last weeks. Additional $PM_{2.5}$ measure obtained during the first week.	✖ Car drivers instructed to operate ventilation as normal	✔ Sampling devices operation and analysis were standardized	✔ Confidence intervals and geometric standard deviation	✔ PM2.5: Average of weight of three filters. CO: Sample period average based on 10-sec intervals UFP: Average of count of particles per sample	✔ $PM_{2.5}$: 197 samples CO: 111 samples UFP: 86 samples
Kingham, 2013[335]	✔ Four commuters carried a complete set of monitors over the.	✔ Sampling was made at the same time each day in the morning (7+40-	✔ One route was followed by the three MT, one bus route.	✔ Car: petrol engine sedan All other modes: as specified	✔	✔	✔ Windows closed, vents set to 'fresh'	✔ Sampling, device operation and	✔ Interquartile range (box-plot graphs).	✔ Trips and 6-sec	✔ CO: Car: 49 trips, Bus: 52, Bike on-road:

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	specified routes, all commuters met in a specified point of the route to complete the second part of the route.	9+00) and evening (16+45-18.05) rush hours	off-road cycle route was followed additionally by bike. Car and road cyclist followed the bus route.								
	✔	Partially	✔	✔	✕	NA	-	✔	✔	✔	✔
Li, 2015[301]	Door-to-door measurement were performed along three defined routes in pairs of MT, being bicycle always one of the modes	Measurements between 7-9.30am and 14-16.30pm, during 6 weekdays. A round of measurements constituted four measurements by bike and four concurrent measurements by the other modes along the three routes.	Three routes were followed by all modes of transport in pairwise design. Route 1 had 1-5 multi-storey residential buildings at both sides with 8 lanes; Route 2 had high-rise buildings with 10 lanes and Route 3 had 2-5 storey buildings with dense shade tree cover at both sides and 2 lanes	Bus and taxis: taken randomly. Bus were diesel fueled, and ventilation settings were not controlled Taxis: gasoline fueled. Bicycle: Used right side of the lane Pedestrian: Lateral middle of the pavement	Door to door (including waiting times in stations)		Not specified	Sampling, device operation and analysis were standardized	Standard deviation	1-s measurements, averaged as six-day measurement campaigns	Bus, subway, taxi and walking: 12 trips per route. Cycling: 48.
Liu, 2015[326]	✕ 120 healthy young students, no smokers and no history of cardiovascular disease were classified according to their usual commuting style. They were monitored three times during 1-hr commuting time with personal monitors of	✔ One hour (9-10am) commute for each volunteer in an assigned MT	✕ Actual commuting route	✔ Subway: electrically powered Bus: Gas powered Car: gasoline-powered; not specified whether the commutes were standardized	✕ Included measurements in stations	✔	✔ A/C used in car, bus, subway and subway station	✔ Sampling and analyses ere standardized, not clear device operation	✔ Standard deviation	✔ Hour measurements	✔ Subway: 30 participants, bus, 30, car: 30, walking: 30

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	both air pollution and electrocardiography	✔	Partially	✔	✔	✔	✔	✔	✔	✔	✔
McNabola, 2008[285]	Volunteers carried in parallel the personal monitors in pairs by two different modes of transport over the same of the two routes set at peak morning and afternoon hours	During weekdays between 8-9am and between 5-6pm. The pair of volunteers started at the same time, but finished at different time, depending on the traffic	Two routes followed by all modes of transport. Route 1 was a broad major congested road. Route 2 was a canyon like road with variable traffic	Car: Route 1: diesel-fueled. Route 2: diesel-fueled. Bus: closed-shell, double-decker, diesel-fueled; ventilation beyond the control of volunteer, no A/C available, seat at random Bicycle: traveled on cycle lanes adjacent to footpath Walk: along the center of the footpath adjacent to incoming (AM) or outgoing (PM) traffic on each route	Sampling at the beginning of the journey and closed at the end of the trip		Windows and vents closed, no A/C	Sampling, devices operation and analysis were standardized	Standard deviation	Trip concentration	Car=45 samples, cyclists: 42, bus: 27, walk: 37
	✔	✕	✕	✔	?/ ✔	NA	✕	✔	✔	✔	✔
Morabia, 2009[336]	Each of the 20 volunteers were asked to complete a car, subway and walking trip arm, each one planned to last 8-hours	Modes were not simultaneous	Specific road for each mode	Trips were standardized and trips details recorded in minute-by-minute diaries	Not clear whether car and MMT modes included waiting times		Participants reported in a minute-by-minute diary whether the windows were closed or open and internal circulation was on or off	Sampling, device operation and analysis were standardized	Geometric standard deviation	Averages based on 1-min measurements	Car: 7941 minutes, Subway: 6299, Waking: 5929
Moreno, 2015[337]	✔	Partially	Partially	✔	✕	?	-	✔	✔	✔	✔
	Two commuters made simultaneous but separated round trips by one MT over a set route	Measurements made in pairs by MT. The journeys started at 10AM, and round trips were made.	The main route was a 4.2km route (one way) with a city center section (congested, canyon-like) and a suburban section (broader	Walking: sidewalks of the main route Tram: selected route, included walking section Bus: diesel fueled, the traveler placed in the central part of the vehicle Metro: Set lines monitored, included waiting times	Bus, tram and metro included walking sections pre-, during-, and post commute	Not specified	NA	Sampling, device operation and analyses were standardized	Standard deviation	Average per MT	78 trips performed, not specified trips per mode

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
			with wide sidewalks). Walking section of tram trip differed slightly from the others. Metro route did not overlap with the main route.								
Nyhan, 2014[286]	✗ 55 young healthy non-smoking volunteers carried personal monitors 2 to 7 times since before morning commute (between 8-9) to work until just before evening commute	Partially Participants commuted to work between 8-9AM	✗ Actual commuting routes	✗ Not standardized	Partially Pre- and post-commute walking was asked to be kept at minimum	✔	✗ Not standardized	✔ Instructions were provided to volunteers. Sampling, devices operation and analysis were standardized	✔ Standard deviation	✔ Sampled full commutes	✔ Bus: 28 commutes, Train: 24, Pedestrian: 37, Cyclists: 33
Onat, 2013[316]	✔ Two volunteers traveled along the selected sections of the same route twice a day carrying the personal monitors	Partially Measurements were obtained at rush (8-10.30am) and non-rush (12-14pm) hours Two researchers participated in data collection, no simultaneity assumed for all modes	Partially Same route for all modes, walking route was only 1.5km, bus was 5km, and metro-bus and car were 10km	✔ <u>Car</u>: Euro 4 engine <u>Metro-bus</u>: Engine 4-5; windows closed and A/C on. <u>Bus</u>: pre-Euro engine; windows open and not A/C <u>Walk:</u> Over a side of the route, with timing specified	- Not specified	NA	✔ <u>Car</u>: Windows closed, A/C on or A/C off and recirculation on	✔ Sampling, device operation and data analysis were standardized	✔ Standard deviation	✔ Trips (data logging of 30s)	✔ Bus: 8 trips, Metro-bus: 7, Car A/C on: 10; Car A/C off: 10, Walking: 10
Ramos, 2016[302]	✔ A route was followed by one out of five modes of transport per day in five measurement times	✗ Each MT was followed each monitoring day. Measurements made at 8AM, 11AM, 14PM, 17:30PM and 21PM	✔ A 7-km route for all the modes	✔ <u>Bus:</u> A/C available <u>Metro</u>: A/C available, windows closed. <u>Motorcycle</u>: diesel, fueled <u>Bicycle</u>: two cyclists carried side by side with the monitoring devices <u>Car</u>: Gasoline fueled	? Not specified	✔	✔ Windows closed and A/C and fan ventilation off	✔ Sampling and device operation were standardized	✔ Standard deviation	✔ Average per mode	✔ Three trips per mode

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
Rank, 2001[338]	✔ Two teams of two cyclists and two car drivers drove a slow route carrying the samplers for 4 hours in two days	✔ Sampling between 7.40-9.40am and 10-12pm	✔ Measurements on the same route	✔ Car: two B-class cars from 1990. Bicycle trips standardized.	✔	NA	✔ No air recirculation allowed during the experiment	✔ Sampling and samples analyses were standardized	✔ Standard deviation	✔ Concentration at each sample measured is provided	✔ Car: 42rounds; Bicycle: 32.
Saksena, 2008[317]	✔ Two teams of two researchers made retour trips over the same route in two different modes of transport each time.	Partially Pair modes on time were: car AC on and bus; mobike and car AC off; mobike and walk. Repeated in rush and non-rush hours.	✔ The procedure was repeated on each road on consecutive days between two bus stops.	✔ Trips were standardized for all modes. Bus: diesel fueled and use A/C. Car: one car used for the measurements, not provided specifications Mobike and walk: Timing and route specified, no further specifications provided	✔ Monitors were turned off when changing direction or mode	✔	✔ A/C on or off, depending on the pair mode	✔ Sampling, device operation and analysis were standardized	✔ Standard and geometric standard deviation	✔ Trip averages	✔ Bus: 16, Car: 32, Mobike: 32, Walking: 16
Suárez, 2014[304]	✔ Two volunteers at a time commuted over a defined route carrying a personal monitor each one by one MT, then switched the monitor and travelled along the route again Transport mode and order of samplers was assigned randomly	Partially Sampling between 8-9am, two commuters per sampling session, therefore not at the same time.	✔ Same fixed routes for all modes	✔ Car: three different cars were used, all gasoline powered and with catalytic converters Bus: diesel buses All commutes were standardized.	✘ Bus and subway commutes included walking portions, pedestrians included street crossing and waiting periods.	✘ Each commuter carried a different monitor	✔ Windows closed, no recirculation, though ventilation and heating at used at will	✔ Sampling, devices operation and analysis were standardized	✔ Standard deviation	✔ Commute averages	✔ PM2.5 Bicycle: 16trips, Bus: 17, Car: 17, Subway: 17 UFP: Bicycle: 14trips, Bus: 18, Car: 18, Subway: 18
Van Wijnen, 1995[287]	✔ Young, healthy non-smoking volunteers covered selected routes during approximately 1 hour carrying the sampling equipment either by car (n=4) or bicycle (n=4)	✔ Departure and arrivals were synchronized on each run In January, routes were followed between 8-10am. In May, were followed between 9-10am and one route was also followed	✔ Fixed routes selected, routes were followed simultaneously. If the car took a tunnel route, the cyclists followed one of the inner city routes.	✔ Petrol car without catalytic convertor. Bicycle commutes were standardized.	✔	✔	✔ No instructions about ventilation, although windows were kept closed during the trips	✔ Devices operation and analysis were standardized	✔ Standard deviation	✔ Commute averages	✔ Bicycle=90; Car=69; Walking=36

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
		between 3-6pm. A third route was also followed during morning and evening in August.									
Vellopolou, 1998[339]	✕ 50 non-smoking participants who commuted at least 4 times per week to and from work carried the personal monitors during one working day and recorded their concurrent time allocation to different activities	✕ Participants registered a diary with times of commuting (each participant recorder exposure during one working day between 7-8am and 18-19pm).	✕ Actual commuter routes	✕ Not standardized	✕ All modes while commuting, modes recorded.	NA	✕ Not standardized	✔ Volunteers were instructed about the sampling and devices operation standards.	✔ Standard deviation	✔ Average CO concentration per period, averaging instantaneous measurements recorded every 60s	✔ Used data from 44 participants. Car: 20 trips, bus: 19, motorbike: 4, walking: 1
Vouitsis, 2014[340]	✔ Monitoring was performed simultaneously in pairs of modes of transport on one of the tree routes defined, four times a day.	Partially Each monitoring trip consisted of full trip. Measurements between 7.30-10, 10-12am, 1-3pm, 3-5pm. Two transportation modes were measured simultaneously; car in all pairs alternating ventilation settings between trips	✔ Three fixed routes with different traffic load and configuration.	Partially Car: Gasoline engine car. Bus ventilation settings not specified. Cyclists: No further information provided	✕ Full trip from origin to destination	✔	✔ In one configuration, car was driven with cabin ventilation on the second level, cabin air recirculation turned off and windows closed.	✔ Sampling, device operation and analysis were standardized.	✔ Standard deviation	✔ Average per commute and trip,	? Bicycle: 28 trips; Bus: 24; Car: 28 It is unclear the actual number of commutes used for analyses
Williams, 2016[341]	✕ One researcher carried the monitoring device during 20 24-hour periods during his normal activities and filled a diary of activity patterns	✕ Actual commuting time	✕ Actual commuting routes	✕ Car: One petrol fueled	? Not specified whether pre or post commuting legs were included	NA	✔ Car driven either with low fan setting, recirculate on and windows fully closed; or no	✔ Sampling and device operation were standardized	✔ Standard deviation	✔ 30-seconds average per mode	✔ Car [WO]: 719 30-s averages Car [V]: 809 Walking: 491 Cycling: 491 Bus: 917 Train: 297

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes recirculation and windows open	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	✔	Partially	✖	Partially	-	NA	✔	✔	✔	✔	✔
Wu, 2013[311]	Volunteers as passengers carried the personal monitors by different modes of transport twice a day in three time periods, two in spring and one in summer	Al measurements obtained at between 7-12am and 14-19pm, but data of on-road modes (walking, bicycle and motorcycle) were available only in summer whereas data of in-cabin (bus, taxi, metro) were available both in summer and spring	Routes differed between modes of transport. Three bus lines over different routes.	Bus: diesel-fueled Taxi: compressed natural gas Ventilation settings for bus and metro, beyond the control of volunteers Motorcycle: fueled by regular unleaded gasoline Walk, cycle and motorcycle modes were performed over the same route, no further data provided	Not specified		Windows opened and A/C off or windows closed and A/C on during the second cycle.	Sampling, device operation and analysis were standardized	Standard deviation	Trip average	Bus=101 samples., taxi: 53, metro: 33, walking: 54, bicycle: 35, motorcycle: 34
	✔	✖	Partially	✔	✖	✔	NA	✔	✔	✔	✔
Yan, 2015[314]	A pair of researchers followed each route by one MT several times a day carrying the personal monitors. Each MT was measured in different day periods.	Different measurement campaigns were performed per mode - Walking Dec 10-16 every other hour from 8.00 to 21.00 - Bus and subway: 18-23 Dec[Bus: 8.00, 12.00 and 18.00hrs][Subway between 14 to 16.00 hr.]	One route for each MT, the routes overlapped on some sections	Bus: with and without AC, but no comparisons were made according to this criterion Subway: two specific lanes with above and underground sections Walking: time and route specified	For bus trips, it included waiting times and walks to approach the stations/stops. For subway trips, from entering subway station entrances			Sampling, device operation and analyses were standardized	Standard deviation	Trip average	Pedestrians: 39samples/trips, bus commuters: 17 Subway: 5 sets

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Simultaneous pollutants	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
Zuurbier, 2010[296]	✔	Partially	Partially	✔	✔	✔	✔	✔	✔	✔	✔
	One route was followed during 47 days evenly spaced along a year by three modes of transport, each mode on approximately one third of the days. A second route with low traffic was followed by bicycle.	Samples between 8.00 to 10.00 hours on Tuesdays and Thursdays. On each sampling campaign, modes were simultaneous for diesel and gasoline car or electric and diesel bus	One fixed high-traffic route for cars, buses and high-traffic bicycle route. A second fixed route for low-traffic bicycle route.	Diesel and gasoline-fueled cars, Diesel and electric trolley buses (ventilation not controlled); not A/C available, windows closed, not smoking allowed; diesel buses retrofitted with particulate filters.			Windows closed and air conditioning at moderate level	Sampling, device operation and analysis were standardized.	Standard deviation	PM10: Daily averages PM2.52-hour averages from one-sec readings	Diesel bus: 13, electric bus: 13, gasoline car: 14, diesel car: 14, high-traffic bicycle: 15, low-traffic bicycle: 15

MMT: Massive motorized transport. CO: Carbon monoxide, BC: Black carbon, NO_2: Nitrogen dioxide. PM: Particulate matter. A/C: Air conditioned. NS: Not specified. NA: Not applicable "?" Unclear ✖ either if unclear or unspecified for ventilation settings, because it is assumed that it was not standardized. – not specified.

Appendix 52. Quality of the studies[¶]

Author, year (Reference)	Modal comparison	Background factors	Heterogeneity	Same time – All modes	Same route – All modes	Ascertainment of exposure	Sample size and dispersion
Adams, 2001 [307] Adams, 2002 [328]	E	**	**	*	**	**	*
Boogaard, 2009 [305]	E	**	*	*		**	*
Brauer, 1999 [329]	E	*	*			**	*
Briggs, 2008 [318]	E	**	**	**	**	**	*
Chertok, 2004 [330]	O	**	NA	*	NA	*	
De Bruin, 2004 [331]	O	*	NA	NA	NA	*	*
de Nazelle, 2012 [295]	E	**	**	*	**	**	*
Dirks, 2012 [297]	O		NA	*	*	**	*
Dons 2011 [298] Dons 2012[299]	O	**	*	NA	NA	**	*
Dor, 1995 [320]	E		**			**	
Duci, 2003 [332]	E	**		*	*	**	*
Farrar, 2001 [312]	O	*	*	NA	NA	**	
Gee, 1999 [333]	E/O	*	*	**	*	**	*
Georgoulis, 2002 [334]	O	*	NA	NA	NA	*	
Goel 2015 [325]	E	**	*		*	**	*
Gulliver, 2004 [319]	E	**	**	**	**	**	*
Gulliver, 2007 [313]	E	**	**	**	**	**	*
Huang, 2012 [300]	E	**	*		**	**	*
IntPanis, 2010 [284]	E	**	**	*	**	**	*
Kaur, 2005 [306] Kaur, 2009 [310]	E	**	**	**	*	**	*
Kingham 2013 [335]	E	*	**	**	**	**	*
Li, 2015 [301]	E	**	**	*	**	**	*
Liu, 2015 [326]	O	*	*	**	NA	*	*
McNabola, 2008 [285]	E	*	**	*	**	**	*
Morabia, 2009 [336]	E	**	**			*	*
Moreno 2015 [337]	E	*	*	*	*	**	*
Nyhan, 2014 [286]	O	**	**	*	NA	**	*
Onat, 2013 [316]	E	*	*	*	*	**	*
Ramos 2016 [302]	E		*		**	*	*
Rank, 2001 [338]	E	**	**	**	**	**	*
Saksena, 2008 [317]	E	*	**	*	**	**	*
Suárez, 2014 [304]	E	**	**	*	**	**	*
Van Wijnen, 1995 [287]	E	*	**	**	**	**	*
Vellopolou, 1998 [339]	O	*	*	*	NA	**	*
Vouitsis, 2014 [340]	E	**	*	*	**	**	*
Williams 2016 [341]	O		*	NA	NA	**	*
Wu, 2013 [311]	E	**	**	*		**	*
Yan, 2015 [314]	E	*	**		*	**	*
Zuurbier, 2010 [296]	E	**	**	*	*	**	*

E: Experimental. O: Observational [¶] Details of the tool used for the assessment is provided in **Appendix 48** (page 197).

Appendix 53. Distribution of pollutants exposure levels according to MT

Pollutant	MT	Median	25th percentile	75th percentile	Minimum measurement	Study	Maximum measurement	Study	Number of studies	Comparisons with exposure above ambient air quality standards*		References
BC, µg/m³	🚲	6.3	3.6	7.9	1.0	Williams 2016	10.5	Vouitsis 2014	5	-/8		295,299,301,340,341
	🚌	6.6	5.5	7.3	2.4	Williams 2016	8.0	Li 2015	6	-/10		295,299,301,337,340,341
	🚗	7.7	4.9	10.9	1.7	Williams 2016	16.7	de Nazelle 2012	5	-/10		295,299,301,340,341
	🚗 [V]	4.2	4.0	4.3	3.8	Vouitsis 2014	4.4	Williams 2016	2	-/4		340,341
	🚊	7.0	2.4	8.2	0.5	Williams 2016	12.7	Li 2015	4	-/7		299,301,337,341
	🚶	5.5	4.4	5.8	1.2	Williams 2016	9.6	Moreno 2015	5	-/10		295,299,301,337,341
CO, ppm	🚲	1.1	0.3	1.5	0.1	Ramos 2016	2.3	Van Wijnen 1995	6	0/16	0%	287,295,297,300,302,306
	🚌	5.9	0.9	9.8	0.1	Ramos 2016	15.0	Duci 2003	12	15/40	38%	295,297,300,302,306,317,320,331,332,334,337,339
	🚗	6.1	1.3	16.9	0.7	Dirks 2012	24.1	Duci 2003	8	10/22	45%	295,297,306,320,331,332,334,339
	🚗 [V]	2.4	0.7	4.8	0.2	Ramos 2016	18.5	Saksena 2008	4	1/16	6%	287,300,302,317
	🚊	0.9	0.2	3.4	0.1	Ramos 2016	4.4	Duci 2003	7	0/19	0%	297,302,320,331,332,334,337
	🚲 [M]	2.2	0.5	7.4	0.3	Ramos 2016	18.6	Saksena 2008	6	3/14	21%	297,302,317,331,334,339
	🚶	3.0	0.9	10.0	0.4	Dirks 2012	12.6	Duci 2003	10	5/19	26%	287,295,297,306,317,320,331,332,337,339
Coarse particles, µg/m³	🚲	65.7	48.0	74.0	21.0	Rank 2001	141.0	Vouitsis 2014	7	17/24	71%	284,286,296,302,333,338,340
	🚌	55.0	40.8	69.3	14.0	Ramos 2016	338.0	Gee 1999	7	13/20	65%	286,296,302,317,326,333,340
	🚗	74.0	43.0	84.0	43.0	Vouitsis 2014	84.0	Vouitsis 2014	1	2/3	67%	340
	🚗 [V]	46.5	35.8	65.0	5.9	Briggs 2008	408.0	Saksena 2008	10	13/28	46%	284,296,302,313,317-319,326,338,340
	🚊	52.0	41.6	64.0	31.5	Liu 2015	80.0	Ramos 2016	3	7/12	58%	286,302,326
	🚲 [M]	61.0	56.0	93.0	41.0	Ramos 2016	580.0	Saksena 2008	2	9/11	82%	302,317
	🚶	38.2	22.1	50.2	19.1	Gulliver 2007	495.0	Saksena 2008	6	2/7	29%	286,313,317-319,326
Fine particles, µg/m³	🚲	42.3	25.5	71.7	6.0	Boogaard 2009	347.0	Goel 2015	14	33/43	77%	284-286,295,296,300,302,304-307,311,325,340
	🚌	54.6	38.7	116.7	21.4	Nyhan 2014	315.0	Goel 2015	16	37/40	93%	285,286,295,296,300,302,304,306,307,311,314,316,325,326,337,340
	🚗	35.6	29.5	41.5	13.1	Morabia 2009	180.0	Goel 2015	6	10/13	77%	295,306,307,325,336,340
	🚗 [V]	36.0	26.0	56.8	3.0	Briggs 2008	122.0	Boogaard 2009	15	30/39	77%	284,285,296,300,302,304,305,311,313,316,318,319,325,326,340
	🚊	44.0	34.8	64.7	19.6	Morabia 2009	270.8	Adams 2001	11	18/20	90%	286,302,304,307,311,314,316,325,326,336,337
	🚲 [M]	125.0	58.0	224.0	39.0	Ramos 2016	257.0	Goel 2015	3	12/12	100 %	302,311,325
	🚶	56.7	25.7	119.1	6.6	Briggs 2008	278.0	Goel 2015	14	15/20	75%	285,286,295,306,311,313,314,316,318,319,325,326,336,337

Appendix 54. Distribution of pollutants exposure levels according to MT

Pollutant	MT	Median	25th percentile	75th percentile	Minimum measurement	Study	Maximum measurement	Study	Number of studies	Comparisons with exposure above ambient air quality standards*		References
NO_2, $\mu g/m^3$	🚲	92.5	41.4	98.0	33.5	Chertok 2004	156.0	Van Wijnen 1995	3	0/6	0%	[287,312,330]
	🚌	60.4	58.3	69.6	58.3	Farrar 2001	69.6	Farrar 2001	2	0/3	0%	[312,330]
	🚗	34.4	28.2	40.5	28.2	Farrar 2001	40.5	Chertok 2004	2	0/2	0%	[312,330]
	🚗 [V]	116.5	59.0	225.5	59.0	Van Wijnen 1995	277.0	Van Wijnen 1995	1	1/4	25%	[287]
	🚍	20.3			20.3	Chertok 2004			1	0/1	0%	[330]
	🚶	70.8	35.6	106.0	35.6	Williams 2016	106.0	Van Wijnen 1995	2	0/2	0%	[287,330]

🚲 Cyclists 🚶 Pedestrians 🚗 Car driven <u>without</u> controlled ventilation settings 🚗 [V] Car driven <u>with</u> controlled ventilation settings 🚌 Bus 🚍 Massive motorized transport 🚲 [M] Motorcycle. CO: Carbon monoxide, BC: Black carbon, NO_2: Nitrogen dioxide. ppm=parts per million. * The standards used were: coarse particles: 50$\mu g/m^3$ in 1hr; fine particles: 2.5$\mu g/m^3$ in 1hr; NO_2: 200$\mu g/m^3$ in 1hr, CO: 8.73ppm (maximum daily 8hour mean) [290]

Appendix 55. Distribution of ratio of pollutants exposure level among modes of transport, compared to (A) cyclists or (B) pedestrians' exposure

MT	Pollutant	Higher exposure among	Median	25th percentile	75th percentile	Minimum ratio	Study	Maximum ratio	Study	Number of studies	Comparisons with ratio of exposure higher than 1*		References
Reference category: active transport (cyclists or pedestrians)													
			1.00	0.79	1.41	0.10	Vouitsis 2014	6.26	Gee 1999	29	57/109	52%	285,286,295-297,299-302,304,306,307,311,312,314,316,317,320,325,326,329-333,337,339-341
	All		1.22	0.90	1.76	0.55	Morabia 2009	4.27	de Nazelle 2012	16	30/42	71%	295,297,299,301,306,307,312,325,329-332,336,339-341
[V]			0.95	0.66	1.54	0.21	Briggs 2008	7.55	Ramos 2016	19	39/86	45%	284,285,287,296,300,302,304,305,311,313,316-319,325,326,338,340,341
			0.67	0.49	1.13	0.31	Duci 2003	10.34	Adams 2001	20	21/55	38%	286,297,299,301,302,304,307,311,314,316,320,325,326,329-332,336,337,341
[M]			0.99	0.86	1.38	0.69	Ramos 2016	33.5	Dirks 2012	7	16/32	50%	297,302,311,317,325,331,339
Reference category: cyclists													
	Coarse	(HT)	1.04			1.04	Zuurbier 2010			1	1/1	100%	296
(HT)†	Fine		0.68	0.39	1.01	0.39	Adams 2001	1.01	Zuurbier 2010	2	1/3	33%	296,307
	BC		1.09	0.82	1.50	0.71	Vouitsis 2014	2.26	Williams 2016	5	5/8	63%	295,299,301,340,341
	CO		1.59	1.33	3.25	0.73	Kaur 2005	4.98	Ramos 2016	5	7/9	78%	295,297,300,302,306
	Coarse		0.83	0.61	1.41	0.10	Vouitsis 2014	6.26	Gee 1999	6	9/20	45%	286,296,302,329,333,340
	Fine		1.03	0.84	1.41	0.43	Ramos 2016	2.33	Vouitsis 2014	13	17/31	55%	285,286,295,296,300,302,304,306,307,311,325,329,340
	NO_2		1.68	1.41	1.80	1.41	Farrar 2001	1.80	Chertok 2004	2	3/3	100%	312,330
	BC		1.57	1.25	1.76	0.85	Li 2015	1.96	de Nazelle 2012	5	7/9	78%	295,299,301,340,341
	CO		1.17	1.08	2.72	1.00	Kaur 2005	4.27	de Nazelle 2012	3	4/4	100%	295,297,306
	Coarse		0.81	0.66	1.10	0.60	Vouitsis 2014	1.30	Vouitsis 2014	2	¼	25%	329,340
	Fine		1.14	0.92	1.21	0.80	Vouitsis 2014	1.36	Adams 2001	5	8/12	67%	295,306,307,329,340
	NO_2		0.95	0.68	1.21	0.68	Farrar 2001	1.21	Chertok 2004	2	1/2	50%	312,330
	BC	[V]	1.03	0.40	4.22	0.40	Vouitsis 2014	4.22	Williams 2016	2	2/3	67%	340,341
	CO	[V]	3.12	2.33	4.15	1.00	Van Wijnen 1995	7.55	Ramos 2016	3	12/12	100%	287,300,302
[V]	Coarse		0.77	0.60	1.29	0.33	Vouitsis 2014	2.24	Rank 2001	5	9/22	41%	284,296,302,338,340
	Fine		0.95	0.72	1.24	0.37	Vouitsis 2014	2.50	Boogaard 2009	9	12/30	40%	284,285,296,300,302,304,305,311,340
	NO_2		0.66	0.60	1.12	0.60	Van Wijnen 1995	1.12	Van Wijnen 1995	1	1/3	33%	287
	BC		1.23	0.67	1.43	0.47	Williams 2016	1.85	Li 2015	3	4/6	67%	299,301,341
	CO		0.59	0.43	1.13	0.33	Dirks 2012	4.00	Ramos 2016	2	2/5	40%	297,302
	Coarse		0.66	0.57	1.06	0.43	Ramos 2016	1.25	Ramos 2016	3	4/12	33%	286,302,329

MT	Pollutant	Higher exposure among	Median	25th percentile	75th percentile	Minimum ratio	Study	Maximum ratio	Study	Number of studies	Comparisons with ratio of exposure higher than 1*		References
	Fine	🚲	0.97	0.62	1.16	0.36	Wu 2013	10.34	Adams 2001	6	6/12	50%	286,302,304,307,311,329
	NO_2	🚲	0.60			0.60	Chertok 2004			1	0/1	0%	330
	CO	🚲 [M]	3.05	2.18	3.92	1.84	Ramos 2016	4.28	Ramos 2016	1	4/4	100%	302
🚲[M]	Coarse	🚲	0.89	0.83	0.91	0.69	Ramos 2016	1.04	Ramos 2016	1	1/10	10%	302
	Fine	🚲	0.86	0.82	0.95	0.73	Goel 2015	1.07	Ramos 2016	3	2/8	25%	302,311,325
	BC	🚲	0.85	0.80	0.96	0.67	de Nazelle 2012	1.19	Williams 2016	4	1/6	17%	295,299,301,341
	CO	🚲	0.78	0.70	0.87	0.67	Dirks 2012	0.92	Van Wijnen 1995	4	0/4	0%	287,295,297,306
🚶	Coarse	🚲	0.77	0.71	0.83	0.71	Brauer 1999	0.83	Nyhan 2014	2	0/2	0%	286,329
	Fine	🚲	0.82	0.72	0.96	0.67	Goel 2015	1.0	Brauer 1999	7	1/9	11%	285,286,295,306,311,325,329
	NO_2	🚶	1.09	1.06	1.12	1.06	Chertok 2004	1.12	Van Wijnen 1995	2	2/2	100%	287,330
							Reference category: pedestrians‡						
	BC	🚌	1.04			1.04	Moreno 2015			1	1/1	100%	337
🚌	CO	🚶	0.92	0.83	1.23	0.74	Duci 2003	3.25	Dirks 2012	7	10/24	42%	297,317,320,331,332,337,339
	Coarse	🚶	0.66	0.53	0.79	0.53	Saksena 2008	0.79	Liu 2015	2	0/2	0%	317,326
	Fine	🚶	0.89	0.76	1.26	0.60	Goel 2015	1.63	Moreno 2015	5	5/11	45%	314,316,325,326,337
🚗	CO	🚗	1.90	1.82	2.25	1.49	Duci 2003	2.90	Vellopolou 1998	4	9/9	100%	297,331,332,339
	Fine	🚶	0.66	0.55	0.77	0.55	Morabia 2009	0.77	Goel 2015	2	0/2	0%	325,336
	CO	🚗	2.18			2.18	Saksena 2008			1	1/1	100%	317
🚗 [V]	Coarse	🚶	0.75	0.68	0.95	0.21	Briggs 2008	1.13	Gulliver 2004	5	1/6	17%	313,317-319,326
	Fine	🚶	0.67	0.38	0.76	0.24	Goel 2015	1.03	Gulliver 2004	6	1/9	11%	313,316,318,319,325,326
	BC	🚗 [V]	1.32			1.32	Moreno 2015			1	1/1	100%	337
🚍	CO	🚶	0.37	0.34	0.70	0.31	Duci 2003	1.29	Moreno 2015	4	2/8	25%	320,331,332,337
	Coarse	🚶	0.63			0.63	Liu 2015			1	0/1	0%	326
	Fine	🚶	0.53	0.49	0.85	0.37	Goel 2015	1.53	Moreno 2015	6	3/9	33%	314,316,325,326,336,337
	CO	🚲 [M]	2.19	1.50	2.37	1.26	Vellopolou 1998	33.50	Dirks 2012	4	5/5	100%	297,317,331,339
🚲[M]	Coarse	🚲 [M]	1.17	1.17	1.17	1.17	Saksena 2008	1.17	Saksena 2008	1	1/1	100%	317
	Fine	🚲 [M]	1.08	0.98	1.14	0.88	Goel 2015	1.18	Goel 2015	1	3/4	75%	325
🚶(HT)†	BC	🚍	1.23	0.83	1.81	0.83	Moreno 2015	1.81	Moreno 2015	1	2/3	67%	337
	CO	🚍	1.29	1.14	1.57	1.14	Moreno 2015	1.57	Moreno 2015	1	3/3	100%	337

🚲 Cyclists 🚶 Pedestrians 🚗 Car driven without controlled ventilation settings 🚗 [V] Car driven with controlled ventilation settings 🚌 Bus 🚍 Massive motorized transport [M] Motorcycle. CO: Carbon monoxide, BC: Black carbon, NO_2: Nitrogen dioxide. ppm=parts per million. *Ratio of exposure higher than one means that the exposure of the MT is larger

than the exposure of the reference category (cyclists or pedestrians). †Comparison is cyclists/pedestrians in high traffic route (HT) vs. cyclists/pedestrians in low traffic route. ‡From 11 studies where cyclists were not measured, we used pedestrians as reference category

Appendix 56. Meta-analysis of ratio of pollutants exposure level according to MT

MT	Pollutant	Ratio	95% CI		I^2	Comparisons*	References
Reference category: Cyclists							
(HT)	Coarse	1.04	0.88	1.23	100%	1	296
	Fine	1.01	0.53	1.91	100%	1	296
	BC	1.29	0.95	1.75	96%	6	299,301,340,341
	CO	1.40	1.12	1.74	63%	7	300,302,306
	Coarse	1.12	0.75	1.67	93%	18	286,296,302,340
	Fine	1.05	0.94	1.19	90%	22	285,286,296,300,302,304,306,311,325,340
	NO_2	1.53	1.27	1.85	0%	2	312
	BC	1.38	1.15	1.65	91%	7	299,301,340,341
	CO	1.07	0.83	1.38	0%	2	306
	Coarse	0.92	0.64	1.34	94%	3	333,340
	Fine	1.05	0.89	1.25	80%	5	306,340
	NO_2	0.68			100%	1	312
	BC	1.32	0.13	13.25	99%	2	302,340,341
	CO	2.85	2.53	3.22	57%	10	287,300
[V]	Coarse	0.71	0.55	0.91	88%	18	284,296,302,340
	Fine	0.95	0.84	1.07	95%	30	284,285,296,300,302,304,305,311,340
	NO_2	0.77	0.48	1.25	88%	3	287
	BC	1.04	0.67	1.60	99%	6	299,301,341
	CO	1.00	0.21	4.74	0%	4	302
	Coarse	0.70	0.59	0.83	0%	11	286,302
	Fine	0.73	0.46	1.14	85%	8	286,302,304,311
	CO	3.00	0.53	16.96	0%	4	302
[M]	Coarse	0.75	0.55	1.04	0%	10	302
	Fine	0.84	0.79	0.90	0%	8	302,311,325
	BC	0.92	0.81	1.03	79%	5	299,301,341
	CO	0.91	0.78	1.05	0%	3	287,306
	Coarse	0.83	0.67	1.03	100%	1	286
	Fine	0.82	0.71	0.94	66%	7	285,286,306,311,325
	NO_2	1.12	0.79	1.58	100%	1	287
Reference category: Pedestrians							
	BC	1.04	0.55	1.95	100%	1	337
	CO	0.92	0.86	0.99	74%	21	317,331,332,337
	Coarse	0.66	0.45	0.98	86%	2	317,326
	Fine	0.90	0.76	1.06	79%	10	314,316,325,326
	CO	1.77	1.64	1.92	36%	7	331,332
	Fine	0.77			100%	1	325
	CO	2.18	0.03	141.99	100%	1	317
[V]	Coarse	0.66	0.47	0.95	97%	6	317-319,326
	Fine	0.54	0.39	0.73	95%	9	316,318,319,325,326
	BC	1.32	0.73	2.39	100%	1	337
	CO	0.48	0.34	0.70	97%	7	331,332,337
	Coarse	0.63	0.61	0.65	100%	1	326
	Fine	0.58	0.45	0.74	70%	7	314,316,325,326
	CO	1.75	1.22	2.52	47%	2	317,331
[M]	Coarse	1.17	0.94	1.46	100%	1	317
	Fine	0.97	0.86	1.11	0%	4	325
	BC	1.26	0.82	1.92	72%	3	337
(HT)	CO	1.33	1.10	1.61	32%	3	337

🚲/🚶 (HT) Cyclists/pedestrians exposure over a high traffic route, the reference is low traffic route. 🚶 Pedestrians 🚗 Car driven without controlled ventilation settings [V] Car driven with controlled ventilation settings 🚌 Bus 🚍 Massive motorized transport 🏍 [M] Motorcycle. BC: Black carbon. CO: Carbon monoxide. NO_2 Nitrogen dioxide. 95% CI: 95% level confidence interval. IQR: Interquartile range (between 25^{th} and 75^{th} percentiles) I^2 describes the percentage of variation across comparisons that corresponds to heterogeneity rather than chance[292]. The highest the I^2 indicates a higher heterogeneity. *Some meta-analyses were performed with less comparisons that the corresponding exposure ratios, because of incomplete reporting of sample size and variance, required for estimation of pooled ratios.

Appendix 57. Parameters of pollutant inhalation/dose uptake estimation

Author	Pollutant parameters	Respiratory parameters	Time parameters	Inhaled dose	Uptake dose
de Nazelle, 2012 [295]	Average exposure concentration	Estimated; inhalation rates estimated using EPA algorithms, based on average measured energy expenditure (METS) Energy expenditure was measured during the trips in different modes	Duration of trip exposure (minutes)	Inhaled dose (µg*trip)=inhalation rate * concentration (arithmetic mean) * duration of exposure * conversion factor for each pollutant	
		Mode / Inhalation rates (L/min)		CO (µg/trip) / BC (µg/trip) / $PM_{2.5}$(µg/trip)	
		🚲 41	25	3.2 / 8.7 / 32.8	
		🚶 34.1	49	4 / 10.70 / 35.1	
		🚌 20.1	20.1	3 / 5.3 / 18	
		🚗 19.9	19.9	8.9 / 10.4 / 19.7	
Dirks, 2012 [297]	Average exposure concentration	Assumed; based on previous literature. Exercise factor was estimated as the ratio of minute ventilation factor for the mode vs. Sedentary modes (12L/min)	Recorded commute time (hours)	Dose (ppm*h)= concentration of pollutant (ppm) * commute time (h) * exercise factor	
				Pollutant: CO (ppm*hr.)	
		Mode / Exercise factor (times)	Route 1 / Route 2 / Route 3	Route 1 / Route 2 / Route 3	
		🚲 3	0.73±0.004	1.4 ±0.2	
		🚶 4	0.82 ±0.04 / 1.11±0.12	1.2 ±0.7 / 1.6 ±1.3	
		🚌 1	0.52 ±0.07 / 0.80 ±0.08 / 0.80 ±0.10	0.7±0.2 / 0.2 ±0.2 / 0.3 ±0.3	
		🚗 1	0.433 ±0.12 / 0.58 ±0.16 / 0.33±0.08	0.4±0.3 / 1.2 ±0.4 / 0.3 ±0.2	
		🚇 1	0.63±0.05	0.2 ±0.4	
Dons 2011 [298] Dons 2012 [299]	Average exposure concentration	Assumed, based on previous literature [VE (L/min)]	Time recorded per mode (5-min observations)	Dose ratio (µg/min). Dose defined using minute ventilation per activity and per transport mode.	
		Mode / Male / Female		Morning rush hour (7-10AM) / Evening rush hour (4-7PM) / Non-rush hour	
		🚲 59.1 / 46.2	1167	0.46 / 0.52 / 0.32	
		🚶 49.2 / 39.8	1161	0.61 / 0.65 / 0.46	
		🚌 16.1 / 13.0	190	0.73 / 0.94 / 0.89	
		🚗 13.4 / 11.3	3835	Ref / Ref / Ref	
		🚇 16.1 / 13.0	686	2.57 / 2.38 / 1.92	

Author	Pollutant parameters	Respiratory parameters	Time parameters	Inhaled dose	Uptake dose
Huang, 2012 [300]	Average exposure per trip	Assumed, based on previous literature. Based on activity level of every MT: moderate intensity activity for AT and light intensity for motorized transport Mode: Inhalation rate ($*10^{-2}$ m^3/min) 2.60 1.10 1.10	Trip duration (minutes) Heavy traffic times: 35.29; 37.23; 26.56 Light traffic times: 32.79; 24.65; 18.75	Average whole trip exposure=trip averaged concentration * trip duration * inhalation rate Traffic time: Heavy / Light / Heavy / Light / Heavy / Light $PM_{2.5}$ (μg): 43.2 ±5.87; 33.68 ±5.71; 30.32 ±17.37; 15.27 ±6.46; 15.78 ±11.06; 6.78 ±4.93 CO (mg): 2.11 ±0.35; 1.31 ±0.32; 1.52 ±0.76; 0.66 ±0.16; 2.16 ±0.83; 0.97 ±0.20	
IntPanis, 2010 [284]	$PM_{2.5}$ or PM_{10} mass	Estimated, based on the equation VE=breathing frequency * tidal volume. These were measured with a portable cardiopulmonary indirect breath-by-breath calorimetry system, while standing and during exercise. Minute ventilation (L/min) Mode: Male / Female 59.1 ±13.7 / 46.2 ±10.6 13.4 ±1.7 / 11.3 ±1.8	Journey duration (minutes) City: Male / Female Brussels: 15.4 ±1.3 / 17.6±1.9 LLN: 16.3 ±1.2 / 14.7 Mol: 18.8 ±2.8 / 21.1±2.0 Brussels: 16.2±3.6 LLN: °1 / 10.8 Mol: 10.2±1.24	Inhaled pollutants=$PM_{2.5}$ or PM_{10} mass * minute ventilation PM_{10} (μg/km): 11.5±4.5; 8.4±1.6; 8.5±0.2; 1.6±0.6; 0.9±0.1; 1.2±0.2 $PM_{2.5}$ (μg/km): 3.4±1.3; 3.8±0.8; 5.2±0.2; 0.6±0.2; 0.5±0.1; 0.7±0.1	Lung deposited fraction based on deposition factor (deposition fraction decrease with particle size and increase with tidal volume, increases with exercise). Mass based deposition fraction for $PM_{2.5}$ and PM_{10} was obtained from evidence: 0.23 ±0.03 PM_{10} (μg/km): 2.6; 1.9; 1.9; 0.4; 0.2; 0.3 $PM_{2.5}$ (μg/km): 0.8; 0.9; 1.2; 0.1; 0.1; 0.1
Li, 2015 [301]	Average concentration per	Assumed, based on previous literature	Journey duration (hours)	Inhalation dose (μg)=average concentration in a microenvironment or a journey (μg/m^3) * respiratory rate * duration exposure during a journey (h)	

Author	Pollutant parameters	Respiratory parameters		Time parameters	Inhaled dose	Uptake dose	
	microenvironment or journey	Mode	Respiratory rate (m^3/h)		Pollutant: Black carbon (µg)		
		🚆	0.47	0.44	0.95±0.29		
		🚌	0.47	0.21	1.50±0.39		
		🚗	0.47	0.17	0.68±0.33		
		🚶	0.63	0.45	1.58±0.29		
		🚲	0.70	0.30	1.36±0.37		
McNabola, 2008 [285]	Exposure concentration	NS		Average trip time Mode / Time (minutes) 🚶 25-30 🚲 20 🚗 20-45 🚌 20-45		Total absorption of pollutants in the lung cumulatively during the exposure duration: based on HRT model† $PM_{2.5}$ total absorption/deposition (µg) 17.35 23.06 16.73 21.83	
Nyhan, 2014 [286]	PM exposure concentration	Estimated; Minute ventilation was estimated based on heart rate, following the equation VE=exp(c+m*HR). Heart rate (HR) was monitored with Actiheart units placed on participants` chest. C is the intercept (male 1.03 and female 0.57), m is the slope (0.021 male and 0.023 female).		Mean trip time (minutes)	Inhaled dose=derived VE * PM exposure concentration * time (min). Total inhaled dose obtained by summing up the dose each two minutes over the entire commute.		Total dose used to estimate HRT model† Same respiratory parameters as inhaled pollutant estimation, based on heart rate.
		Mode	Relative change in heart rate compared to baseline (bpm):		PM_{10} (µg/trip)	$PM_{2.5}$ (µg/trip)	PM_{10} (µg/trip)	$PM_{2.5}$ (µg/trip)
		🚲	+47.2	37.3 ± 9.5	65.0 ± 45.5	52.6±44.1	45.6 ±29.8	36.8±36.5
		🚶	+36.1	40.8 ± 9.2	40.1 ± 27.1	26.7±27.3	28.10±18.9	18.7±17.2
		🚌	+8.7	34.9 ± 10.1	20.3 ± 11.4	11.0±6.8	14.2±8.0	7.7±5.10
		🚆	+8.0	25.7 ± 8.2	21.4 ± 11.1	20.6±12.7	14.9±7.8	14.4±11.1

Author	Pollutant parameters	Respiratory parameters		Time parameters				Inhaled dose				Uptake dose
		Assumed, based on EPA handbook estimates		Average trip time				Dose (µg/km)=(average concentration of the pollutant*minute ventilation*time in round trip)/distance of the route				
		Mode	Minute ventilation (L/min)	8AM	11AM	14PM	17.30PM	21PM	Hour	CO	$PM_{2.5}$	PM_4	PM_{10}	
Ramos, 2016 [302]	Average concentration of the pollutant								8	39.17	2.57	2.94	3.43	
									11	4.34	1.88	2.08	2.32	
		🚌	13.9	52±2.8	55±8.8	52±12	46±4.8	38±15	14	3.22	2.45	2.69	3.04	
									17.30	10.55	3.0	3.21	3.60	
									21	3.77	1.56	1.69	1.88	
									8	84.45	4.89	5.64	6.15	
									11	40.25	2.36	2.53	3.09	
		🚐	13.9	91±7.4	81±5.9	87±19	64±21	63±26	14	45.26	4.40	4.71	5.69	
									17.30	42.33	3.15	3.31	3.77	
									21	6.84	2.42	2.50	2.74	
									8	43.95	2.16	2.28	2.60	
									11	28.79	1.82	1.90	2.17	
		🚗	13.9	37±2.4	34±2.7	39±3.9	46±2.5	32±10	14	31.31	1.99	2.07	2.31	
									17.30	33.92	1.83	1.89	2.06	
									21	7.35	1.08	1.12	1.21	
									8	63.52	28.01	29.13	33.09	
									11	64.18	23.81	24.25	25.35	
		🚲	55.9 (Assumed as high intensity activity)	82±33	86±10	88±1.9	94±17	51±60	14	38.13	15.38	15.77	25.35	
									17.30	78.22	25.23	25.97	27.83	
									21	0.02	13.04	16.31	16.88	
Van Wijnen, 1995 [287]		Measured. Exhaled air was measured continuously with a gas-meter while covering the routes by car or by bicycle during 20-30min; heart rate measured semi-continuously with heart rate monitor												1-h averaged uptake of contaminants of participants on the inner city routes, adjusted for the amount of exhaled air per minute - µg
		Mode	Mean exhaled air volume (L/min)											CO (µg) / NO_2 (µg)
		🚲	28.7 ± 4.4											2632-3217 / 146-199
		🚌	12.3 ± 1.8											2734-3672 / 48-64

Author	Pollutant parameters	Respiratory parameters		Time parameters			Inhaled dose				Uptake dose
		Assumed, based on inhalation rates reported in the literature		Journey duration (minutes)			Average inhaled dose=inhalation rates*average concentration*trip time (min)				
		Mode	Respiratory rates (L/min)	Route 1	Route 2	Route 3	Route	$PM_{2.5}$ (μg/trip)	PM_{10} (μg/trip)	BC (μg/trip)	
Vouitsis, 2014 [340]	Average exposure concentration	🚲	41	26	27	30	1	85.5	149.3	11.1	
							2	50.3	63.2	-	
							3	50.2	58.6	4.5	
		🚌	20.1	28	37	34	1	56.3	78.2	4.1	
							2	77.8	157.2	4.6	
							3	28.2	36.9	2.1	
		🚗	19.9	24	22	25	1	33.4	40.2	4.3	
							2	23.8	32.1	4.7	
							3	16.4	21.2	2.4	
		🚗 [V]	19.9	21	22	23	1	20.5	24.5	1.7	
							2	11.3	13.5	1.7	
							3	6.8	7.3	1.7	
		Estimated; minute ventilation estimated based on heart rates measured while commuting (heart rate monitors). Also, participants performed submaximal bicycle ergometer test to measure heart rate and minute ventilation					Inhaled dose estimated with mean ventilation rates				
Zuurbier, 2010 [296]	Median exposure corrected by background concentration	Mode	Average minute ventilation (L/min)	Doses estimated as values of 2-hr mean			PM2.5 (μg/hr.)		PM10 (μg/hr.)		
		🚌[Diesel]	12.7				55.7		46.1		
		🚌[Electric]	12.7				45.6		43.2		
		🚗[Diesel fueled]	11.8				51.9		32.0		
		🚗[Gasoline fueled]	11.8				62.3		30.0		
		🚲[High traffic]	23.5				100.4		50.2		

Author	Pollutant parameters	Respiratory parameters		Time parameters		Inhaled dose	Uptake dose
		🚲[Low traffic]	23.5	93.3	52.5		

🚲 Cyclists 🚶 Pedestrians 🚗 Car driven without controlled ventilation settings 🚗 [V] Car driven with controlled ventilation settings 🚌 Bus 🚍 Massive motorized transport. L/min: liters per minute. m^3/h: cubic meters per hour. bpm: beats per minute. †Human respiratory tract model (HRT) model includes concentration of the pollutant, duration of exposure, breathing parameters, physical and chemical properties of solubility and diffusivity of the pollutant.

Appendix 58. Gains of years of life expectancy per age-group due to exposure to air pollution and physical inactivity, compared between any MT vs. bicycle (A) or walk (B) commuting of a 7km route per week*

Mode of transport	Age-group (years)	Comparisons	Gains in YLE due to air pollution exposure †			Gains in YLE due to physical activity †			Net gain in YLE			Comparisons in favor of AT‡		References
			Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile			
Baseline scenario=Cyclists														
🚌	20-39	29	0.09	0.06	0.30	-0.69	-0.72	-0.67	-0.59	-0.65	-0.42	27	(93%)	285,286,295,296,300,302,304,306,307,311,325,340
	40-64		0.09	0.05	0.26	-0.61	-0.61	-0.58	-0.53	-0.57	-0.36	26	(90%)	
	65+		0.05	0.03	0.16	-0.36	-0.37	-0.33	-0.31	-0.35	-0.21	26	(90%)	
🚗	20-39	11	0.16	0.11	0.19	-1.62	-1.62	-1.57	-1.46	-1.52	-1.40	11	(100%)	295,304,306,307,340
	40-64		0.14	0.10	0.17	-1.44	-1.44	-1.35	-1.29	-1.35	-1.22	11	(100%)	
	65+		0.09	0.06	0.10	-0.85	-0.85	-0.74	-0.76	-0.80	-0.66	11	(100%)	
🚗 [V]	20-39	30	0.25	0.13	0.37	-1.57	-1.67	-1.55	-1.36	-1.44	-1.21	30	(100%)	284,285,296,300,302,305,311,340
	40-64		0.22	0.12	0.32	-1.40	-1.46	-1.40	-1.21	-1.28	-1.08	30	(100%)	
	65+		0.13	0.07	0.19	-0.81	-0.83	-0.81	-0.65	-0.74	-0.61	30	(100%)	
🚊	20-39	11	0.09	0.06	0.36	-0.72	-0.72	-0.69	-0.57	-0.66	-0.35	11	(100%)	286,302,304,307,311
	40-64		0.08	0.05	0.32	-0.61	-0.62	-0.61	-0.50	-0.56	-0.30	11	(100%)	
	65+		0.05	0.03	0.19	-0.36	-0.37	-0.35	-0.30	-0.33	-0.17	11	(100%)	
🚲[M]	20-35	8	0.38	0.35	0.98	-1.69	-1.72	-1.69	-1.29	-1.34	-0.72	7	(88%)	302,311,325
	40-64		0.33	0.30	0.76	-1.46	-1.46	-1.32	-1.11	-1.16	-0.58	7	(88%)	
	65+		0.19	0.18	0.34	-0.83	-0.83	-0.55	-0.62	-0.65	-0.24	7	(88%)	
Total - Motorized transport	20-39	89	0.19	0.09	0.36	-1.55	-1.72	-1.69	-1.20	-1.41	-0.59	86	(97%)	
	40-64		0.17	0.08	0.32	-1.35	-1.46	-1.32	-1.01	-1.23	-0.53	85	(96%)	
	65+		0.10	0.05	0.19	-0.74	-0.83	-0.55	-0.55	-0.70	-0.31	85	(96%)	
🚶	20-39	8	-0.30	-0.56	-0.14	5.30	-4.17	-3.96	4.87	4.50	5.28	0	(0%)	285,286,295,306,311,325
	40-64		-0.25	-0.47	-0.13	4.62	-3.28	-2.99	4.35	3.81	4.88	0	(0%)	
	65+		-0.11	-0.25	-0.08	3.21	-1.47	-0.99	3.05	2.04	3.55	0	(0%)	
Baseline scenario=Pedestrians														
🚌	20-39	11	0.66	0.45	1.51	-4.17	-4.88	-4.91	-3.35	-3.44	-2.66	11	(100%)	314,316,325,326,337
	40-64		0.56	0.38	1.14	-3.23	-3.75	-4.05	-2.72	-2.84	-1.85	11	(100%)	
	65+		0.28	0.20	0.45	-1.41	-1.57	-2.02	-1.18	-1.22	-0.54	11	(100%)	
🚗	20-39	2	1.44	0.27	2.61	-4.88	-4.66	-4.66	-3.44	-4.65	-2.23	2	(100%)	325,336
	40-64		1.11	0.23	1.98	-3.75	-3.79	-3.89	-2.64	-3.82	-1.47	2	(100%)	

Mode of transport	Age-group (years)	Comparisons	Gains in YLE due to air pollution exposure †			Gains in YLE due to physical activity †			Net gain in YLE			Comparisons in favor of AT‡		References
			Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile			
🚗 [V]	65+	9	0.48	0.13	0.82	-1.57	-1.66	-2.10	-1.09	-1.89	-0.30	2	(100%)	[313,316,318,319,325,326]
	20-39		0.86	0.15	0.93	-4.66	-4.01	-4.17	-3.81	-4.35	-3.74	9	(100%)	
	40-64		0.73	0.13	0.78	-3.79	-3.28	-3.51	-3.07	-3.76	-3.01	9	(100%)	
	65+		0.39	0.08	0.42	-1.66	-1.47	-1.79	-1.28	-2.01	-1.25	9	(100%)	
🚌	20-39	9	0.48	0.34	0.95	-4.01	-4.88	-4.91	-3.45	-3.75	-3.06	9	(100%)	[314,316,325,326,336,337]
	40-64		0.41	0.29	0.80	-3.28	-3.75	-4.05	-2.86	-3.15	-2.48	9	(100%)	
	65+		0.25	0.15	0.43	-1.47	-1.57	-2.02	-1.22	-1.64	-1.04	9	(100%)	
🚲[M]	20-35	4	2.08	1.55	2.58	-4.84	-4.84	-4.84	-3.43	-3.79	-2.66	4	(100%)	[325]
	40-64		1.58	1.16	1.95	-3.45	-3.45	-3.45	-2.84	-3.20	-1.85	4	(100%)	
	65+		0.64	0.46	0.80	-1.12	-1.12	-1.12	-1.21	-1.67	-0.54	4	(100%)	
Total - Motorized transport	20-39	35	0.87	0.42	1.61	-4.23	-4.66	-4.01	-3.43	-3.79	-2.66	35	(100%)	
	40-64		0.73	0.37	1.21	-3.45	-3.79	-3.23	-2.84	-3.20	-1.85	35	(100%)	
	65+		0.37	0.19	0.48	-1.47	-1.90	-1.12	-1.21	-1.67	-0.54	35	(100%)	

C=Comparisons. YLE: Years of life expectancy. *Parameters of estimation are shown in Appendix 49 (page 198). Baseline life table was estimated from mortality rates for the indicated age-groups reported by the Global Burden of Disease in 2013 for both sexes for the country of each study included in the analysis (Belgium, Chile, China, Greece, India, Ireland, Netherlands, Portugal, Spain, Taiwan, Turkey, United Kingdom and United States) [343]. †A positive gain means years of life expectancy gained in comparison to the reference category (cyclists or pedestrians). A negative gain means years of life expectancy lost in comparison to the reference category (cyclists or pedestrians). ‡In favor of AT means that losses in life expectancy due to lower physical activity (scenario A: cycling, scenario B, walking) are larger than gains due to higher inhaled dose of fine particles.

Appendix 59. Sensitivity analysis of gains of years of life expectancy per age-group due to exposure to air pollution and physical inactivity, compared between any MT vs. bicycle (A) or walk (B) commuting of a 3.5km route per week*

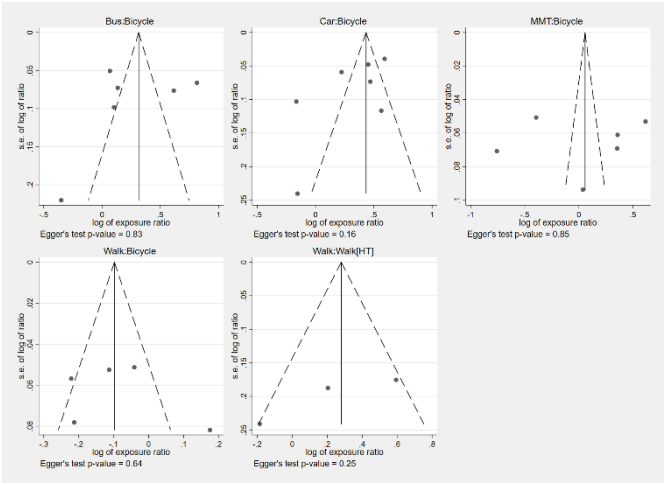
Mode of transport	Age-group (years)	Comparisons	Gains in YLE due to air pollution exposure †			Gains in YLE due to physical activity †			Net gain in YLE			Comparisons in favor of AT‡	
			Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile		
Baseline scenario=Cyclists													
	20-39	29	0.05	0.03	0.15	-0.28	-0.30	-0.28	-0.24	-0.27	-0.15	25	(86%)
🚌	40-64	29	0.04	0.03	0.13	-0.25	-0.25	-0.24	-0.21	-0.23	-0.13	25	(86%)
	65+	29	0.03	0.02	0.08	-0.15	-0.16	-0.14	-0.13	-0.15	-0.08	25	(86%)
	20-39	11	0.08	0.06	0.10	-0.69	-0.69	-0.67	-0.61	-0.64	-0.58	11	(100%)
🚗	40-64	11	0.07	0.05	0.08	-0.62	-0.62	-0.58	-0.54	-0.57	-0.51	11	(100%)
	65+	11	0.05	0.03	0.05	-0.38	-0.38	-0.33	-0.33	-0.35	-0.29	11	(100%)
	20-39	30	0.13	0.07	0.19	-0.67	-0.71	-0.66	-0.56	-0.60	-0.49	30	(100%)
🚗 [V]	40-64	30	0.11	0.06	0.16	-0.60	-0.63	-0.60	-0.50	-0.54	-0.44	30	(100%)
	65+	30	0.07	0.04	0.10	-0.36	-0.37	-0.36	-0.28	-0.32	-0.26	30	(100%)
	20-39	11	0.05	0.03	0.18	-0.30	-0.30	-0.28	-0.23	-0.27	-0.12	11	(100%)
🚌	40-64	11	0.04	0.02	0.16	-0.25	-0.26	-0.25	-0.20	-0.23	-0.10	11	(100%)
	65+	11	0.03	0.01	0.09	-0.15	-0.16	-0.15	-0.12	-0.13	-0.06	11	(100%)
	20-39	8	0.19	0.18	0.50	-0.72	-0.73	-0.72	-0.52	-0.54	-0.23	6	(75%)
🚲[M]	40-64	8	0.17	0.15	0.38	-0.63	-0.63	-0.56	-0.45	-0.47	-0.19	6	(75%)
	65+	8	0.10	0.09	0.17	-0.37	-0.37	-0.24	-0.26	-0.27	-0.09	6	(75%)
Total -	20-39	89	0.10	0.04	0.18	-0.66	-0.73	-0.72	-0.48	-0.58	-0.24	83	(93%)
Motorized	40-64	89	0.08	0.04	0.16	-0.58	-0.63	-0.56	-0.41	-0.51	-0.21	83	(93%)
transport	65+	89	0.05	0.02	0.09	-0.33	-0.37	-0.24	-0.23	-0.30	-0.13	83	(93%)
	20-39	8	-0.14	-0.25	-0.06	1.86	1.82	1.97	1.67	1.27	1.79	0	(0%)
🚶	40-64	8	-0.12	-0.21	-0.06	1.61	1.49	1.67	1.39	1.05	1.59	0	(0%)
	65+	8	-0.05	-0.12	-0.04	0.95	0.60	1.08	0.83	0.45	1.04	0	(0%)
Baseline scenario=Pedestrians													
	20-39	11	0.34	0.23	0.80	-1.87	-1.87	-1.79	-1.47	-2.12	-0.82	11	(100%)
🚌	40-64	11	0.29	0.20	0.59	-1.46	-1.49	-1.37	-1.17	-1.78	-0.57	11	(100%)
	65+	11	0.15	0.10	0.23	-0.69	-0.72	-0.49	-0.56	-0.96	-0.15	11	(100%)
	20-39	2	0.77	0.14	1.40	-2.25	-2.27	-2.22	-1.67	-2.00	-1.64	2	(100%)
🚗	40-64	2	0.59	0.12	1.05	-1.76	-1.90	-1.62	-1.38	-1.76	-1.35	2	(100%)
	65+	2	0.24	0.07	0.42	-0.80	-1.03	-0.57	-0.65	-1.02	-0.63	2	(100%)

Mode of transport	Age-group (years)	Comparisons	Gains in YLE due to air pollution exposure †			Gains in YLE due to physical activity †			Net gain in YLE			Comparisons in favor of AT‡	
			Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile		
	20-39	9	0.46	0.08	0.50	-2.13	-2.13	-2.07	-1.51	-1.66	-1.30	9	(100%)
[V]	40-64	9	0.39	0.07	0.42	-1.77	-1.83	-1.77	-1.27	-1.42	-1.07	9	(100%)
	65+	9	0.20	0.05	0.22	-0.85	-1.07	-0.85	-0.60	-0.80	-0.51	9	(100%)
	20-39		0.25	0.18	0.49	-1.80	-1.87	-1.79	-1.11	-1.40	-0.84	9	(100%)
	40-64	9	0.21	0.15	0.42	-1.49	-1.61	-1.46	-0.78	-1.00	-0.58	9	(100%)
	65+		0.13	0.08	0.22	-0.72	-0.88	-0.69	-0.24	-0.33	-0.16	9	(100%)
	20-39		1.12	0.83	1.38	-2.22	-2.22	-2.22	-1.50	-1.69	-1.08	4	(100%)
[M]	40-64	4	0.84	0.62	1.04	-1.62	-1.62	-1.62	-1.26	-1.44	-0.77	4	(100%)
	65+		0.33	0.24	0.41	-0.57	-0.57	-0.57	-0.59	-0.82	-0.26	4	(100%)
Total -	20-39		0.46	0.22	0.86	-1.91	-2.13	-1.80	-1.50	-1.69	-1.08	35	(100%)
Motorized	40-64	35	0.39	0.19	0.64	-1.62	-1.77	-1.46	-1.26	-1.44	-0.77	35	(100%)
transport	65+		0.19	0.10	0.25	-0.72	-0.93	-0.57	-0.59	-0.82	-0.26	35	(100%)

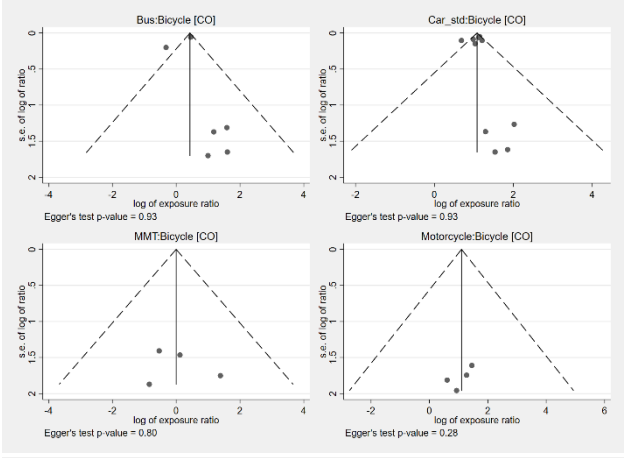
*Relative risk of physical activity changes to 0.8, due to less time of physical activity [303]

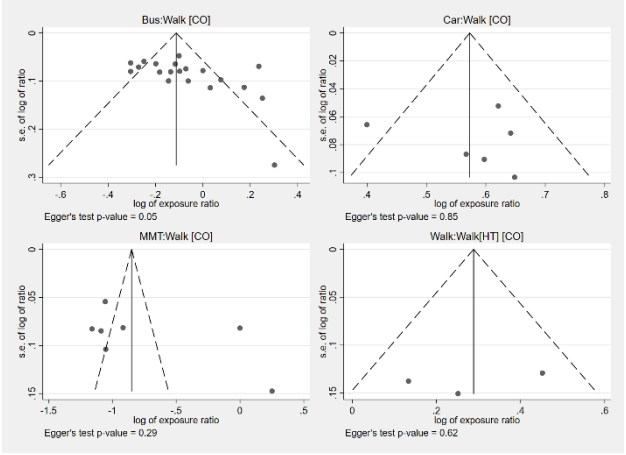
Appendix 60. Funnel plots for publication bias assessment, according to mode of transport and pollutant

a) BC

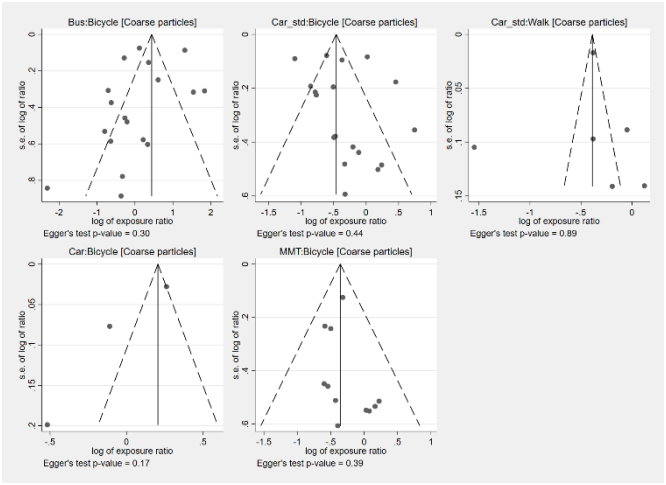


b) CO

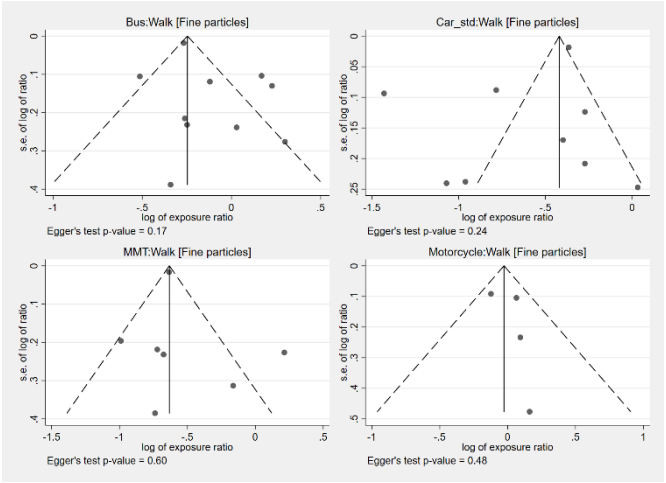


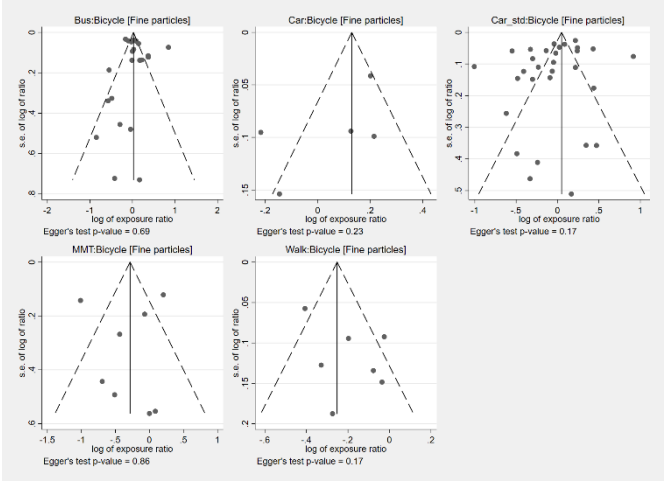


c) Coarse particles

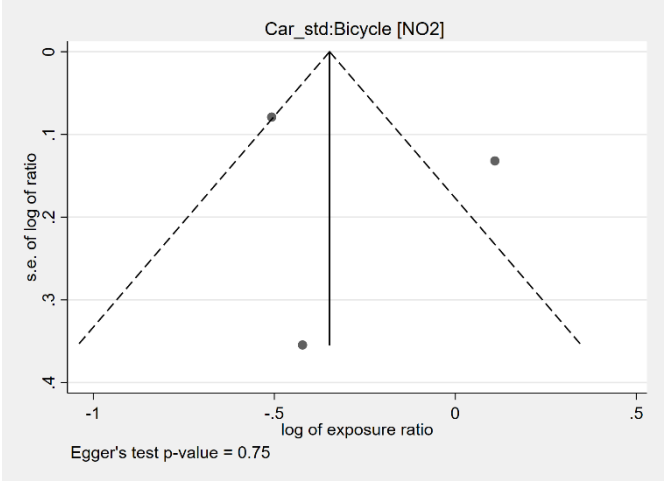


d) Fine particles





e) NO2


Car_std:Bicycle [NO2]
s.e. of log of ratio
0
.1
.2
.3
.4
-1
-.5
0
.5
log of exposure ratio
Egger's test p-value = 0.75

3.1.2 Exposure and inhaled dose of ultrafine particles according to mode of transport: systematic review

Magda Cepeda, Josje D. Schoufour, Rosanne Freak-Poli, Chantal Koolhaas, Klodian Dhana, Mònica Guxens, Oscar H. Franco

ABSTRACT

Introduction: There is increasing interest concerning the toxicity of ultrafine particles, however differences in exposure to ultrafine particles according to mode of transport and differences in inhalation dose remain unclear. In this systematic review we aimed to compare the exposure to ultrafine particles according to mode of transport, calculate the potential differences in the inhalation and dose, and examine if these differences differed according to World Health Organization (WHO) region.

Methods: We searched ten online datasets from inception to January 27th 2018 without language or temporal restrictions. We searched for cohort, cross-sectional, and experimental studies that compared the exposure to ultrafine particles was measured in at least one motorized (car, massive motorized transport (MMT), tram, bus) and at least one active (pedestrian and cyclist) mode of transport. We excluded studies that measured air pollution exposure exclusively with biomarkers, or on the basis of simulated data, reviews, comments, consensuses, editorials, guidelines, in-vitro studies, meta-analyses, ecological studies, and protocols. We extracted average exposure and commuting time per mode of transport. Within each study, we calculated exposure ratios using active commuters as reference categories. We also calculated potential inhalation of ultrafine particles per mode of transport and the corresponding inhalation ratios per study. The exposure and inhalation ratios were summarized as medians and interquartile ranges, for all studies and stratified according to WHO region.

Results: Out of 2,277studies identified, we included 28 studies. Car and bus commuters were more likely to exhibit higher exposure to ultrafine particles than active commuters (median ratio=1.23 [interquartile range (IQR)=0.72, 1.46] and 1.11 [IQR=0.72, 1.46], respectively), while MMT and tram commuters were less likely to exhibit higher exposure (0.86 [IQR=0.45, 1.48] and 0.62 [0.38, 0.79]). Reducing air interchange through closed windows and air conditioning reduced the exposure among car commuters (0.79 [IQR=0.47, 1.08]). However, active commuters (i.e. those walking or biking) inhaled a larger dose of ultrafine particles than motorized commuters, due to higher breathing rate and commuting time.

Conclusions: Car and bus commuters are exposed to more ultrafine particles, but active commuters inhaled more pollutants. However, the inhalation of ultrafine particles was larger among active commuters.

INTRODUCTION

The negative health effects of exposure to particulate matter with a diameter below 10 and 2.5µm have been widely documented.[282,344] Nevertheless, within the complex mixture of air pollution, particles of smaller size, such as ultrafine particles (diameter < 0.1µm),[345] may have a toxicity independent of the effects of larger particles. Such toxicity is partly attributed to its enhanced deposition efficiency, given its small size, what contributes to reach more organs and tissues in the body.[346] Additionally, because ultrafine particles account for up to 80% of the particulate matter number,[347-349] these have a larger interaction surface carrying toxic components.[345] Most of the ultrafine particles are known as accumulation mode (size about 0.03-0.5µm), which are mostly produced in traffic during the combustion of diesel and petrol engines.[345,347,348] Ultrafine particles can also be found as nucleation mode particles (size below 0.03µm),[347] which are mostly generated in the environment under warm and dry conditions and high concentration of sulfur dioxide.[347,349] The concentration of ultrafine particles is strongly determined by traffic characteristics,[50,350] and up to 50% of the daily exposure occurs in proximity to traffic, for example while commuting.[345,351]

Ultrafine particles besides having a high deposition efficiency are also slowly cleared and retained longer than coarser particles, leading to chronic tissue damage under repeated exposure.[345]. However, it remains unclear if the mode of transport confers any advantage in terms of exposure to ultrafine particles. For example, car and bus commuters would be more exposed to freshly emitted particles given their position in the traffic line.[50] However, since pedestrians and cyclists have a higher respiratory rate than motorized transport commuters, they would inhale more particles. Previously, we showed that the mortality risk due to the increased exposure to air pollutants with a diameter of less than 2.5 micrometers ($PM_{2.5}$) would be offset by the benefits of increased physical activity [352] and a meta-analysis of health impact assessment studies identified that the gains of active commuting were driven mostly by physical activity.[353] Also, de Nazelle et al performed a systematic review comparing the exposure to ultrafine particles,[50] but they did not examine the potential differences in inhalation dose and it was limited to studies performed in Europe. Therefore, we performed a systematic review with the aim to compare the exposure to ultrafine particles according to mode of transport and calculated the potential differences in the inhalation and dose.

METHODS

Search strategy

We conducted a systematic review of studies that compared the exposure to ultrafine particles between modes of transport among adult commuters. Ten databases (Embase, Medline, Cinahl, Cochrane, Web of science, Scopus, PubMed, Google Scholar, Proquest, and Scielo) were searched in cooperation with a medical information specialist to identify relevant studies; the search was last conducted on January 27th 2018. Terms related to air pollution (e.g. “air pollution”) or key terms of a detailed list of air pollutants (e.g. “ufp”, “particulate matter”, etc.) were combined with key terms of mode of transport (e.g. “traffic” “subway”, “car”, “bicycle”, “walk”). Full search strategies are provided in Appendix 61 (page 250).

Selection criteria

We selected all studies, independently of study design (observational and experimental) where personal ultrafine particle air pollution exposure was measured while commuting by at least one active and at least one motorized mode of transport. Ultrafine particles were defined as particles size below 0.01µm. Modes of transport were defined as walking, cycling, bus, massive motorized transportation (MMT, i.e. subway, metro and train), car (private or public). The mode of transport 'car' was stratified in two categories: "car[V]" for those that reported controlled ventilation settings (windows closed, air conditioning (A/C) on or off and/or air recirculation modes on or off) and "car" for those without controlled ventilation settings or not specified.

Furthermore, we selected studies conducted among healthy human adults. We excluded studies where exposure was measured exclusively by biomarkers or studies solely based on simulated data. We also excluded reviews, comments, consensuses, editorials, guidelines, in vitro studies, meta-analyses, ecological studies and protocols. We include all studies independently of language or temporal limits.

Study selection

All titles and abstracts were reviewed by two independent investigators to select those that would fulfill the selection criteria. Then, we retrieved the full text of all studies selected in this initial appraisal. Two authors reviewed independently the full text, to select those studies that fulfilled the selection criteria. We solved disagreements through discussion and consulting a third independent author. We also extracted the references of all the selected studies and of previous systematic reviews to find other potentially relevant references. Finally, we contacted experts in the field to identify further relevant references to consider.

Data extraction and quality assessment of the evidence

Data from each article was extracted in a purposely designed form. Data extracted included overall study design, measurement period, modes of transport, monitoring devices and range of detection, commuting time and number of measurements performed, city and country of the study. Additionally, we extracted the levels of exposure to ultrafine particles, according to mode of transport. If available, we extracted the most disaggregated summary and dispersion measures by season, day of measurement campaign, day period of monitoring (e.g. morning vs. afternoon), and type of route. If more than one summary measure was provided per strata, we extracted preferably arithmetic means, then geometric means, and then median. If available, we also extracted the summary and dispersion measures of inhalation dose and deposition (per hour or per trip), along with the model or procedures for calculation and the parameters used for the estimation. If more than one article was available for one study, we extracted data from the most complete report.

We also assessed the study quality in terms of comparability of the exposure measured between modes of transport, and the information provided to assess such comparability. We used a modified version of the Quality Newcastle-Ottawa scale of the studies for assessing the quality of observational studies (Appendix 48 (page 197)).

Statistical analysis

In order to improve the comparability of the exposure to ultrafine particles according to mode of transport across the selected studies, we calculated exposure ratios according to mode of transport within each study. The exposure ratios were calculated using the exposure measurements provided for each mode of transport, using the exposure of active commuters' (pedestrian or cyclists) as the reference. When both cyclists and pedestrians were measured, we used cyclists' as the reference. We prioritized the calculation of exposure ratios between modes over the same route, when more than one route was measured. Nevertheless, if no active commuter was measured in a given route, we used as reference the exposure of the active commuter with the highest exposure, as long as it was in the same city and in approximate the same period of time. We summarized the exposure ratios as medians and interquartile ranges per mode of transport, and we calculated the percentage of exposure ratios that were above one.

We also calculated inhalation ratios, using inhalation doses estimated with the exposure levels extracted from each study. The inhalation ratios were calculated for the same comparisons as in the previous step, except for two studies,[335,354] where no exposure levels were provided, but only exposure ratios. The inhaled doses were calculated using the formula: average exposure concentration (reported by authors; #/m^3) x minute ventilation (VE) (m^3/hr). We used VE suggested by U.S. EPA 2011[293] for each mode of transport. The inhalation ratios were also summarized as medians and interquartile range, according to mode of transport. To account for the different time that commuters of each mode of transport would require to complete the same route, we performed a sensitivity analysis recalculating the inhalation dose with the following formula: average exposure concentration (reported by authors; #/m^3) x minute ventilation (VE) (m^3/hr) x trip time (hour). To derive trip times per mode of transport we used Google Maps to calculate the time that would take to complete a route of approximately 3km in Rotterdam, the Netherlands. The calculated route can be followed by any mode of transport, except by bus, but we assumed the same time as MMT. The inhalation doses were also summarized as explained above.

We performed a subgroup analysis, in order to assess whether the exposure and inhalation ratios would differ according to the geographic region were the study were performed; we plotted the exposure and inhalation ratios using different vignettes for the region according to the WHO.[355] We also summarized the exposure and inhalation ratios according to WHO region.

Finally, we meta-analyzed the exposure ratios using random effect models;[291] heterogeneity was assessed with I^2, which describes the percentage of the variation across comparisons that is due to heterogeneity rather than chance [292]. We used funnel plots and Egger's test to assess publication bias. All tests were two- tailed and p-values below 0.05 were considered statistically significant. All analyses were performed in Stata (version 15.0).[72]

RESULTS

Overall studies characteristics and reporting quality

We included 28 studies in this systematic review (Figure 16 (page 244)). Six studies were performed in four American countries (AMR), 18 studies in nine European countries (EUR) and four studies in four West Pacific Region countries (WPR) (Table 20 (page 247)). No studies from

other WHO regions were identified. Bus commuting was examined in 18 studies, car commuting in 25 studies (in 10 without standard ventilation settings and in 19 with standard ventilation settings), MMT commuting in 9 studies, tram commuting in 4 studies, pedestrians' in 17 studies, and cyclists' in 17. In one study cyclists and pedestrians' exposure was merged in one category [356] and in other the exposure of all motorized commuters was merged in one category.[357] In one study motorcyclists were included, but no data on ultrafine particles was reported.[358] Overall exposure was highest among car commuters, followed by bus, cyclists, MMT, car [V], and tram commuters (Appendix 64 (page 254)). Pedestrians experienced the lowest exposure.

The study design was experimental in 24 studies and observational or mixed in the remaining. The experimental studies consisted in planned commutes where exposure was measured, with scripted routes and/timing. Observational studies consisted in measures obtained from volunteers during their regular commutes. Complete information about commuting standards was provided in 18 studies (Appendix 65 (page 255) and Appendix 66 (page 256)). The same routes were followed by all commuters in 13 studies and in four all commuters started the commute at the same time, whereas in 19 the commuters started around the same time frame or consecutively.

Comparison of exposure and inhaled dose of ultrafine particles according to mode of transport

Car commuters were more likely to experience higher exposure to ultrafine particles than active commuters (14/20 comparisons; median (IQR): 1.23 (0.98-1.61)), followed by bus (15/26; 1.11 (0.72-1.46)), and MMT commuters (5/11; 0.86 (0.45-1.48)) (Figure 17 (page 245) and Appendix 67 (page 266)). Car [V] and tram commuters were less likely to experience higher exposure to ultrafine particles than active commuters (15/44 comparisons (0.79 (0.47-1.08)) and 0/4 comparisons (0.62 (0.38-0.79)), respectively). Nevertheless, active commuters were more likely to inhale more ultrafine particles than car, car [V], and tram commuters in all comparisons. In 20/26 comparisons active commuters inhaled more particles than bus commuters (0.69 (0.42-0.86)) and in 10/11 than MMT commuters (0.51 (0.26-0.87)).

Pedestrians commuting in low traffic routes experienced a higher exposure to ultrafine particles than pedestrians commuting in high traffic routes or cyclists in 10/39 comparisons (0.83 (0.63-1.00)) (Figure 17 (page 245) and Appendix 67 (page 266)). Cyclists commuting in high traffic routes experienced higher exposure than cyclists commuting in low traffic routes in 2/2 comparisons (0.63 (0.45-0.81)).

Inhalation ratios while taking into account the time that would take the commuters to complete the same route were similar to those of the main analysis, as long as the reference mode of transport was cyclists (Appendix 68 (page 267)). When the reference was pedestrians, the inhalation ratio is farther from one, as pedestrians inhaled more particles due to their larger commuting time (about 30 minutes per trip) than motorized commuters (about 15 minutes).

Exposure ratios according to WHO region

Bus commuters were more likely to experience higher exposure than active commuters and the magnitude of the exposure ratio was larger in studies performed in AMR countries (median ratio = 1.93 (IQR = 1.11, 2.85), 4 studies) than in studies performed in EUR countries (0.91 (IQR =

0.72, 1.14), 11 studies) and WPR countries (1.3 (0.62, 1.57), 4 studies) (Appendix 68 (page 267)). Additionally, whereas most of the studies that measure car commuter exposure were performed in EUR and AMR (9/10 studies), car [V] commuters exposure was mostly measured in EUR studies (12/18 studies).

Meta-analysis and publication bias

The heterogeneity of meta-analyzed exposure ratios was close to 100% in all comparisons; compared to the median of exposure ratios, the direction was similar in all comparisons. Given the large heterogeneity observed we provide these findings only for informative purposes (Appendix 62 (page 252) and Appendix 63 (page 253)). We found evidence of publication bias for the comparison car [V] vs. active transport ($p<0.001$), car vs. active transport ($p=0.005$), walk vs. active transport ($p=0.041$) (Appendix 62 (page 252) and Appendix 63 (page 253)).

DISCUSSION

In general, car and bus commuters not using standard ventilation settings (i.e. windows closed and air conditioning on) had higher exposure to ultrafine particles than active commuters, while massive motorized transport (MMT) and tram commuters had lower exposure. Exposure ratios according to mode of transport were heterogeneous, due to factors that affect the emission rates, including local traffic load, speed, and fleet composition; factors of the built environment that affect the proximity to traffic, such as availability of cycle-routes; and factors that affect the dispersion, such as attributes of the built environment and meteorological factors. Nevertheless, under a hypothetical scenario of commuting over a standard route, active commuters inhaled more ultrafine particles than motorized commuters, due to higher breathing rate and commuting time.

Differences in exposure according to mode of transport were mostly determined by the proximity to traffic,[50,318] explained by the fast decay of ultrafine particles concentration once emitted. Consequently, the emissions of surrounding traffic explain the higher exposure of car and bus commuters.[359] In contrast, MMT exposure was generally lower than that of active commuters because they usually ride underground.[304,337,360,361] However, public transport commuters (i.e. bus, MMT, and tram) experienced high exposure levels while approaching and waiting at stations and stops. Car [V] commuters had a lower exposure ratio than car commuters by reducing the rate of air interchange between cabin and on-road by closing windows and turning on the air conditioning.[356,362] However, the largest reduction of car [V] commuters exposure was when using air recirculation and particulates filters, since these features contribute to remove ultrafine particles filtered inside the cabin at a low but steady rate from products of combustion [284] or surrounding emissions,[335,350] or rapidly filtering when bus doors were opened at stops.[314,363] Enclosed bus stops reduced the exposure to idling buses emissions.[358] Poorly ventilated MMT stations increased the exposure [314] to particles filtered from above-ground or generated in the abrasion of wheels, rails, and breaks. [314,360,364,365]

Exposure among active commuters was characterized by concentrations peaks [364] in proximity to highly emitting sources, either mobile, such as passing vehicles and mopeds,[305,359,360,363] especially in high traffic routes;[296] or static, such as construction dust,

restaurants or smoking by the curbside.[314,358,364] Cyclists and pedestrians had increased exposure when rode along to the motorized traffic due to lack of separate cycle routes and at intersections and elevated crossings.[305,358] Choosing alternative less trafficked routes reduced the exposure of active commuters (about 19% among pedestrians and 37% among cyclists; (Appendix 67 (page 266))).[305,354,356]

Local attributes and methodological characteristics explained part of the heterogeneity in the comparison of ultrafine particles' exposure according to mode of transport. Among local attributes, traffic, meteorological factors, and built environment influenced the concentration of ultrafine particles in the commuter microenvironment. Among meteorological factors, the exposure was high during rush traffic hours and around midday, especially in summer time, due to photochemical nucleation events [314,356] that occur under high sunlight and temperature and low relative humidity.[314,363] The exposure was also high under low ambient temperature, which favors the formation of particles at vehicle exhausts [363] and the persistence of the particles by low mixing heights and temperature inversions [356,363] and under high wind speed, which favors the dispersion and dilution of particles,[363] but also its resuspension.[347] Among built environment attributes, the exposure was high in canyon-like routes, where both accumulation mode and precursors of nucleation mode particles were trapped.[366] Methodological characteristics that influenced the heterogeneity of the comparisons were mainly the comparability of exposure between commuters and the range of particles size detected by the devices used to measure the exposure. First, the exposure comparability between commuters was low in the studies included in this review, because only two studies compared all modes of transport in the same route and commuting time and in 19 studies all commuters started the commute in the same time frame. The large temporal and geographical variability of ultrafine particles concentrations reduce the comparability of exposure between commuters when these are not measured in almost similar time and space. Nevertheless, these differences may also reflect real life scenarios, such as active commuters choosing quieter or shorter routes. Second, the lower limit of particles size detected by the devices used in 14 studies was 0.01μm, which is more sensitive to detect particles generated in nucleation events than devices with lower limit of 0.02μm, which were used in the remaining studies. This feature may become a source of bias in settings with frequent nucleation events.[347,363] Although particles generated by diesel and gasoline combustion are mostly larger than 0.02μm,[363] particles generated in nucleation events are smaller and may remain unnoticed in some commuters microenvironment as these coagulate and grow,[345] to become detectable only among commuters traveling by the curbside.[347]

The inhalation ratios were less heterogeneous; as active commuters would inhale more ultrafine particles than motorized in all regions. Our estimations of inhaled dose based on standard assumptions were in agreement with those calculated in nine studies.[284,285,295,302,340,367-370] (Appendix 70 (page 270)) Also, according to our assumptions, public transport commuters would inhale some more particles than private transport commuters due to the slightly higher breathing rate attributed to account for the active period while approaching the stations and stops. We also accounted in our sensitivity analysis for the larger time that would take pedestrians to complete route compared to motorized commuters and cyclists. As a consequence, the inhalation rates where pedestrians' inhalation was reference were farther from one than those where cyclists'

inhalation was reference. Nevertheless, the inhalation dose of cyclists may be underestimated in our analysis because it is usually assumed that pedestrians and cyclists have the same breathing rate, corresponding to moderate intensity activity. However, recent studies suggest that cyclists' breath rate may be higher than that of pedestrians, especially in routes with a high physical demand.[358,371]

A key feature of the toxicity of ultrafine particles is its high deposition efficiency, depending on its particle size and composition.[348,372] However, it also depends on individuals characteristics, such as water uptake, density, breathing pattern (oral vs. nasal), and lung morphology,[349,373] among others.[345] In six studies that examined the deposition of ultrafine particles, bus commuters,[337,356,374] pedestrians,[284,360] and cyclists [375] experienced the highest deposition, what is partly explained because they were exposed to smaller particles. Nevertheless, differences according to mode of transport may also vary depending on the particles size that predominates in the microenvironment. For example, active commuters over highly trafficked routes were exposed to bigger ultrafine particles, probably accumulation mode particles, what implied a lower lung deposited surface area than among active commuters over low trafficked routes.[356] Moreover, Kumar et al [348] suggested than in spite the exposure to ultrafine particles is higher in Asia; the relative deposition would be higher in Europe. This, because the nucleation mode particles are more prevalent in Europe, whereas the accumulation mode particles predominate in Asia. These differences in particles size could be partly explained by European regulations to reduce the emission of accumulation mode particles, which absorb the precursors of accumulation mode particles.[348] Differences in the toxicity potential of particles due to particles size and composition reduces the external validity between epidemiological studies performed in regions with diverse modal distribution of ultrafine particles [376] and could explain the large heterogeneity in the association of ultrafine particles with health outcomes.

Our findings suggest there might be differences in exposure ratios of car and bus commuters according to the WHO region of the study. Such differences could be attributed to lack of maintenance and access to cleaner and newer vehicle technologies that reduce the emission of ultrafine particles [377] and the unequal reduction of Sulphur from diesel fuel and lubricant oils.[347] However, it is unclear if the characteristics of the vehicles used in the studies contributed to explain these differences, because the reporting of ventilation settings standards in buses was heterogeneous. Although we aimed for a comprehensive review of the literature by including studies irrespective of study region, 18 out of 28 studies were performed in EUR, whereas six were performed in AMR and four in WPR. Therefore, our findings not only underrepresent regions outside Europe, but may also not be generalizable to regions such as African, South-East Asian, or Eastern Mediterranean. The observed evidence of publication bias, according to the funnel plots, may have reflected such lack of generalizability. Future studies and international collaboration are required to better understand the exposure determinants and the health consequences of exposure to ultrafine particles in other regions. Additionally, given the large heterogeneity of the studies, the meta-analysis of exposure ratios is provided only for informative purposes. Nevertheless, we aimed to provide a comprehensive assessment of the available evidence; therefore, the observed sources of heterogeneity should be accounted for local-wise policies.

Previous studies concluded that air pollution exposure is not the most important reason to select a route among active commuters (e.g. the fastest route is of higher importance [378]). Additionally, active commuter's exposure is not constant even if travelling in less trafficked routes,[371] as the exposure increases in proximity to highly emitting hotspots.[378] Therefore, while pursuing to increase the shift from motorized to active commuting, policies in urban infrastructure should prioritize pedestrian and cyclists' corridors separated from highly trafficked roads and hotspots. Additionally, improving the attractiveness and safety perception of active transport not only contributes to reduce the exposure to air pollution, but also contributes to reduce the risks of accidents and enhances health by increasing levels of physical activity,[353,379] what might lead to further health benefits including increases in life expectancy and reduction of mortality.[353,380] Other societal gains of corridors include reduced noise exposure, increased exposure to green environments, and enhanced self-efficacy and social cohesion, among others.[353,380,381] As for the future, it is uncertain whether the contribution of new fuels, such as biodiesel and ethanol and electric, and vehicle technologies will reduce the emissions of ultrafine particles.[345] Therefore, it may be insufficient to aim for a reduced proximity of active commuters to traffic,[371] as stakeholders could aim for a higher efficiency in the use of public space with policies aimed to also reduce the preference for the use of private motorized transport.

In conclusion, car and bus commuters had a higher exposure to ultrafine particles than active commuters while MMT commuters were more likely to exhibit a lower exposure. Use of ventilation settings that reduced the air interchange between in-vehicle and on-road contributed to reduce the exposure of car and bus commuters. However, active commuters would inhale a larger dose of ultrafine particles, independently of the differences in exposure between active and motorized commuters. Although the burden of commuting actively due to exposure to traffic-related air pollution may not be larger than the advantages of added physical activity,[352] it is necessary to grant active commuters with urban environment that reduces their proximity to traffic emissions. Reducing emissions of ultrafine particles require strategies aimed to reduce the reliance on private motorized transport.

Figure 16. Flowchart of the systematic review

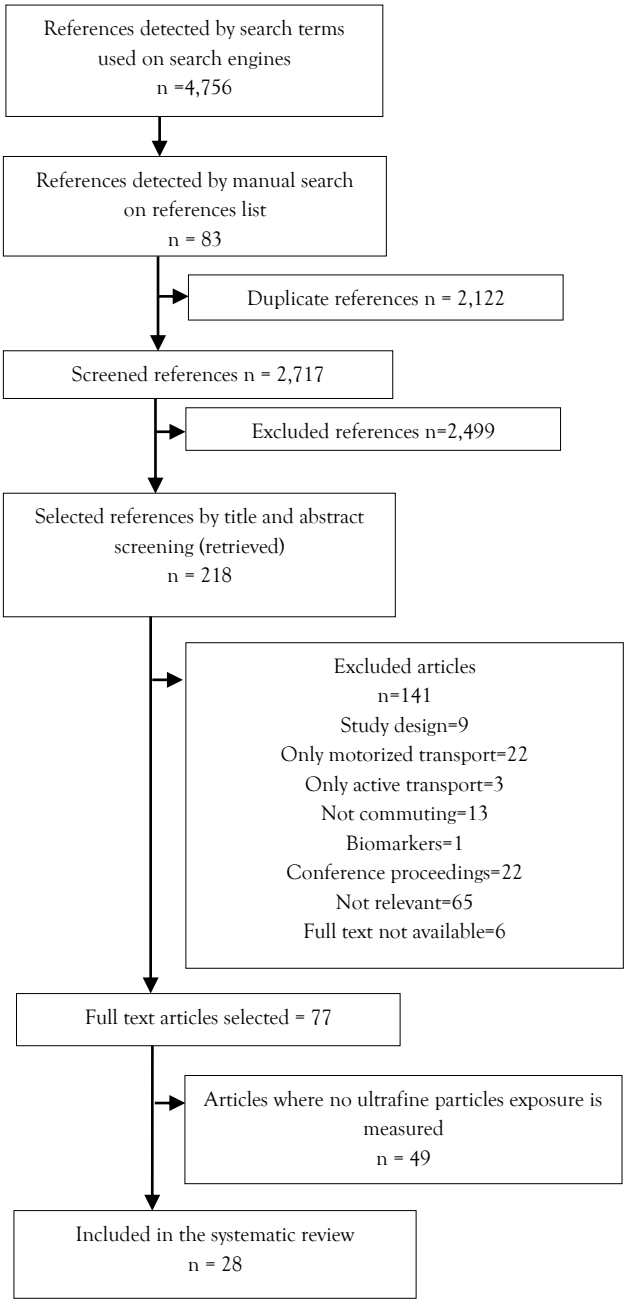
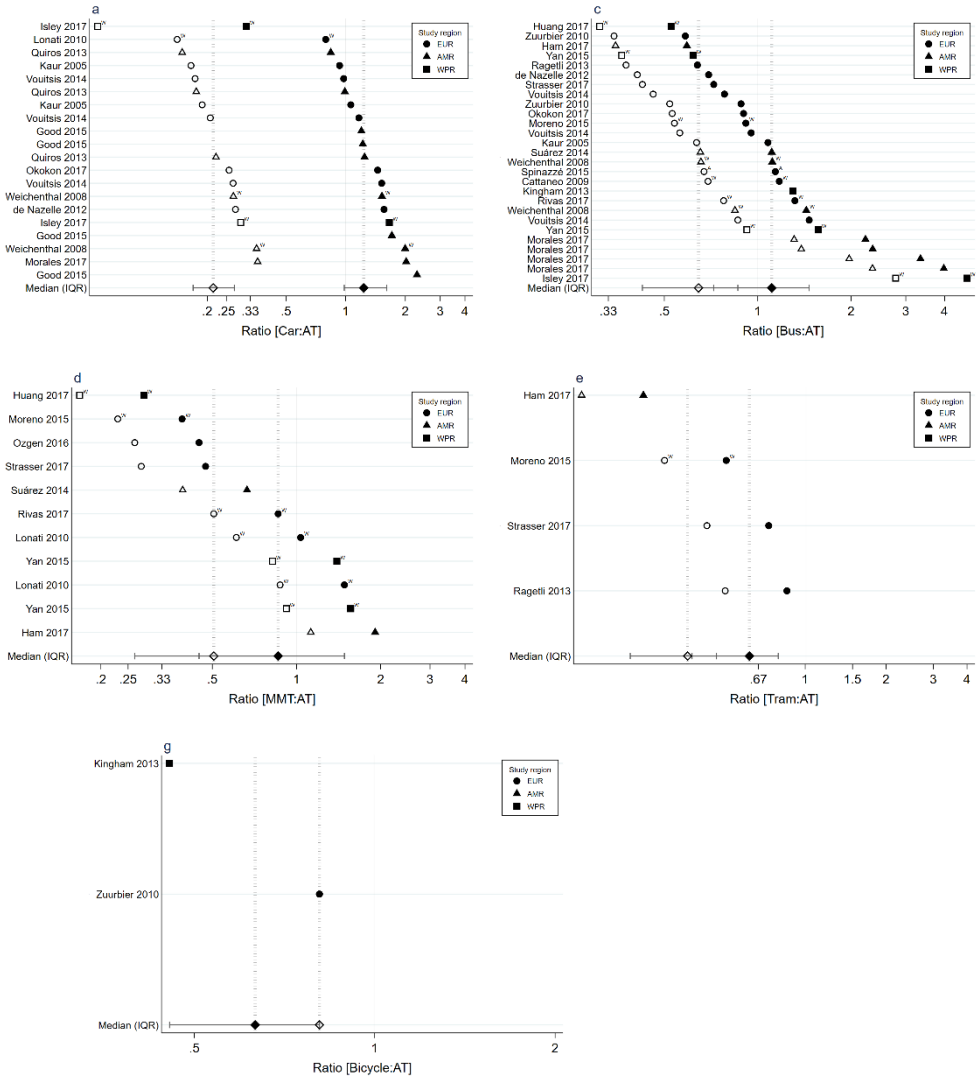
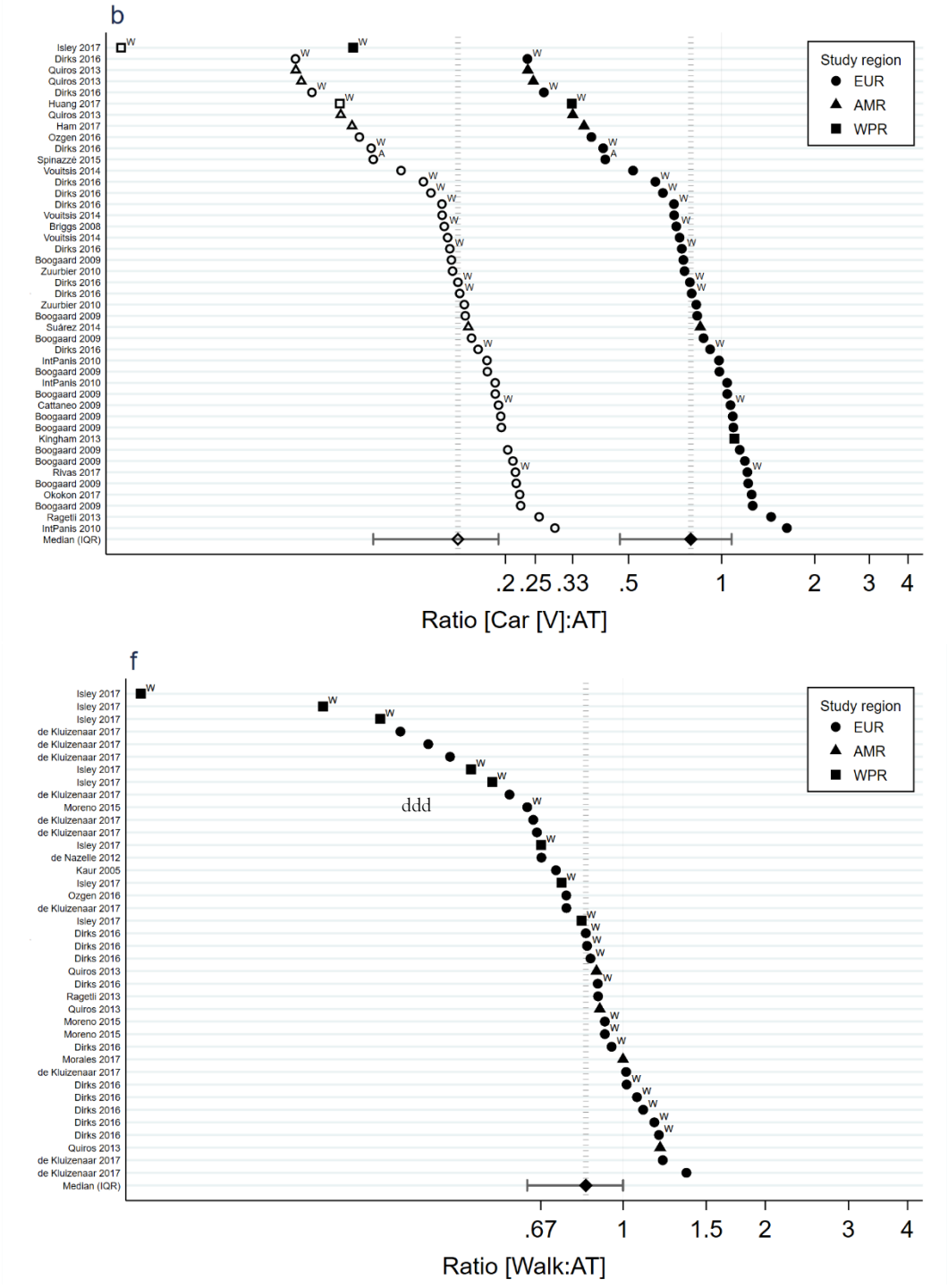


Figure 17. Ratio of exposure and dose inhaled of ultrafine particles according to motorized modes of transport





Full symbols correspond to exposure ratios. Hollow symbols correspond to dose ratios. All ratios are calculated using bicycle as reference, unless stated otherwise with labels in the ratio as follows: W: Walk as reference, A: Active transport as reference (the authors combined walk and bicycle exposure). Car [V]: Car driven under standard ventilation conditions. MMT: Massive motorized transport. AT: Active transport (bicycle or walk). Study region: EUR: European countries, AMR: American countries, WPR: Western Pacific Region countries. IQR: Interquartile range (25th to 75th percentiles).

Table 20. General characteristics of the studies included in the review

Author, year (Reference)	Size range detection	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
Boogaard, 2009 [305]	0.01-1µm	Real time condensation particle counter (CPC model 3007, TSI Inc)	Bicycle	Car [V]	Eleven days (except Fridays) in late August-October 2006	[Apeldoorn, Delft, Den Bosch, Eindhoven, Groningen, Haarlem, Maastricht, Nijmegen, The Hague, Utrecht, Zwolle]; Netherlands
Briggs, 2008 [318]	>0.02 µm	TSI P-Track Ultrafine Particle Counter 8525	Walk	Car [V]	Seven weekdays during May and June 2005	London; UK
Cattaneo, 2009 [382]	TSI CPC 3007: 0.01-1µm P-Track 8525: 0.02-1µm	TSI Condensation particle Counter 3007 & TSI P-Track Ultrafine Particle Counter 8525	Walk	Car[V]	One week in April 2005	Milan, Italy
Dirks 2016 [383]	0.02-1µm	P-Trak*	Walk	Car[V]	November 2015	Bradford, UK
Good 2015 [354]	0.01-1µm	Disc Mini, Matter Aerosol AG, Switzerland	Bicycle [Low and high traffic route]	Car	September 2012-February 2014	Fort Collins; USA
Ham 2017 [374]	0.01-1µm	DiSCmini (Matter Aerosol)	Bicycle	Bus Car MMT [Train] Tram [Light rail]	April 2014 and November 2015	Sacramento; USA
Huang 2017 [360]	0.01-1µm	Condensation particle counter: TSI Condensation particle Counter 3007	Walk	Bus Car [Std] MMT	April 10th to June 24th 2013	Singapore; Singapore
IntPanis, 2010 [284]	0.02-1µm	P-Trak UFP counter (TSI 8525)	Bicycle	Car[V]	8 days, June 2009	[Brussels, LLN, Mol]; Belgium
Isley 2017 [384]	0.01-0.3 µm	Philips Aerasense Nanotracer	Walk	Bus Car Car [V]	19th to 20th October 2015	Suva; Fiji
Kaur, 2005 [306] Kaur, 2009 [310]	0.02-1µm	TSI P-Track Ultrafine Particle Counter 8525	Bicycle Walk	Bus Car	Four week field campaign 28April/23May 2003	London; UK
Kingham 2013 [335]	0.01-1µm	TSI Condensation particle Counter 3007	Bicycle [Off and on road]	Car [V] Bus	February-March 2009	Christchurch, New Zealand
De Kluizenar 2017 [357]	0.01-0.3 µm	DiSCmini, Matter Aerosol	Bicycle Walk	Motorized transport (unspecific)	3 sessions of 5 days in June 2015	Eindhoven, The Netherlands

Author, year (Reference)	Size range detection	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
Lonati 2010 [385]	0.02-1µm	Personal condensational particle counter: P-Trak UFP counter (TSI 8525)	Walk	Car MMT [Subway and Train]	Not specified	Milan, Italy
Morales 2017 [358]†	>0.02nm	DiSCmini (Matter Aerosol, Wohlen, Switzerland)	Bicycle Walk	Bus [BRT, hybrid bus diesel/electric), bus]	July-August 2015	Bogotá; Colombia
Moreno 2015 [337]	0.01-0.3 µm	PNC: NanoTracer (Philips Aeraswnse Nanotracer)	Walk [high and low traffic]	Car Bus MMT [Subway] Tram	39 weekdays between October-November 2014	Barcelona, Spain
de Nazelle, 2012 [386]	0.01-1µm	TSI Condensation particle Counter 3007	Bicycle Walk	Bus Car	Four weeks beginning May 28th 2009	Barcelona; Spain
Okokon 2017 [359]	0.02-1µm	Personal condensational particle counter: P-Trak UFP counter (TSI 8525)	Bicycle	Bus Car Car [V]	The: 5-13th April 2011 Rot: 10-19th May 2011 Hel: 7-17th June 2011	Helsinki; Finland Rotterdam; The Netherlands Thessaloniki; Greece
Ozgen 2016 [361]	0.02-1µm	Personal condensational particle counter: P-Trak UFP counter (TSI 8525)	Bicycle Walk	Car [V] MMT	July 2010	Milan; Italy
Quiros 2013 [387]	CPC3007: 0.01-1µm (pedestrians and cyclists) P-Track 8525: 0.02-1µm (driving)	Condensation particle counter: TSI Condensation particle Counter 3007 for walking and cycling. Water based Condensation Particle Counter WCPC (TSI Model 3785, TSI, Inc., Shoreview, MN, USA) & personal condensational particle counter: P-Trak UFP counter (TSI 8525) for driving and beach-side measurements	Bicycle Walk	Car Car [V]	March 22nd to April 21st 2011	Santa Monica; USA
Ragettli 2013 [362]	0.01-0.3	Diffusion Size Classifier (minidisc)	Bicycle Walk	Bus Car [V] Tram	13 days in March and 5 in September 2011	Basel; Switzerland

Author, year (Reference)	Size range detection	Method of measurement	Mode of transport		Monitoring period	City; Country
			Active transport	Motorized transport		
Rivas 2017 [388]	0.02-1µm	P-Track model 8525 (TSI Inc, USA)	Walk	Car [V] Bus MMT [Underground]	40 sampling days between February 25th to June 17th 2016	London; UK
Spinazzè 2015 [356]	>0.02µm	DiSCmini (Matter Aerosol, Wohlen, Switzerland)	Bicycle Walk [high and low traffic areas]	Bus Car [V]	Winter/spring/summer/autumn 2014	Como; Italy
Strasser 2017 [375]	0.01-0.3 µm	minDISC diffusion size classifier (Dr. Martin Fierz, Fachhochschule Nord-westschweiz, Windisch, Switzerland)	Bicycle	Bus Car [V] MMT Tram	7 days between October 2015 and June 2016	Vienna; Austria
Suárez, 2014 [304]	0.02-1µm	Personal condensational particle counter: P-Trak UFP counter (TSI 8525)	Bicycle	Bus Car [V] MMT	Winter-spring 2011, summer-autumn 2012	Santiago de Chile; Chile
Vouitsis, 2014 [389]	0.02-1µm	Personal condensational particle counter: P-Trak UFP counter (TSI 8525)	Bicycle	Bus Car Car [V]	April 2011	Thessaloniki; Greece
Weichenthal 2008 [363]	0.02-1µm	Personal condensational particle counter: P-Trak UFP counter (TSI 8525)	Walk	Bus Car	April-November 2006	Montreal, Canada
Yan, 2015 [314]	0.01-1µm	Condensation particle counter: TSI Condensation particle Counter 3007	Walk	Bus [A/C on and off] MMT [Subway and ground]	10-23 December 2011	Beijing; China
Zuurbier, 2010 [296]	0.01-1µm	Condensation particle counter: TSI Condensation particle Counter 3007	Bicycle [Low and high traffic route]	Bus [Diesel and electric] Car ([V] Diesel and gasoline)	June 2007-June 2008	Arnhem; Netherlands

*Assumed P-Trak 8525. †Motorcycles were included in this study, but no data on ultrafine particles was reported. BRT: Bus Rapid Transit.

SUPPLEMENTARY MATERIAL

Appendix 61. Search terms per search engine

Embase.com: ('air pollution'/de OR 'air pollutant'/exp OR 'air pollution indicator'/de OR 'environmental exposure'/de OR 'exhaust gas'/de OR acetylene/de OR benzene/de OR '1, 3 butadiene'/de OR 'carbon monoxide'/de OR dust/de OR ethane/de OR ethylbenzene/de OR ethylene/de OR 'airborne particle'/de OR 'nitrogen dioxide'/de OR 'particulate matter'/de OR toluene/de OR xylene/de OR 'polycyclic aromatic hydrocarbon'/exp OR combustion/de OR 'black carbon'/de OR 'volatile organic compound'/de OR ((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEXT/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEXT/1 dioxide*) OR pm1 OR 'pm2 5' OR pm10 OR 'pm 1' OR 'pm 2 5' OR 'pm 10' OR soot OR toluene* OR xylene* OR ufp* OR 'black carbon' OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex):ab,ti) AND ('traffic and transport'/de OR 'motor vehicle'/exp OR 'railway'/de OR traffic/de OR bicycle/de OR 'car driving'/exp OR 'motorized transport'/de OR walking/de OR travel/de OR pedestrian/de OR (traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) NEAR/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*):ab,ti) AND (commut* OR telecommut* OR ((travel) NEAR/3 (work*)))

Medline (OvidSP): ("air pollution"/ OR exp "Air Pollutants"/ OR "environmental exposure"/ OR "Vehicle Emissions"/ OR acetylene/ OR benzene/ OR "Benzene Derivatives"/ OR "butadienes"/ OR "carbon monoxide"/ OR dust/ OR ethane/ OR ethylenes/ OR "nitrogen dioxide"/ OR "particulate matter"/ OR toluene/ OR "Polycyclic Hydrocarbons, Aromatic"/ OR ((air ADJ3 (clean*)) OR ((environment* OR personal) ADJ3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon ADJ monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* ADJ3 matter*) OR (nitro* ADJ dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic ADJ3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile ADJ3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex).ab,ti.) AND ("Transportation"/ OR exp "Motor Vehicles"/ OR "railroads"/ OR Bicycling/ OR walking/ OR travel/ OR (traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) ADJ3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*).ab,ti.) AND (commut* OR telecommut* OR ((travel) ADJ3 (work*)))

Cinahl (ebsco): (MH "air pollution+" OR MH "Air Pollutants+" OR MH "environmental exposure+" OR MH "Benzene Derivatives+" OR MH "carbon monoxide+" OR MH dust+ OR MH ethylenes+ OR MH "particulate matter+" OR MH toluene+ OR MH "Polycyclic Hydrocarbons, Aromatic+" OR ((air N3 (clean*)) OR ((environment* OR personal) N3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon N1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* N3 matter*) OR (nitro* N1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic N3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile N3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND (MH "Transportation+" OR MH "Motor Vehicles+" OR MH "railroads+" OR MH cycling+ OR walking+ OR travel+ OR (traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) N3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*)) AND (commut* OR telecommut* OR ((travel) N3 (work*)))

Cochrane: (((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEXT/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEXT/1 dioxide*) OR pm1 OR 'pm2 5' OR pm10 OR 'pm 1' OR 'pm 2 5' OR 'pm 10' OR soot OR toluene* OR xylene* OR ufp* OR 'black carbon' OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex):ab,ti) AND ((traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) NEAR/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*):ab,ti) AND (commut* OR telecommut* OR ((travel) NEAR/3 (work*)))

Web-of-science: TS=((((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEAR/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEAR/1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND ((traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) NEAR/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*)) AND (commut* OR telecommut* OR ((travel) NEAR/3 (work*))))

Scopus: TITLE-ABS-KEY((((air W/3 (clean*)) OR ((environment* OR personal) W/3 expos*) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon W/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* W/3 matter*) OR (nitro* W/1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic W/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile W/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND ((traffic* OR subway* OR tram OR tramway* OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail* OR automobile* OR bicycle* OR motorcycle* OR cycling OR walk* OR bus OR busses OR buses OR foot OR bike OR transport* OR vehicle* OR ((commut*) W/3 (mode OR type OR way OR public OR strateg*)) OR travel* OR pedestrian* OR passenger* OR driver*)) AND (commut* OR telecommut* OR ((travel) W/3 (work*))))

PubMed: ("air pollution"[mh] OR "Air Pollutants"[mh] OR "environmental exposure"[mh] OR "Vehicle Emissions"[mh] OR acetylene[mh] OR benzene[mh] OR "Benzene Derivatives"[mh] OR "butadienes"[mh] OR "carbon monoxide"[mh] OR dust[mh] OR ethane[mh] OR ethylenes[mh] OR "nitrogen dioxide"[mh] OR "particulate matter"[mh] OR toluene[mh] OR "Polycyclic Hydrocarbons, Aromatic"[mh] OR ((air AND (clean*[tiab])) OR ((environment*[tiab] OR personal) AND expos*[tiab]) OR pollut*[tiab] OR microenvironment*[tiab] OR exhaust*[tiab] OR emission*[tiab] OR acetylene*[tiab] OR benzene*[tiab] OR butadiene*[tiab] OR (carbon ADJ monoxide*[tiab]) OR co OR carbonmonoxide*[tiab] OR Coarse OR dust OR ethane OR ethylbenzene*[tiab] OR ethylene*[tiab] OR ethene*[tiab] OR particle*[tiab] OR (particul*[tiab] AND matter*[tiab]) OR (nitro*[tiab] ADJ dioxide*[tiab]) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene*[tiab] OR xylene*[tiab] OR ufp*[tiab] OR "black carbon" OR (polycyclic AND (hydrocarbon*[tiab] OR carbon*[tiab])) OR pah OR pahs OR combust*[tiab] OR (volatile AND compound*[tiab]) OR VOCs OR voc OR tVOCs OR tvoc OR btex)) AND ("Transportation"[mh] OR "Motor Vehicles"[mh] OR "railroads"[mh] OR Bicycling[mh] OR walking[mh] OR travel[mh] OR (traffic*[tiab] OR subway*[tiab] OR tram OR tramway*[tiab] OR streetcar OR metro OR underground OR tube OR train OR car OR cars OR rail*[tiab] OR automobile*[tiab] OR bicycle*[tiab] OR motorcycle*[tiab] OR cycling OR walk*[tiab] OR bus OR busses OR buses OR foot OR bike OR transport*[tiab] OR vehicle*[tiab] OR ((commut*[tiab]) AND (mode OR type OR way OR public OR strateg*[tiab])) OR travel*[tiab] OR pedestrian*[tiab] OR passenger*[tiab] OR driver*[tiab])) AND (commut*[tiab] OR telecommut*[tiab] OR ((travel) AND (work*[tiab]))) AND publisher[sb]

Google Scholar: Pollution | pollutant | pollutants | exhaust | "particulate matter | pah | pahs | combustion | "black carbon" | vacs traffic | vehicle | railway | bicycle | car | driving | motorized | walking | pedestrian | pedestrians | subway | metro | underground | train | cycling commuters | commuting | commuter

Proquest: (ti(Pollution OR pollutant OR exhaust OR "particulate matter" OR pah OR pahs OR combustion OR "black carbon" OR vac OR vacs OR carbonmonoxide OR Coarse OR dust OR btex) OR ab(Pollution OR pollutant OR exhaust OR "particulate matter" OR pah OR pahs OR combustion OR "black carbon" OR vac OR vacs OR carbonmonoxide OR Coarse OR dust OR btex)) AND (ti(traffic OR vehicle OR railway OR bicycle OR car OR driving OR motorized OR walking OR pedestrian OR pedestrians OR subway OR metro OR underground OR train OR cycling) OR ab(traffic OR vehicle OR railway OR bicycle OR car OR driving OR motorized OR walking OR pedestrian OR pedestrians OR subway OR metro OR underground OR train OR cycling)) AND (ti(commuter*) OR ab(commuter*))

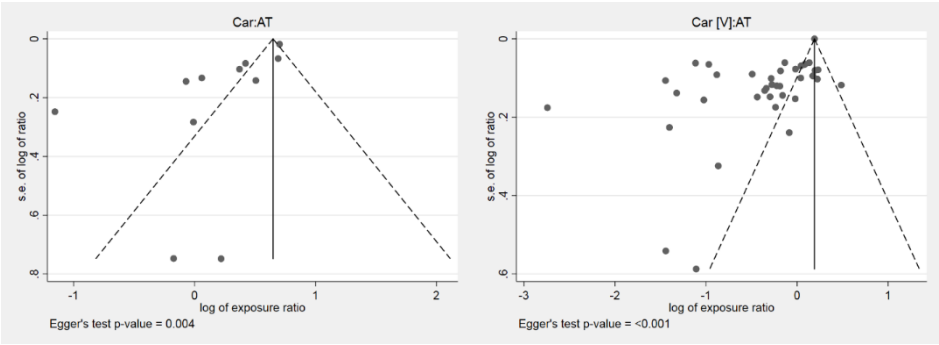
Scielo: (Pollution OR pollutant OR exhaust OR "particulate matter" OR pah OR pahs OR combustion OR "black carbon" OR vac OR vacs OR carbonmonoxide OR Coarse OR dust OR btex) AND (traffic OR vehicle OR railway OR bicycle OR car OR driving OR motorized OR walking OR pedestrian OR pedestrians OR subway OR metro OR underground OR train OR cycling) AND (commuter*)

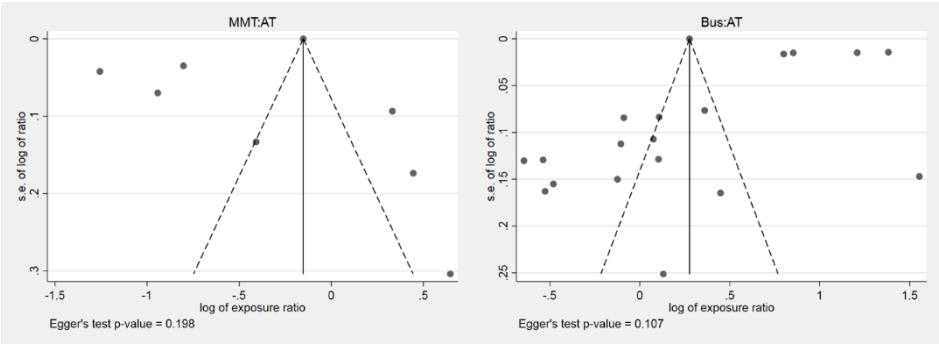
Appendix 62. Meta-analysis of exposure ratios according to mode of transport and WHO region

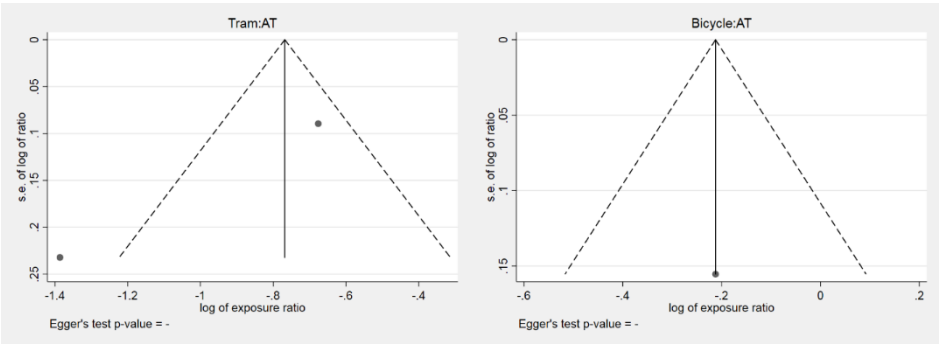
Mode of transport	WHO region	Ratio	(95% CI)	I^2	S	C
Bus	EUR	0.95	(0.75 - 1.20)	93.1	6	7
	AMR	1.78	(1.42 - 2.24)	99.5	4	8
	WPR	1.24	(0.45 - 3.46)	98.0	3	4
	Total	1.30	(0.99 - 1.70)	99.9	13	19
Car	EUR	1.14	(0.87 - 1.50)	72.5	2	3
	AMR	1.75	(1.48 - 2.06)	73.3	3	6
	WPR	0.73	(0.14 - 3.73)	97.0	1	2
	Total	1.26	(1.00 - 1.57)	92.3	6	11
Car [V]	EUR	0.80	(0.70 - 0.93)	97.3	9	31
	AMR	0.38	(0.21 - 0.69)	86.7	3	5
	WPR	0.15	(0.03 - 0.72)	98.7	2	2
	Total	0.68	(0.57 - 0.80)	98.1	14	38
MMT	EUR	0.53	(0.31 - 0.92)	99.6	3	3
	AMR	1.09	(0.39 - 3.05)	90.1	2	2
	WPR	0.85	(0.24 - 3.01)	99.3	2	3
	Total	0.74	(0.49 - 1.12)	99.4	7	8
Tram	EUR	0.51		100.0	1	1
	AMR	0.25		100.0	1	1
	WPR	-				
	Total	0.37	(0.18 - 0.74)	87.7	2	2
Walk	EUR	0.80	(0.69 - 0.92)	94.5	5	25
	AMR	1.00	(0.96 - 1.04)	0.0	2	4
	WPR	0.40	(0.23 - 0.71)	95.6	1	8
	Total	0.70	(0.61 - 0.81)	95.7	8	37

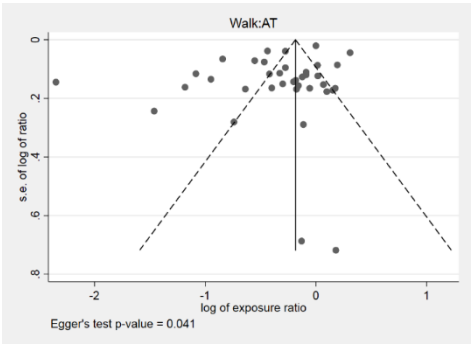
S = Number of studies; C = Summary data extracted per study for comparisons

Appendix 63. Funnel plots and evaluation of publication bias according to meta-analyses









Appendix 64. Exposure to ultrafine particles according to mode of transport and WHO region (units: particles/cm^3)

Mode of transport	WHO region	Median	IQR	S	C
Bus	EUR	31,451	14,055 – 52,300	11	18
	AMR	89,950	25,328 – 139,500	4	8
	WPR	22,471	15,211 – 44,990	4	4
	Total	31,451	15,000 – 66,845	19	30
Car	EUR	58,950	40,000 – 87,545	5	10
	AMR	31,495	13,200 – 38,348	4	6
	WPR	13,966	4,456 – 23,476	1	2
	Total	39,174	23,476 – 78,000	10	18
Car [V]	EUR	21,639	9,159 – 29,722	13	39
	AMR	7,830	3,500 – 7,900	3	5
	WPR	7,672	911 – 14,433	3	2
	Total	16,515	8,848 – 25,000	19	46
MMT	EUR	10,804	6,857 – 26,500	5	8
	AMR	42,250	42,000 – 42,500	2	2
	WPR	19,405	12,542 – 21,740	2	3
	Total	19,405	8,609 – 30,000	9	13
Tram	AMR	13,312	11,783 – 18,818	3	5
	WPR	5,500	5,500 – 5,500	1	1
	Total	-			
	EUR	12,547	10,008 – 18,818	4	6
Walk	EUR	13,400	9,300 – 27,400	11	43
	AMR	20,470	12,700 – 28,400	3	6
	WPR	9,490	4,335 – 13,924	3	11
	Total	12,450	9,300 – 25,700	17	60

S = Number of studies; C = Summary data extracted per study for comparisons

Appendix 65. Quality of the studies included in the systematic review

Author, year (Reference)	Modal comparison	Background factors	Heterogeneity	Same time – All modes	Same route – All modes	Ascertainment of exposure	Sample size and dispersion
Boogaard, 2009 [305]	E	**	*	*	-	**	*
Briggs, 2008 [318]	E	**	**	**	**	**	*
Cattaneo, 2009 [382]	E	*	*	*	*	*	*
Dirks 2016 [383]	E	-	*	**	**	**	*
Good 2015 [354]	E/O	**	NA	*	*	**	*
Ham 2017 [374]	O	-	NA	*	NA	**	*
Huang 2017 [360]	E	**	**	*	**	**	*
Int Panis, 2010 [284]	E	**	**	*	**	**	*
Isley 2017 [384]	E	**	*	-	-	*	*
Kaur, 2005 [306] Kaur, 2009 [310]	E	**	**	**	*	**	*
Kingham 2013 [335]	E	*	**	**	**	**	*
De Kluizenar 2017 [357]	O	NA	-	NA	NA	**	*
Lonati 2010 [385]	E	-	-	-	-	**	*
Morales 2017 [358]	E	**	*	*	*	**	*
Moreno 2015 [337]	E	*	-	*	*	**	*
de Nazelle, 2012 [295]	E	**	**	*	**	**	*
Okokon 2017 [359]	E	-	**	*	**	**	*
Ozgen 2016 [361]	E	*	**	*	**	**	*
Quiros 2013 [387]	E	**	*	*	**	**	*
Ragettli 2013 [362]	E	**	**	*	**	**	*
Rivas 2017 [388]	E	**	**	-	-	**	*
Spinazzè 2015 [356]	E	**	*	*	-	**	*
Strasser 2017 [375]	E	**	**	*	**	-	*
Suárez, 2014 [304]	E	**	**	*	**	**	*
Vouitsis, 2014 [340]	E	**	*	*	**	**	*
Weichenthal 2008 [363]	E/O	**	*	*	*	**	*
Yan, 2015 [314]	E	*	**	-	*	**	*
Zuurbier, 2010 [296]	E	**	**	*	*	**	*

E: Experimental. O: Observational

Appendix 66. Quality of reporting of studies included in the review

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	✔	Partially	✖	✔	✖	✔	✔	✔	✔	✔
Boogaard 2009 [305]	Volunteers carried monitoring devices over 12 predefined routes of approx.. 10-20min duration in 11 cities	Sampling between 12:00 and 19:00PM. Not specified if started at the same time	Shortest route per mode from origin to destination, in a radius of 02.5km within city center	Car: petrol-fueled	Routes consisted in before-, during and after-transport	Windows closed, A/C off and fanned ventilation on moderate setting	Sampling, device operation and analysis were standardized	Standard deviation	1-min averages	Car: 126 routes Bicycle: 120 routes
	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Briggs 2008 [318]	Monitors were carried over 48 routes to simulate typical trips (between 601-1361m in total length).	Each route was walked once (or twice for a very short route), and driven repeatedly until the walk was completed	Walking route followed the road route as closely as possible. Retour route was made by the same side of the car.	Car: Diesel-fueled; car was previously ventilated Walk: followed road route as closely as possible, taking centerline of sidewalks	Monitoring data not related directly with experiments was excluded	Windows closed, A/C off and fanned ventilation system on a moderate setting	Sampling, device operation and analysis were standardized.	Standard deviation	Trip average	46 pairs of samples (car and walking)
	✔	Partially	Partially	?	✔	✔	✔	✔	✔	?
Cattaneo 2009 [382]	Volunteers carried the monitoring devices over four routes, one indoor and one outdoor microenvironments, during at least 15 minutes each	Data collected between 7-11am, 11am-3pm and 4-8pm. Not specified whether started at the same time	Bus and car trips are approximately the same Walking route is runs over a portion of car route. Metro is different from all the routes.	Bus: Diesel fueled Car: Petrol fueled with three-way catalyst Standards on walking, bus and metro commuting not provided.	A time-activity diary was used to classify the microenvironments	Closed windows, ventilation system turned on and air-recycling deactivated	Sampling, device operation and analysis were standardized.	Standard deviation	1-min sampling	4005 1-min data collected, not specified per mode of transport
	Yes	Partially	Yes	NS	NS	Yes	Yes	Yes	Tes	Yes
Dirks 2016 [383]	Three volunteers commuted over a predefined route, one driving and two walking on opposite sides of the road.	Yes, both modes were programmed to be followed at the same time. Measurements performed in the morning and in the afternoon at the same time.	The same route was followed in both modes	Not clear if standard commuting conditions for pedestrians	Not clear if the car commuter also monitored the walking section of the commute	Recirculation mode turned off, previously fully ventilated (not clear if windows closed) Because of sampling period (November) and location, assumed yes.	Sampling and device operation was standard	Standard deviation	10-s observations	120 10s observations
	Partially	Partially	No	Partially	Yes	No	Yes	Yes	Yes	Yes

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
Good 2015 [354]	Volunteers commuted from home to workplace (minimum distance of 2.4km); each participant contributed with 8 commute days within 4-12 weeks period.	Participants contributed four days-worth of cycling commute and four days-worth of car commute. All commutes between 7-9hrs and 1630 to 18hrs, on Tuesdays and Thursdays	Routes were preselected by researches and featured either high traffic roads (direct route) or alternative roads.	Besides time/route parameters, participants commuted on their own cars and own pace. Adherence to route was examined with GPS	It is not clear if car commutes monitoring included exposure while approaching the cars.	Participants provided this information in a diary	Sampling, device operation and analysis were standardized	Confidence intervals of mean and cumulative difference	Average per trip	110 commutes per mode, for PNC measurements
	No	No	No	No	No	No	Yes	Yes	Tes	Tes
Ham 2017[374]	Volunteers wore monitors during their typical commuting trips between April 2014 and November 2015. Each participant contributed with 3 to 8 monitoring days.	Typical commuting trips, all were monitored between 7+30am-9+00am and 4+00 to 5+30 pm on typical workdays (Monday to Friday)	Typical commuting trips; participants were asked to keep a diary	Typical commuting trips, up to four volunteers were monitored simultaneously.	Trips were monitored since leaving home until reaching office and vice versa	Data of ventilation settings was retrieved in a trip diary	Sampling and device operation and data analysis were standardized	Standard deviation	Exposure per trip	Personal vehicle: 101 trips; bus: 20 trips; 9; light trail: 9 trips; train: 19 trips; bike: 12 trips
		Partially	Partially		Yes	Yes	Yes	Yes	Yes	Sample size
Huang 2017 [360]	Participants followed predefined routes on specified time, consecutive modes of transport (Taxi, MRT, Bus, MRT, Walk)	Measurements on weekdays from 16 to 19h during 30days between April 2013 to June 2013. The trips are followed consecutively	All modes have the same start and end point, but routes vary in some sections	Bus: measurements at the middle of the bus, near the exit door. Buses are air-conditioned and driven with closed windows. MRT (Subway): measures in the middle of the carriage, if possible. Pedestrian: Measures by the middle of curbside, about 1.5m away from road.	The complete commute included door-to-door measurements, but exposures are provided according to microenvironment	Taxi: Measures at the middle of the back seat, windows closed, air conditioning on recirculation	Sampling and device operation and data analysis were standardized	Standard deviation	Average per trip	Bus: 23 trips, MRT; 45 trips, Taxi: 23 trips Walk: 23 trips
	Yes	Partially	✔	✔	✔	✔	✔	✔	✔	✔
Int Panis 2010 [284]	55 healthy non-smoker volunteers were driven as passengers over one selected route, and then asked to ride the same route by bike	The bike commute followed the car route with a minimum difference (between 3-8 min).	Three routes were followed in each city with mixed traffic load.	Same car for all the commutes, not specified fuel type. Bicycle: The specified for timing and route	Measurements only while commuting	Windows closed, A/C off and fanned ventilation system in mode 1	Sampling and devices operation and analysis were standardized.		Averages based on 1-s readings	43 pairs of bike-car trips

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
		The Brussels road was cycled twice.								
	Yes	No	No	Yes	No	✔	Partially	Yes	Yes	Yes
Isley 2017 [384]	Routes were monitored in a monitoring campaign of 8 hours in two days	Not specified, apparently consecutive	Not specified	Bus: windows open Not specified for pedestrians	Not specified	Two car trips, windows closed and A/C on; two taxi trips with from window open and seated in the back, one trip with windows closed	Devices operation and analyses were standardized, not clear sampling standards	Interquartile ranges	One-minute averages	Minutes per mode Bus: 90; Car: 20; Walk: 1182; Taxi: 151
	✔	✔	Partially	✔	-	✖	✔	✔	✔	✔
Kaur 2005 [306]/Kaur 2009 [310]	Groups of four volunteers were randomly assigned to travel along two set routes by five modes of transport	Three timings (morning [8.30am], lunch [12.00pm] and afternoon [3.15pm]). An additional early evening [5.15pm] measurement during the first week for $PM_{2.5}$	Two routes were randomly travelled. Route 1 was heavily trafficked. Route 2 had a mixture of congested sections and backstreets with very little traffic; this route was completely followed only on foot or bicycle, due to circulation restrictions for car and taxi, and bus riding only over part of the route.	Car: petrol car with three way catalyst and diesel black cab. Bus: diesel buses Bicycle: followed bus lanes over the routes Walking: The specified for timing and route	Not specified	Car drivers instructed to operate ventilation as normal	Sampling devices operation and analysis were standardized	Confidence intervals and geometric standard deviation	UFP: Average of count of particles per sample	$PM_{2.5c}$ 197 samples CO: 111 samples UFP: 86 samples
	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Kingham 2013 [335]	Four commuters carried a complete set of monitors over the specified routes, all commuters met in a specified point of the route to complete the second part of the route.	Sampling was made at the same time each day in the morning (7+40-9+00) and evening (16+45-18.05) rush hours	One route was followed by the three MT, one off-road cycle route was followed additionally by bike. Car and	Car: petrol engine sedan All other modes: as specified		Windows closed, vents set to 'fresh' and fan set 2/4.	Sampling, device operation and analysis were standardized	Interquartile range (box-plot graphs).	Trips and 6-sec measurements	Car: 42 trips; Bus: 12; Bike on-road: 44; Bike off-road: 34

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
			road cyclist followed the bus route.							
	✕	NA	NA	NA	-	NA	✔	✔	✔	
De Kluizenar 2017 [357]	Exposure of 12 participants during 5 consecutive days, including week and weekend-days. Four participants were monitored in each of 5 sessions, performed between Wednesday and Monday, in June 2015. Participants used the air pollution monitor and a GPS during the measurement period to derive microenvironments.	Not applicable	Not applicable	No, modes of transport were derived from GPS measured data.	Not specified	Not applicable	Sampling, device operation and data analyses were standardized	IQR	Mean UFP concentration per micro-environment and activity	54,029 minutes, 1.9% walking, 2.6% cycling and 3.7% motorized transport
	?	-	✕	-	-	-	✔	Partially	✔	✔
Lonati 2010 [385]	Concentration of fine and ultrafine particles was measured in several indoor and outdoor microenvironments	Not specified	Different routes reported per mode of transport	Not specified	Not specified	NA	Sampling, device operation and data analysis were standardized	IQR	Microenvironment average from 1-min measurements	Walking: 48min, Car: 160; Subway = 60; train=230
	✔	Partially	Partially	Partially	✕	✕	✔	✔	✔	✔
Morales Betancourt 2017 [358]	Volunteers followed a predetermined route in all sampling campaigns	Participants started the trip at the same time. Nevertheless, not all the modes were followed in all campaigns	The same route was followed by all modes of transport in the same campaign	The volunteers followed scripted routes at selected times, but information about trip standards are not provided.	The measurements of public transport included the time approaching the public transport stations	Not specified	Sampling, device operation and analyses were standardized.	Geometric standard deviation	30s exposure per mode	BRT Bus, S1 = 12 trips; Bus S2 = 9; Bus S3 = 6; Hybrid bus S2 = 6; Car S2 = 6; Bicycle S3= 6; Bicycle S2 = 9; Pedestrian s2 = 4; Pedestrian s3 = 2; Bicycle s1 = 12; Pedestrian s1 = 12
	✔	Partially	Partially	✔	✕	-	✔	✔	✔	✔
Moreno 2015 [337]	Two commuters made simultaneous but separated	Measurements made in pairs by MT. The journeys	The main route was a 4.2km route (one way)	Walking: sidewalks of the main route	Bus, tram and metro included walking	NA	Sampling, device operation and	Standard deviation	Average per MT	78 trips performed, not

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
	round trips by one MT over a set route	started at 10AM, and round trips were made.	with a city center section (congested, canyon-like) and a suburban section (broader with wide sidewalks). Walking section of tram trip differed slightly from the others. Metro route did not overlap with the main route.	Tram: selected route, included walking section Bus: diesel fueled, the traveler placed in the central part of the vehicle Metro: Set lines monitored, included waiting times	sections pre-, during-, and post commute		analyses were standardized			specified trips per mode
	✔	Partially	Partially	✔	✘	✘	✔	✔	✔	✔
de Nazelle 2012 [386]	Commuters carried the monitoring devices in pairs of two different modes of transport over two selected 'round trip' commute routes.	Measurements at peak (8-10h, 13-15h, 17-20h) and off peak(10-13h, 15-17h) times Measurements performed in pairs of two different modes	The two routes were approximately the same, with variations for car and bus commuters Included high traffic roads, street canyons	Routes and commutes were standardized for all the modes. Bike lane location ranging from the middle to a side of the road Car: diesel-fueled car	Full trip from origin to destination, including walk to bus stop or car park	Driver's windows open	Sampling and device operation and data analysis were standardized	Standard deviation	Trip averages from 10-s and 1-s measurements	Walk=48 trips, Bike=54, Bus=34; Car=36
	✔	Partially	Partially	Yes	Yes	Partially	Yes	Yes	Yes	Yes
Okokon 2017 [359]	Measurements were conducted during the morning and afternoons simultaneously in two modes of transport during six days	Measurements were performed over car trip and simultaneously either bus or bicycle. Only workdays.	In each city, three to six high trafficked routes were selected. Each route of approximately 8km. Nevertheless, not the same route	Bicycle: Three-gear electric bicycle; commuters followed cycle route if available or commuted alongside motorized traffic Bus: no predefined place in the vehicle, seat chosen according to availability Car: 1.6-l gasoline engine younger than 3 years. Monitor placed in the middle of the backseat.	Measures only while in the vehicles	In half of the rides the windows were closed and ventilation in moderate level without recirculation. On remaining rides windows were open and air conditioning turned off	Sampling and device operation and data analysis were standardized	Standard deviation	Average exposure per mode	Total unidireccional trips: Bicycle: 84; Bus: 72; Car [V]: 94; Car: 69

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
				in the middle of the backseat.						
	✔	Partially	✔	✔	✔	✔	✔	✔	Yes	Yes
Ozgen 2016 [361]	Measurements on three weekdays in two weeks. 2hour campaign per day, the route was travelled consecutively with each mode of transport.	Measurements were not simultaneous, but were made consecutively within a 2hour time frame. Each trip lasted 30min for one round trip walking and 2 round trips on the other modes. Trips between 3am and 5pm in the same order: car, bike, walk, subway.	2km long route. Route is a city-center trafficked 4-lane road, bordered by 4 and 5-story buildings.	Car: petrol fueled and equipped with air filtration system. Bicycle: right-most lane, because no cycle lane available. Walking: on sidewalk, at 2m approx. from roadway. Subway: Only underground measurements, including waiting in platforms.	Surface transport modes: Car Walking, cycling and one underground (Subway)	Windows were closed; air conditioning was turned on, set on cool air and moderate ventilation.	Sampling, device operation and analyses were standardized	Standard deviation	Average per trip	Six round trips walking, 12 round trips for other modes of transport. Analyses unit were 1-min measures obtained from the monitor.
	Yes	Partially	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Quiros 2013 [387]	One roadway segment was followed by two researchers carrying each sampling devices in scripted roundtrips	Nine sampling days; three sessions of two hours (morning (7+30 to 9+30); afternoon (13+30-14+30) and evening (17 to 19)). Two modes were followed at a time. Per session, two walking trips, four cycling, six driving with window open and four with standard ventilation settings	Same route for all measures	No standards for commuting are specified		In standard ventilation settings, windows closed, air conditioning recirculation on	Sampling, device operation and analyses were standardized	Geometric standard deviation	Average per trip	Nine trips per mode
	Yes	?/Partially	✔	✔	NS	✔	✔	✔	✔	✔
Ragetli 2013 Substudy 2 [362]	Repeated measurements were carried out on five modes of transport over a set road	Sampling was performed at set times on weekday rush hours (7-9AM, 4.30-6.30PM),	Same road followed for all modes of transport. It is a broad route with	Bus: diesel or compressed gas natural, mechanically ventilated with windows closed. car lane		Windows closed, air-conditioning off and ventilation system moderated.		Standard deviation	One-minute average median per commute	Walk = 40 trips Bicycle = 51 Bus = 53 Tram = 47 Car = 84

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
		weekday non-rush hours (10-11.30AM and 2-3.30PM), and weekend (10AM-3PM). Weekdays measurements were collected consecutively for at least 4 times (2 in each direction) for each mode and time. Car measurements were taken the day before or after the other modes. Same day for all weekend measurements.	multiple car lanes and availability of tram lines, and bus/tram stops.	Tram: Electrically powered, not mechanical ventilation available and windows occasionally open. Car: 2 gasoline fueled with controlled ventilation settings. Pedestrians: Sidewalks are partly separated from the street by parking lane Cyclists: Share the right-mots side of the						
	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Rivas 2017 [388]	Volunteers followed four scripted routes with same destination and origin from four areas from four deprivation groups	Sampling on weekdays during morning (starting at 7+45), afternoon (12+00) and evening (16+30); not specified if all routes started at the same time	The fastest route was monitored for each mode of transport	Car: Petrol-fueled Bus: passenger seated in first or second row behind rear door in the lower deck or standing of no seats available Underground: passenger located in midpoint carriage Walking monitored while approaching the bus or underground stations		Windows closed, ventilation set at 50% fan velocity with no air recirculation	Sampling, device operation and analyses were standardized	GSM	Average per trip	Car: 82 trips, Bus: 42 trips, Underground: 87 trips; Walking: 130 trips
	No	Partially	No	Partially	Yes	Tes	Yes	Yes	Tes	Yes
Spinazzè 2015 [336]	Measurements of pollution were obtained sequentially following different modes of transport	Modes of transport were measured consecutively.	The modes were followed consecutively in a loop, this not the same route for all	Car: Petrol fueled; monitor located at passenger seat. Not standards provided for cyclists and pedestrians. Bus:		Windows closed, circulation fan on and recirculation fan on; fan speed low	Sampling, device operation and analyses were standardized	IQR	Average per mode	16 trips per mode

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
				diesel fueled with diesel particulate filter, driven with windows open, ventilation and air condition settings were not standardized						
	✔	Partially	✔	✔	NS	✔	✔	✖	✔	✔
Strasser 2017 [375]	Measurements on 7 days over typical commuter route between Oct2015/June2016.	Modes of transport measurements were consecutive, measured once or twice between 8amd and 12m, in time intervals of 2h; within a similar time period	The route is approximately the same for all modes	Standardized commuting conditions. Subway: underground 2/3 of the route. Train: Partially air-conditioned, open windows. Bus: air-conditioned, diesel powered. Tram: Not air-conditioned, open windows. Bicycle: route runs next to traffic.	Not specified	Car: gasoline powered, air-conditioned, air flow set low, windows closed and cabin ventilated with ambient air	Sampling, device operation and analyses were standardized	Not provided	Average per commute	Subway: 11 commutes, bus: 11 commutes, tram: 11 commutes, car: 4 commutes, bicycle: 5 commutes
	✔	Partially	✔	✔	✖	✔	✔	✔	✔	✔
Suárez 2014 [304]	Two volunteers at a time commuted over a set route carrying a personal monitor each one by one MT, then switched the monitor and travelled along the route again. Transport mode and order of samplers was assigned randomly.	Sampling between 8-9am, two commuters per sampling session, therefore not at the same time.	Same fixed routes for all modes	Car: three different cars were used, all gasoline powered and with catalytic converters Bus: diesel buses All commutes were standardized.	Bus and subway commutes included walking portions, pedestrians included street crossing and waiting periods.	Windows closed, no recirculation, though ventilation and heating at used at will	Sampling, devices operation and analysis were standardized	Standard deviation	Commute averages	PM2.5 Bicycle: 16trips, Bus: 17, Car: 17, Subway: 17 UFP: Bicycle: 14trips, Bus: 18, Car: 18, Subway: 18
	✔	Partially	✔	Partially	✖	✔	✔	✔	✔	?
Vouitsis 2014 [389]	Monitoring was performed simultaneously in pairs of modes of transport on one of the tree routes set, four times a day.	Each monitoring trip consisted of full trip. Measurements between 7.30-10, 10-12am, 1-3pm, 3-5pm. Two transportation modes were measured simultaneously; car in all pairs alternating ventilation settings between trips	Three fixed routes with different traffic load and configuration.	Car: Gasoline engine car. Bus ventilation settings not specified. Cyclists: No further information provided	Full trip from origin to destination	In one configuration, car was driven with cabin ventilation on the second level, cabin air recirculation turned off and windows closed.	Sampling, device operation and analysis were standardized.	Standard deviation	Average per commute and trip,	Bicycle: 28 trips; Bus: 24; Car: 28 It is unclear the actual number of commutes used for analyses

Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
		simultaneously; car in all pairs alternating ventilation settings between trips								
	Partially	✕	✕	✔	?	✕	✔	✔	✔	✔
Weichenthal 2008 [363]	A researcher followed one 29.5km route three weekdays per week during morning and evening commutes, by three modes of transport	The modes were consecutive on time	The route was followed in three sections, each by one mode of transport	Walk: Along the route, included waiting in the bus stop Bus: Along the route, diesel-fueled Car: The researcher was asked to drive as normal, gasoline fueled	Not specified if transit times between modes, besides the walking-bus transit, were measured	As per usual, according to driver's preference	Sampling, device operation and analyses were standardized	Standard deviation	Average exposure per transit period	80 morning and 80 evening commutes
	✔	✕	Partially	✔	✕	NA	✔	✔	✔	✔
Yan 2015 [314]	A pair of researchers followed each route by one MT several times a day carrying the personal monitors. Each MT was measured in different day periods.	Different measurement campaigns were performed per mode - Walking Dec 10-16 every other hour from 8.00 to 21.00 - Bus and subway: 18-23 Dec[Bus: 8.00, 12.00 and 18.00hrs][Subway between 14 to 16.00 hr.]	One route for each MT, the routes overlapped on some sections	Bus: with and without AC, but no comparisons were made according to this criterium Subway: two specific lanes with above and underground sections Walking: time and route specified	For bus trips, it included waiting times and walks to approach the stations/stops. For subway trips, from entering subway station entrances		Sampling, device operation and analyses were standardized	Standard deviation	Trip average	Pedestrians: 39samples/trips, bus commuters: 17 Subway: 5 sets
	✔	Partially	Partially	✔	✔	✔	✔	✔	✔	✔
Zuurbier 2010 [296]	One route was followed during 47 days evenly spaced along a year by three modes of transport, each mode on approximately one third of the days. A second route with low traffic was followed by bicycle.	Samples between 8.00 to 10.00 hours on Tuesdays and Thursdays. On each sampling campaign, modes were simultaneous for diesel and gasoline car or electric and diesel bus or cyclists over high and low traffic route	One fixed high-traffic route for cars, buses and high-traffic bicycle route. A second fixed route for low-traffic bicycle route.	Diesel and gasoline-fueled cars, Diesel and electric trolley buses (ventilation not controlled); not A/C available, windows closed, not smoking allowed; diesel buses retrofitted with particulate filters. Cyclists along low- and high- traffic intensity routes. Bus:		Windows closed and air conditioning at moderate level	Sampling, device operation and analysis were standardized.	Standard deviation	PM10: Daily averages PM2.52-hour averages from one-sec readings	Diesel bus: 13, electric bus: 13, gasoline car: 14, diesel car: 14, high-traffic bicycle: 15, low-traffic bicycle: 15

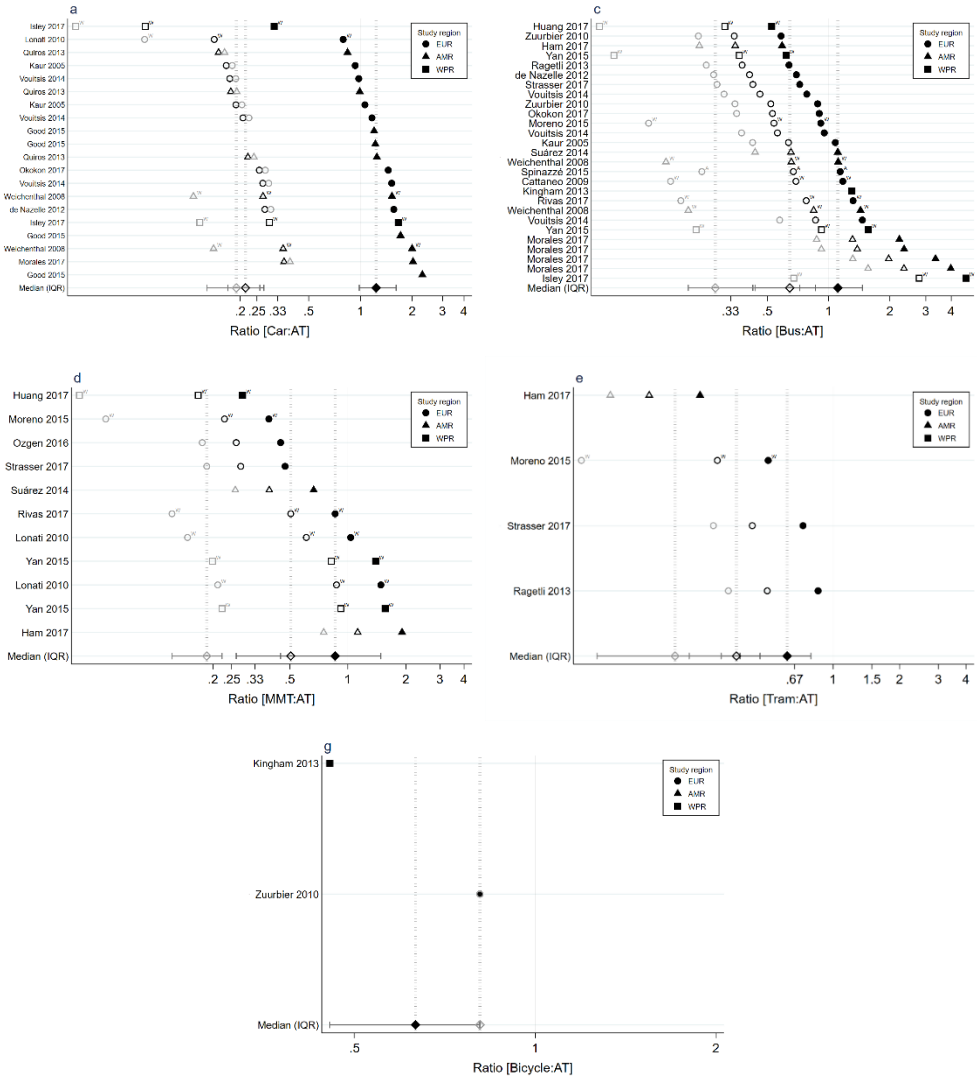
Author (year) Reference	Experiment	Simultaneous modes on time	Same route for all modes	Standard commuting conditions	Only one mode per trip	Control of ventilation settings in car commutes	Air pollution measurements standardized	Precision for summary measurements	Unit of analysis	Sample size
		high and low traffic route		Cyclists along low- and high- traffic intensity routes. Bus:						

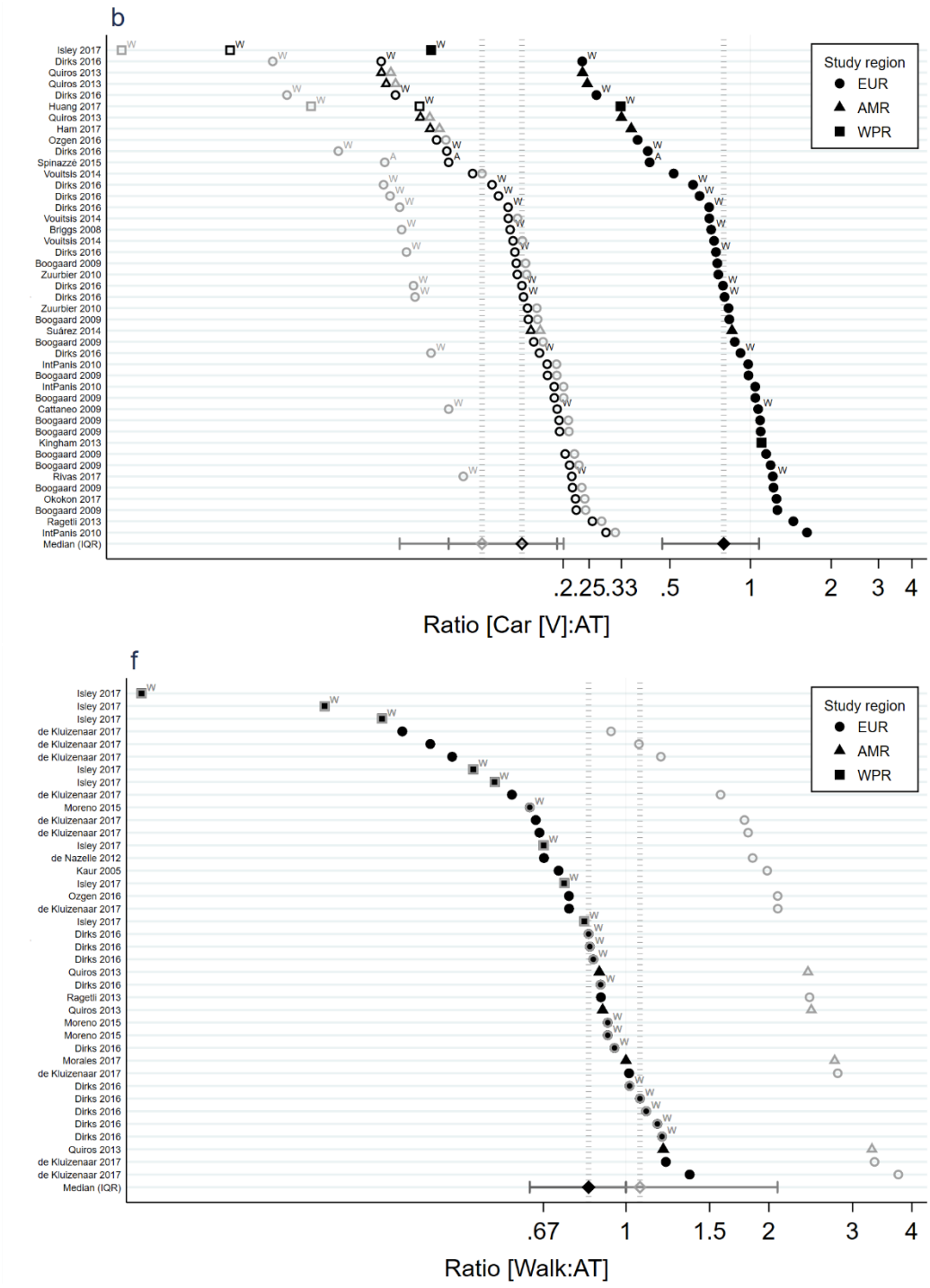
MMT: Massive motorized transport. A/C: Air conditioned. NS: Not specified. NA: Not applicable.? Unclear ✕ either if unclear or unspecified for ventilation settings, because it is assumed that it was not standardized. – not specified.

Appendix 67. Summary of exposure and inhalation ratios according to mode of transport and WHO region

Mode of transport	WHO region	Exposure ratios				Inhalation ratios			
		Median (IQR)	S	C	Ratios above 1 # (%)	Median (IQR)	C	S	Ratios above 1 # (%)
Bus	EUR	0.91 (0.72, 1.14)	11	14	5 (36)	0.53 (0.42, 0.67)	14	11	0 (0)
	AMR	1.83 (1.11, 2.85)	4	8	7 (88)	1.08 (0.65, 1.68)	8	4	4 (50)
	WPR	1.3 (0.62, 1.57)	4	5	3 (60)	0.64 (0.34, 1.85)	4	3	1 (25)
	Total	1.11 (0.72, 1.46)	19	27	15 (56)	0.64 (0.42, 0.86)	26	18	5 (19)
Car	EUR	1.11 (0.95, 1.48)	5	8	5 (63)	0.2 (0.17, 0.26)	8	5	0 (0)
	AMR	1.38 (1.2, 2)	4	10	8 (80)	0.25 (0.18, 0.36)	6	3	0 (0)
	WPR	0.99 (0.32, 1.66)	1	2	1 (50)	0.18 (0.06, 0.3)	2	1	0 (0)
	Total	1.23 (0.98, 1.61)	10	20	14 (70)	0.21 (0.17, 0.27)	16	9	0 (0)
Car [V]	EUR	0.83 (0.7, 1.09)	12	36	13 (36)	0.15 (0.12, 0.19)	36	12	0 (0)
	AMR	0.33 (0.25, 0.36)	3	5	0 (0)	0.06 (0.04, 0.06)	5	3	0 (0)
	WPR	0.33 (0.06, 1.1)	3	3	1 (33)	0.03 (0.01, 0.06)	2	2	0 (0)
	Total	0.79 (0.47, 1.08)	18	44	14 (32)	0.14 (0.07, 0.19)	43	17	0 (0)
MMT	EUR	0.67 (0.45, 1.03)	5	6	2 (33)	0.39 (0.26, 0.61)	6	5	0 (0)
	AMR	1.29 (0.67, 1.91)	2	2	1 (50)	0.76 (0.39, 1.12)	2	2	1 (50)
	WPR	1.39 (0.28, 1.56)	2	3	2 (67)	0.82 (0.17, 0.92)	3	2	0 (0)
	Total	0.86 (0.45, 1.48)	9	11	5 (45)	0.51 (0.26, 0.87)	11	9	1 (9)
Tram	EUR	0.73 (0.51, 0.86)	3	3	0 (0)	0.43 (0.3, 0.5)	3	3	0 (0)
	AMR	0.25 (0.25, 0.25)	1	1	0 (0)	0.15 (0.15, 0.15)	1	1	0 (0)
	WPR	-				-			
	Total	0.62 (0.38, 0.79)	4	4	0 (0)	0.37 (0.22, 0.47)	4	4	0 (0)
Walk	EUR	0.85 (0.66, 1.02)	7	27	8 (30)	0.85 (0.66, 1.02)	27	7	8 (30)
	AMR	0.95 (0.89, 1.1)	2	4	2 (50)	0.95 (0.89, 1.1)	4	2	2 (50)
	WPR	0.5 (0.27, 0.71)	1	8	0 (0)	0.5 (0.27, 0.71)	8	1	0 (0)
	Total	0.83 (0.63, 1)	10	39	10 (26)	0.83 (0.63, 1)	39	10	10 (26)
Bicycle	EUR	0.81 (0.81, 0.81)	1	1	0 (0)	0.81 (0.81, 0.81)	1	1	0 (0)
	AMR	-				-			
	WPR	0.45 (0.45, 0.45)	1	1	0 (0)	-			
	Total	0.63 (0.45, 0.81)	2	2	0 (0)	0.81 (0.81, 0.81)	1	1	0 (0)

Appendix 68. Ratio of exposure and dose inhaled and dose inhaled over a standardized route of ultrafine particles according to motorized modes of transport





Full symbols correspond to exposure ratios. Hollow symbols correspond to dose ratios. Hollow gray symbols correspond to dose ratios over a standard route (sensitivity analysis). All ratios are calculated using bicycle as reference, unless stated otherwise with labels in the ratio as follows: W: Walk as reference, A: Active transport as reference (the authors combined walk and bicycle exposure). Car [V]: Car driven under standard ventilation conditions. MMT: Massive motorized transport. AT: Active transport (bicycle or walk). Study region: EUR: European countries, AMR: American countries, WPR: Western Pacific Region countries. IQR: Interquartilic range (25^{th} to 75^{th} percentiles).

Appendix 69. Mean diameter and lung deposited surface area of ultrafine particles

Author	Study place	Mode of transport	Exposure (#/cm^3)			Mean diameter (nm)	LDSA (mm^2/m^3)		
Ham et al [374]	Sacrament (USA)	Car [V]	7,900 ± 4,700				11 (2-21) [1]		
		Light rail	5,500 ± 3,000				14 (13-15) [1]		
		Bus	13,000 ± 4,400				38 (35-40.5) [1]		
		Bicycle	22,000 ± 11,000				30.5 (25-40) [1]		
		Train	42,000 ± 49,000				37 (30-59) [1]		
Huang et al [360]	Beijing (China)	MMT	12,542 ± 2,577				83 ± 21		
		Car [V]	14,433 ± 3,790				115 ± 39		
		Bus	23,135 ± 14,079				116 ± 30		
		Pedestrians	44,038 ± 6,142				134 ± 23		
Moreno et al [337]	Barcelona (Spain)	MMT	23,000 ± 4,000			90 ±13	94 ± 27		
		Tram	30,000 ± 10,000			66 ± 9	92 ± 27		
		Pedestrians					101 ± 20		
		Outer Diagonal	37,000 ± 6,000			66 ± 9			
		Inner Diagonal	59,000 ± 13,000			56 ± 9			
		Central gridplan	54,000 ± 18,000			54 ± 6			
		Diagonal to La Rambla	54,000 ± 20,000			67 ± 7			
		Bus	54,000 ± 16,000			64 ± 8	125 ± 39		
Ragettli et al [362]	Basel (Switzerland)	Bus	14,055 ± 7,951			50 (44-59) [1]			
		Tram	18,818 ± 8,120			49 (42.5-52) [1]			
		Pedestrian	19,481 ± 11,705			51 (40-55) [1]			
		Bicycle	22,000 ± 0			45 (40-51) [1]			
		Car	31,784 ± 25,555			47 (41-52) [1]			
Spinazze et al [356]	Como (Italy)	Car [V]	9,159 ± 9,048			72.5 ± 17.3	33.5 ± 27.6		
		Active commuters (low traffic)	12,091 ± 9,176			70.1 ± 19.4	66.2 ± 44.8		
		Active commuters (high traffic)	21,778 ± 18,302			64 ± 20.1	41.9 ± 23.2		
		Bus	24,819 ± 13,673			60 ± 12.6	78.2 ± 36.5		
Strasser 2017 [375]	Vienna (Austria)		C1	C2	C3		C1	C2	C3
		Subway	7,233.3	6,480.5	8,608.9		3.56	5.99	4.00
		Car [V]		8,848.4				3.33	
		Tram	11,783.2	10,008.2	13,311.5		5.01	5.01	7.49
		Bus	12,296.0	10,598.3	13,129.9		5.05	6.46	6.32
		Bicycle			18,199.6				35.47

[1]Median and IQR [Approximate, extracted from figure].

Appendix 70. Parameters of pollutant inhalation/dose uptake estimation

Author	Pollutant parameters	Respiratory parameters		Time parameters		Inhaled dose		Uptake dose	
Ham 2017 [374]	Average exposure (µg/mile)	Assumed, based on inhalation rates reported in the literature (L/min)		Average time per trip		Exposure per mile (particles/mile) = ((concentration (particles/cm^3)* time (minutes))/distance (miles))*inhalation rate (m^3/min)			
		Mode	L/min	Mode	Minutes	Mode	Particles/mile (*10^8)	Mode	LDSA (mm^2/m^3)
		Motorized transport	11.8	Car	22	Car	2.2+-1.3	Car	11 (2-21)
				Bus	33	Bus	3.6+-1.3	Bus	14 (13-15)
				Light rail	23	Light rail	1.2+-6.7	Light rail	38 (35-40.5)
				Train	53	Train	5.7+-6.7	Train	37 (39-59)
		Bicycle	23.5	Bicycle	45	Bicycle	3.0+-1.4	Bicycle	30.5 (25-40)
Huang 2017 [360]	Average exposure concentration	Estimated; inhalation rate calculated as $\ln V_E$=0.022(HeartRate)+0.89		Time spent in each microenvironment (minutes)		Inhaled dose (particles) = Exposure*inhalation rate as a function of heart rate*time spent in each microenvironment		Active surface area, measured by a Diffusion Charging Sensor (EcoChem Analytics DC-2000CE) (mm^2/m^3)	
		Mode	Inhalation rates (L/min)						
		Bus	17.9	6.6		$3.3*10^9$		116 ±30	
		Train (MMT)	18.5	4.1		$1.0*10^9$		83 ±21	
		Car	14.3	7.6		$2.1*10^9$		115 ±39	
		Walk	25.0	25.8		$27.8*10^9$		134 ±23	
IntPanis, 2010 [284]	Average exposure	Estimated, based on the equation VE=breathing frequency * tidal volume. These were measured with a portable cardiopulmonary indirect breath-by-breath calorimetry system, while standing and during exercise. Minute ventilation (L/min)		Journey duration (minutes)		Inhaled pollutants=particles * minute ventilation		Lung deposited fraction based on deposition factor (deposition fraction decrease with particle size and increase with tidal volume, increases with exercise).	
								Mass based deposition fraction for ultrafine particles while at rest (car): 0.63 ±0.03and while at bicycle: 0.83 ±0.04.	Sensitivity analysis: Deposition was calculated as a linear function of tidal volume
		Mode	Male	Female	City	Male	Female		
		Bicycle	59.1 ±13.7	46.2 ±10.6	Brussels	15.4 ±1.3	17.6±1.9	5'580.195 ±1'924.800	4'631.562
					LLN	16.3 ±1.2	14.7	2'023.702 ±594.881	1'679.673
					Mol	18.8 ±2.8	21.1±2.0	1'135.046 ±435.493	942.088
		Car	13.4 ±1.7	11.3 ±1.8	Brussels	16.9 ±2.5	16.2±3.6	1'335.467 ±83.365	841.344 965.696
					LLN	10.6 ± 0.3	10.8	305.095 ±83.365	192.210 214.045
					Mol	9.5 ±1.0	10.2±1.24	216.768 ±73.832	136.564 135.956
Moreno 2015 [337]	Particulate number							Estimated; alveolar deposited surface area concentration ($\mu m^2/cm^3$). It is calculated using the formula $S_{AL} \approx 5.4*10^{-2}*N*d_{p,w} \propto I_1$ Where N=pcl/cm^3, $d_{p,w}$ number-averaged particle size (µm), I_1 =total particle charge deposited per unit time inside the Faraday cage	
								Tram	92±27
								Metro	92±21
								Walk	101±20
								Bus	125±39

de Nazelle, 2012 [295]	Average exposure concentration	Estimated; inhalation rates estimated using EPA algorithms, based on average measured energy expenditure (METS) Energy expenditure was measured during the trips in different modes	Duration of trip exposure (minutes)	Inhaled dose (µg*trip)=inhalation rate * concentration (arithmetic mean) * duration of exposure * conversion factor for each pollutant	
		Mode / Inhalation rates (L/min)		(particles/trip)	
		Bicycle: 41	25	$75.6*10^9$	
		Walk: 34.1	49	$89.8*10^9$	
		Bus: 20.1	20.1	$39.8*10^9$	
		Car: 19.9	19.9	$75.6*10^9$	
Okokon 2017 [359]	Average exposure concentration	Estimated; average (female and male) minute ventilation for mode of commuting, at a speed of 19.4km/h	Average commuting time		Estimated; average pollutant intake per one way 8km was calculated using the formula I=E*T*V
		Mode / VE (L/min)	Helsinki / Rotterdam / Thesaloniki	Helsinki / Rotterdam / Thesaloniki	
		Car: 9.87	21/20 / 22/23 / 22/21	2 / 6 / 5	
		Car [V]		8 / 7 / 18	
		Bus: 9.01	23 / 27 / 32	4 / 5 / 15	
		Bicycle: 32	28 / 28 / 27	- / 18 / -	
Quiros 2013 [387]	Average exposure concentration	Estimated; gender-average breathing rates obtained from the literature	Average time per trip	Respiratory exposure = arithmetic concentration (concentration/volume)*length of each trip(time/trip)*gender-average breathing rates	
		Mode / Breathing rates (mL/s)		Morning / Afternoon / Evening	
		Car: 130	4min/trip	$1.39±1.00*10^9$ / $5.98±3.34*10^8$ / $4.73±2.30*10^8$	
		Car [V]: 130	4min/trip	$8.99±1.18*10^8$ / $2.18±2.04*10^8$ / $1.70±0.92*10^8$	
		Pedestrian: 390	25 min/trip	$2.15±1.0^{10}$ / $8.60±2.72*10^9$ / $7.83±2.60*10^9$	
		Cyclist: 580	7 min/trip	$1.09±0.54*10^{10}$ / $4.13±1.93*10^9$ / $4.59±2.13*10^9$	
Strasser 2017 [375]	Average exposure concentration				
Spinazze et al [356]	Average exposure concentration				
Vouitsis, 2014 [340]	Average exposure concentration	Assumed, based on inhalation rates reported in the literature	Journey duration (minutes)		Average inhaled dose=inhalation rates*average concentration*trip time (min)
		Mode / Respiratory rates (L/min)	Route 1 / Route 2 / Route 3		Route 1 / Route 2 / Route 3
		Bicycle: 41	26 / 27 / 30		- / - / 5.4

LDSA: estimated according to the International Commission on Radiological Protection $\mu m^2/cm^3$

Comparison	1	2	3
Subway	3.56	5.99	4.00
Bus	5.05	6.46	6.32
Tram	5.01	5.01	7.49
Car		3.33	
Bicycle			35.47

Mode	LDSA (mm2/m3)
Bus	78.2 ±36.5
Active commuters (low traffic)	66.2 ±44.8
Active commuters (high traffic)	41.9 ±23.2
Car	33.5 ±27.6

		Bus	20.1	28	37	34	2.4	4.8	2.4
		Car	19.9	24	22	25	2.4	2.9	2.1
		Car [V]	19.9	21	22	23	1.3	0.9	1.5
		Estimated; minute ventilation estimated based on heart rates measured while commuting (heart rate monitors). Also, participants performed submaximal bicycle ergometer test to measure heart rate and minute ventilation					Inhaled dose estimated with mean ventilation rates		
		Mode	Average minute ventilation (L/min)				Particles/hr (particles * 10^9)		
Zuurbier, 2010 [296]	Median exposure corrected by background concentration	Bus[Diesel]	12.7	Doses estimated as values of 2-hr mean			34.3		
		Bus[Electric]	12.7				24.3		
		Car[Diesel fueled]	11.8				25.0		
		Car[Gasoline fueled]	11.8				27.5		
		Bicycle [High traffic]	23.5				59.3		
		Bicycle [Low traffic]	23.5				50.5		

L/min: liters per minute. m^3/h: cubic meters per hour. bpm: beats per minute

3.2 Description of air pollution exposure in the Rotterdam Study

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INTRODUCTION

Traffic-related air pollution exposure is a high public health problem, associated with increased mortality and morbidity and shortened life expectancy. [390] The Global Burden of Disease estimated the effects of long-term exposure to ambient particulate matter of size 2.5 or smaller ($PM_{2.5}$) contributed to a loss of 103.1 million (95% uncertainty interval (UI) 90.8 to 115.1) disability-adjusted life years (DALY) and 4.2 million (95% UI 3.7 to 4.8) deaths, which is only one of the pollutants associated to traffic emissions, between 1990 and 2015.[390] They reported that long-term exposure to ambient $PM_{2.5}$ Long-term exposure to ambient $PM_{2.5}$ is ranked as the most important environmental risk factor and fifth cause of mortality worldwide in 2015, with a disproportionate share of the burden borne by population aged 70 years or older. [390] Health effects of traffic-related air pollution exposure are multiple including ischemic heart disease, stroke, cerebrovascular and chronic obstructive pulmonary disease.[390,282] Additionally, an increasing body of evidence links air pollution exposure to insulin resistance and diabetes mellitus.[391-394]

Traffic-related air pollution is a major source of air pollution in urban settings,[46] but it is only one of the multiple traffic-related pollutants strongly correlated between them.[52] For example, proximity to traffic also increases the exposure to noise.[395] This confounds the associations between traffic-related air pollution exposure and several health outcomes, as noise and lower proximity to green spaces has been documented to influence physical activity levels but also hypertension, vascular disease, diabetes, among others. [381,396-400] In order to tackle the burden attributable to air pollution exposure, it is necessary to have a better understanding of the health effects of air pollution by addressing these sources of confounding.

The Rotterdam Study is a large prospective cohort study aimed to examine the incidence of risk factors for neurological, cardiovascular, psychiatric, and other chronic diseases. The population of the Rotterdam Study is recruited from the Ommoord district, a small very well defined geographic area (~4.5 km^2). Therefore, we aimed to calculate the individual exposure to air pollutants among the participants of the Rotterdam Study. In this chapter I describe the assessment of exposures to particulate matter of 10 µm (PM_{10}), 2.5 µm ($PM_{2.5}$), $PM_{2.5}$ absorbance, nitrogen dioxide (NO_2), and nitrogen oxides (NO_x) at the Ommoord district, using land use regression models developed within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project and, for illustrative purposes, I describe the distribution of the exposure levels in one specific visit of the cohort, according to participants characteristics at one follow-up visit.

METHODS

Study design

The Rotterdam Study is a prospective population-based cohort established in 1990 in Rotterdam, the Netherlands,[23] inviting all participants aged 45 years or older living in the Ommoord district (a scheme of the cohort is provided in Figure 1, page 8). In the first cohort (RS-I), 7,983

participants aged ≥55 years were recruited. A second cohort (RS-II) with 3,011 new participants aged ≥55 years started in 2000. A third cohort (RS-III) started in 2006 with 3,932 new participants aged ≥45 years. Follow-up visits are performed every four to five year approximately, so by 2014 were obtained about 41,852 visits among 14,926 participants. For this chapter, we used the information of the participants of the fifth visit of RS-I, third of RS-II, and second of RS-III with updated information of home addresses (n=5,356 out of 7,162 participants). This visit was held between 2009 and 2013.

The Ommoord district is located at the north of Rotterdam, has a land area of 448 hectares (~4.5 km^2). This small district is bounded on the south by a motorway with 3 lanes in each direction and maximal velocity of 100km/h, the river Rotte on the north, and residential neighborhoods on the east and west.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Air pollution exposure assessment

Air pollution concentrations at the participants' home address were estimated using land use regression models developed within the ESCAPE project.[401,402] A multistep procedure was followed in order to ensure the confidentiality of the participants.

Geocoding: As all participants were recruited from population living in the Ommoord district, we obtained a list of all address in the district (about 13,000 unique addresses) from the municipality of Rotterdam and the Rotterdam Study management group. These addresses were geocoded in QGIS using the Google Maps geocoder.[403] These data did not contain personal identifiers or links to the participants of the cohort. About 80% of the addresses were automatically matched with high confidence to spatial points corresponding to build rooftops. The remaining addresses were located nearby the actual address, thus we assigned manually the spatial points to the closest building.

Estimation of air pollution at home's participant addresses: The concentrations of the pollutants were calculated for each geocoded point using the land use regression models for The Netherlands/Belgium developed and validated within the ESCAPE project (described below). Geographic Information System (GIS) predictors were used to estimate the concentration of each pollutant, accordingly, as described below. These calculations were conducted at the Institute for Risk Assessment Sciences (IRAS) from the University of Utrecht.

Backwards extrapolation of the air pollution estimates: Because the concentrations were calculated for the year of models' validation (between February 17th 2009 to February 19th 2010 for The Netherlands/Belgium [404,405]), we first assigned the estimated pollutants concentrations to the records of the cohort participants using as individual identifier the home address reported by

the participant at the follow-up visit. Then, we calculated the exposure for each air pollutant at the home's participant address at the exact day of follow-up visit and fourteen days before that visit applying a backward extrapolation method using the concentrations obtained from fixed-background monitors, according to ESCAPE procedures, also described below.

To calculate the exposure by means of backward extrapolation, first, we collected the daily measurements of the pollutants concentration obtained by seven fixed-background monitors located in The Netherlands (obtained from https://www.lml.rivm.nl/gevalideerd/index.php). The monitors were selected because these had the lowest number of measurement days with missing data among all monitors with available information (monitor 131, 133, 230, 318, 437, 538, and 722). Second, we calculated the average of the daily measurements within the period of the measurement campaigns performed in The Netherland/Belgium (February 17th 2009 to February 19th 2010), per pollutant. Third, we calculated the backward extrapolation factors by dividing the daily data obtained from the fixed background monitors by the average previously calculated, per pollutant. Fourth, we merged the dataset of backward extrapolation factors with the dataset of the participants of the Rotterdam Study at the specified visit, using as individual identifier the date of follow-up visit. To account for lagged air pollution exposure, we assigned to each participant the backward extrapolation factors of fourteen days before the follow-up visit. Fifth, we estimated the exposure of the participant at the home address at the date of the follow-up visit by multiplying the pollutants concentrations estimated with the LUR model times the backwards extrapolation factor corresponding to the date of visit. This step was repeated for each of the 14-days lags per each pollutant. For this illustrative chapter, we express the exposure as the average of the exposure at the day of follow-up and the 14-days lags per pollutant.

Development of the Land Use Regression models by the ESCAPE project: In this section we briefly describe the procedures followed in the ESCAPE project to develop the LUR models for the pollutants exposure calculated in the Rotterdam Study. First, a measurement campaign of particulate matter ($PM_{2.5}$, $PM_{2.5}$ absorbance, and PM_{10}) and nitrogen oxides (NO_2 and NO_x) was performed using mobile monitors deployed in 20 European study areas at 20 to 40 sites for particulate matter and 40 to 80 sites for NO_x, per area, and additional 16 measurement sites for NO_x; second, a number of GIS predictor variables that would explain the concentration of pollutants were calculated at each measurement site; and third, the LUR models were generated by linear regression using as outcomes the measurements obtained in the measurements campaigns and as predictors the GIS variables, background concentrations of the pollutants and the coordinates of the sites monitored, the variable selection followed a predesigned procedure of supervised stepwise selection procedure.

The measurement campaign was performed between October 2008 and April 2011, in each study area were selected approximately 20 sampling sites, representing regional background, urban background and traffic sites [404,405] Rotterdam was among the measurement sites included in the development of the LUR models for The Netherlands/Belgium; the measurement campaigns went between February 17th 2009 to February 19th 2010.[404,405] Traffic sites were overrepresented and the full range of local traffic intensities was included, as more variability was expected between streets. Other sites were considered, such as industries and major ports. Each site was

measured three times for 14 days in cold, warm, and intermediate seasons. The three measurements obtained in each site were averaged to obtain an annual average. A centrally located background reference site was operated for a whole year, the measurements obtained from this monitor were used to adjust for temporal variation the annual average. [404,405] Harvard impactors were used to measure $PM_{2.5}$ and PM_{10}. The measurements of reflectance on $PM_{2.5}$ filters were used to calculate the $PM_{2.5}$ absorbance.[402] Ogawa badges were used for NO_x measurements.[406]

Each measurement site was geocoded with Global Positioning System (GPS) readings at each site visit. At each measurement site, a number of GIS predictor variables from European and local source data were calculated. Circular buffers around each monitoring site were calculated for traffic variables (radii of 25, 50, 100, 300, 500, and 1000 m) and for land use and population (100, 300, 500, 1000, and 5000m).[401,402] Known dispersion patterns of the pollutants were taken into account to select the corresponding buffer size per predictor. The overall sets of European GIS data obtained at each monitoring site was road data, land use data (high density residential land, low density residential land, industry, ports, urban green and natural land), population density data, altitude. Local GIS data included land use, population and household density, altitude and study-area specific variables such as distance to the sea. 401,402

The final LUR models were developed by building a linear regression model using the GIS variables as predictors of the pollutants' concentrations obtained in the measurements campaigns, as well as the geographical coordinates of the measurement sites and background measurements of the pollutants. The models were built using a supervised stepwise selection procedure, to obtain a model that improved the adjusted explained variance (R^2), on the basis of predefined assumptions of the direction of the association with the outcome, collinearity, and model performance (examined by leave-one-out cross validation). A model for each pollutant and study area was developed. [401,402]

LUR models performance: To estimate air pollution exposure among the participants of the Rotterdam Study, we used the LUR models developed for The Netherlands/Belgium region. [401,402] The covariates, coefficient, and model performance of the LUR model for each air pollutant described in the table below.

Pollutant	LUR model	LUR model performance
$PM_{2.5}$[402]	9.46 + 0.42 × (a regional background concentration estimate ($\mu g/m^3$)) + 0.01 × (road length (m) of major roads within a buffer of 50 meters) + $2.28 \cdot 10^{-9}$ × (total traffic load (vehicles·day^{-1}·m) of major roads within a buffer of 1000 meters (sum of (traffic intensity × length of all segments))).	R^2 of the model: 67% R^2 of the model validation was 61%. Forty sites were used for the model development
$PM_{2.5}$ absorbance[402]	0.07 + $2.95 \cdot 10^{-9}$ × (total traffic load (vehicles·day^{-1}·m) of all roads within a buffer of 500 meters (sum of (traffic intensity × length of all segments))) + $2.93 \cdot 10^{-3}$ × (road length of major roads within a buffer of 50 meters) + 0.85 × (a regional background concentration estimate (10^{-5}/m)) + $7.90 \cdot 10^{-9}$ × (surface area (m^2) of all residential land within a buffer of 5000 meters) + $1.72 \cdot 10^{-6}$ × (total heavy-duty traffic load (vehicles·day^{-1}·m) of all roads) within a buffer of 50 meters (sum of (traffic intensity × length of all segments)))	R^2 of the model: 92% R^2 of the model validation: 89%. Forty sites were used for the model development
PM_{10}[402]	23.71	R^2 of the model: 68% R^2 of the model validation: 60%.

	+ $2.16 \cdot 10^{-8}$ × (total traffic load (vehicles·day^{-1}·m) of major roads within a buffer of 500 meters (sum of (traffic intensity × length of all segments))) + $6.68 \cdot 10^{-6}$ × (population (N) within a buffer of 5000 meters) + 0.02 × (road length (m) of major roads within a buffer of 50 meters)	Forty sites were used for the model development
NO_2[401]	7.80 + 1.18 × (a regional background concentration estimate (μg/m^3)) + $2.30 \cdot 10^{-5}$ × (population (N) within a buffer of 5000 meters) + $2.46 \cdot 10^{-6}$ × (total traffic load (vehicles·day^{-1}·m) of all roads within a buffer of 50 meters (sum of (traffic intensity × length of all segments))) + $1.06 \cdot 10^{-4}$ × (road length (m) of all roads within a buffer of 1000 meters) + $9.84 \cdot 10^{-5}$ × (total heavy-duty traffic load (vehicles·day^{-1}·m) of all roads in a buffer of 25 meters (sum of (heavy-duty traffic intensity × length of all segments)) + 12.19 × (inverse distance (m^{-1}) to the nearest road of the central road network + $4.47 \cdot 10^{-7}$ × (total heavy-duty traffic load (vehicles·day^{-1}·m) of all roads within a buffer between 25 and 500 meters (sum of (heavy-duty traffic intensity × length of all segments))	R^2 of the model: 86% R^2 of the model validation: 81%. Eighty sites were used for the model development
NO_x[401]	3.25 + 0.74 × (a regional background concentration estimate (μg/m^3) + $4.22 \cdot 10^{-6}$ × (total traffic load of all roads within a buffer of 50 meters (sum of (traffic intensity × length of all segments))) + $6.36 \cdot 10^{-4}$ × (population (N) within a buffer of 1000 meters) + $2.39 \cdot 10^{-6}$ × (total heavy-duty traffic load of all roads within a buffer of 500 meters (sum of (heavy-duty traffic intensity × length of all segments))) + 71.65 × (inverse distance (m^{-1}) to the nearest major road) + 0.21 × (road length of major roads in a buffer of 25 meters)	R^2 of the model: 87% R^2 of the model validation: 82%. Eighty sites were used for the model development

Statistical analysis

We conducted descriptive analyses of each pollutant. First, we describe the distribution of each pollutant for the Ommoord district as estimated with the LUR models; we calculated the median, mean, range, and the distribution by dividing the range times the mean and multiplying by 100. We compared this distribution to the distribution of the pollutants obtained in the ESCAPE project measurement campaigns for the Netherlands/Belgium region, as reported by Eeftens et al [404] and Cyrys et al [405]. Second, we calculate the correlation between the pollutants at the Ommoord district, using the Pearson correlation coefficient. These are also compared to those estimated in the ESCAPE measurement campaigns. [404,405] Third, we examined the annual distribution of the pollutants calculated among the participants of the Rotterdam Study; we fitted a generalized linear model with a log link to account for the no-linear distribution of the pollutants, using as predictors a natural cubic spline with 7 degrees-of-freedom of the month of follow-up and the year of visit. Then, we plotted the fitted values of the pollutants over date. Finally, we estimated the median and interquartile range of the pollutants according to characteristics of the participants. We tested the distribution according to the following characteristics: age (<60, 60-70, 70-80 and ≥80), sex (men vs. women), season (winter, spring, summer, autumn), income (<2100eur, 2100-3000eur, 3000 to 4200eur, ≥4200eur), education levels (lower general vs. intermediate higher), occupation (yes vs. no), community dwelling (yes vs. no), body mass index (normo-weight (<25kg/m^2), overweight (25-30 kg/m^2) and obese (≥30kg/m^2)), smoking behavior (never, current, former), and medication intake (antihypertensive, statin or antidiabetic). We also stratified in quartiles physical activity (MET-hours/week), systolic and diastolic blood pressure (mmHg), total cholesterol (mmol/L), HDL-cholesterol (mmol/L), glucose (mmol/L), insulin (μIU/mL), and HOMA-IR. For all characteristics, we tested the differences with the U-Mann Whitney test and Kruskal-Wallis test if more than two categories.

For ordered categories, we tested the trend with the p for trend. All analyses were performed using Stata version 14.1 SE (StataCorp LP, College Station, Texas).[72]

RESULTS

Table 21 (page 281) shows the distribution of air pollutants concentrations estimated for the Ommoord district and the distribution of pollutants reported for the measurements obtained within ESCAPE project in the Netherlands/Belgium region. Overall, median exposure was similar between the Ommoord district and the Netherlands/Belgium region, but the dispersion of the distribution was smaller. As observed in the Netherlands/Belgium region, the dispersion of NO_x and NO_2 is larger than that of particulate matter, Also, the correlation between pollutants was similar compared to the Netherlands/Belgium region, although the correlation of $PM_{2.5}$ absorbance with the remaining pollutants was stronger (Table 22 (page 282)). The spatial distribution of air pollutants concentrations estimated for the Ommoord district is shown in Figure 18 (page 288). It shows a larger variability for NO_2 and NO_x, which follows the pattern of the traffic network in the district.

In Figure 19 (page 289) we show the distribution of the 14-days lag average exposure average at the home address of the participants of the Rotterdam Study. The exposure had a substantial temporal variation, with higher values around winter time, which was slightly shifted towards spring for particulate matter. Also, the exposure exhibited a long-term trend towards a reduction of the average levels, which was steeper around 2011 for particulate matter.

Additionally, we show the distribution of the 14-days lag average exposure at home address among participant to PM_{10}, $PM_{2.5}$ and $PM_{2.5}$ absorbance (Table 23 (page)) and NO_2 and NO_x (Table 24 (page 286)), according to participants characteristics at the visit date. The exposure to all pollutants was higher among older participants, among participants with lower general education level, with lower levels of physical activity, and higher systolic and diastolic blood pressure. Specifically, NO_2 exposure was higher among participants with higher insulin and HOMA-IR levels.

DISCUSSION

In this chapter we describe the methods used to estimate the air pollution exposure in one large population-based cohort located in Rotterdam, using the LUR models developed within the ESCAPE project for the region of the Netherlands/Belgium. We found that the dispersion of the distribution of the pollutants was smaller in the Ommoord district than in the region of The Netherlands/Belgium.[404,405] Nevertheless, there were a substantial spatial variation of the pollutants, particularly of NO_2 and NO_x, probably as a consequence of the traffic emissions. Moreover, when we attributed the concentrations at the home address of the participants of the Rotterdam Study, we observed that the 14-days lag average exposure exhibited a large temporal variation, with higher levels of air pollution exposure in winter time.

These findings highlight the issue of a gradient of air pollution exposure, even within relatively small areas, as a consequence of the proximity to roads and avenues. Moreover, these findings are in agreement with the notion that relying on ecologic measures of air pollution exposure, such as single background monitoring stations, may underestimate the influence of

traffic-related air pollution.[405] Consequently, the health effects of air pollution exposure may be biased and confounded, because the actual contribution of traffic to the individual's exposure may not be captured by such background measures. Indeed, air pollutants such as NO_2 and NO_x usually exhibit a large spatial variation, because these rapidly undergo chemical and physical processes in the atmosphere. Therefore, the measures obtained with background monitoring stations may underestimate the actual levels of exposure given the proximity to traffic emitting sources. This is in contrast to PM_{10} and $PM_{2.5}$ concentrations, which are often much more stable in the environment and are better correlated to background measures of particulate matter concentrations. Therefore, the estimation of air pollution exposure in the Rotterdam Study, along with the detailed measurements of biomarkers, genomics, and proteomics data obtained in each visit, will likely contribute to the understanding of the health effects of traffic-related air pollution exposure.

There were differences in the concentrations of pollutants according to certain characteristics of the participants. Overall, the concentration of the pollutants increased as the age and the blood pressure of the participants increased and the education and physical activity levels decreased. Furthermore, the exposure was higher during winter and spring than during summer or autumn. These findings have several interpretations. It is possible that the increase of air pollution exposure according to age, physical activity levels, blood pressure, and insulin-resistance are confounded by a non-random distribution of the participants throughout the year. Indeed, we observed in previous chapters of this thesis that elderly participants were more likely to attend the study center in winter than in summer. Nevertheless, we did not find differences in the distribution of the pollutants according to other variables which are also related to age and age-related comorbidities, such as medication intake. Therefore, we cannot rule out that, at least in part, different levels of air pollution exposure according to participants' characteristics may confound the association between air pollution exposure and relevant health outcomes. Therefore, these characteristics need to be accounted for when exploring the health effects of air pollution exposure in our population. Finally, the fact that exposure levels were higher in winter than in other seasons, as well as the exposure was higher in the highest concentrations of blood pressure and insulin resistance levels, supports the notion of the influence of air pollution exposure in the seasonal pattern of these outcomes.

The LUR models have shown a high ability to detect intra urban contrasts.[405] The choice of the LUR models enhances the prediction of the spatial contrast due to traffic emissions contributing to the air pollution exposure.[407] The oversampling of traffic sites, as well as the detailed selection of measurement sites in the monitoring campaign and the availability of better GIS data contributed to improve the performance of the LUR models.[402] This also explains the enhanced ability of these models to detect a relatively substantial spatial variation of air pollution exposure in a small area, such as the Ommoord district. Regarding the temporal variation, the models are designed to estimate long-term exposure; thus, the estimations obtained for the Rotterdam Study population are yet to be performed in the full cohort. In this chapter, we aimed for a description of the findings in one follow-up visit that includes population from all the cohorts, as an exploratory effort. Thus, future efforts include the assessment of exposure in the remaining visits and the estimation of other relevant geographic covariates, such as noise.

One potential limitation of this estimation is the fact that the exposure is only at home address. It has been shown that the estimation of air pollution exposure at the home-address may lead to an attenuation bias of the health effects estimations of NO_2 exposure up to 12% (95%CI 11-14%), compared to including the work/school exposure.[408] Nevertheless, taking into account that about half of our population was aged 70 years of more in the visit examined in this chapter, the home-address exposure may be more accurate to represent their exposure, compared to younger working/studying population. Nevertheless, it would be valuable, in the future, to at least have information on the time-space-activity patterns of the cohort participants, in order to determine the potential of exposure misclassification as a consequence of not taking into account non-home address exposure. Especially, because a new cohort is currently being recruited, by inviting people aged 40 years and older, for whom not accounting for work/study exposure may be a source of exposure misclassification.[408] Another potential limitation of our approach is that, because the estimations of the exposure are made only in addresses from the Ommoord district, we lose information of participants who move out of the district. Indeed, in spite this population is followed for incident events or until death and may participate in follow-up visits if the participant agrees to, this information cannot be taken into account since the estimation procedure only accounts for the exposure at the Ommoord district home address.

Conclusion

We have shown that there is a relatively high variation of the exposure to air pollution among the participants of the Rotterdam Study, as estimated with land use regression models developed and validated within the ESCAPE project. These estimations can be used in future epidemiological studies aimed to disentangle the role of traffic-related air pollution exposure in a mostly elderly population living in the small and well-defined district. The detailed measurements obtained during each visit, including genetics, imaging and biomarkers, along with these estimations of air pollution exposure, will allow broadening the scope of the risk factors examined within the Rotterdam Study.

Table 21. Distribution of air pollution concentrations estimated in the Ommoord district compared to the adjusted annual average concentrations obtained in the ESCAPE study for The Netherlands/Belgium*

Air pollutant	Ommoord district			ESCAPE (The Netherlands/Belgium)		
	Median (Range)	Mean	Range/ Mean (%)	Median (Range)	Mean	Range/ Mean (%)
PM_{10}	25.6 (24.9 - 31.5)	26	26	n.a.	27.1	56
$PM_{2.5}$	16.7 (16.3 - 19.8)	16.7	21	n.a.	17.7	50
$PM_{2.5}$ absorbance	1.5 (1.3 - 2.4)	1.5	73	n.a.	1.7	123
NO_x	41.8 (31.6 - 106.7)	45.5	165	44.3 (17.5 - 130.8)	n.a.	219
NO_2	31.9 (26.3 - 47.7)	32.7	65	30.2 (12.8 - 61.5)	n.a.	157

The estimations obtained in the ESCAPE The Netherlands/Belgium region were obtained from Eeftens et al [404] and Cyrys et al [405]

n.a. Not available.

Table 22. Correlation of air pollutants estimated with LUR models in the Ommoord district compared to the correlations obtained in the ESCAPE study for The Netherlands/Belgium*

	Ommoord district					**ESCAPE (The Netherlands/Belgium)**				
	PM_{10}	$PM_{2.5}$	$PM_{2.5}$ absorbance	NO_x	NO_2	PM_{10}	$PM_{2.5}$	$PM_{2.5}$ absorbance	NO_x	NO_2
PM_{10}	1.00					1.00				
$PM_{2.5}$	0.73	1.00				0.72	1.00			
$PM_{2.5}$ absorbance	0.96	0.83	1.00			0.74	0.71	1.00		
NO_x	0.94	0.65	0.89	1.00		n.a.	n.a.	n.a.	n.a.	
NO_2	0.74	0.61	0.74	0.83	1.00	0.74	0.57	0.86	0.92	1.00

The estimations obtained in the ESCAPE The Netherlands/Belgium region were obtained from Eeftens et al [404] and Cyrys et al [405]

n.a. Not available

Table 23. Distribution of PM_{10}, $PM_{2.5}$, $PM_{2.5}$ absorbance, and according to characteristics of the participants of the Rotterdam Study

Covariate	n (%)	PM_{10} (µg/m^3)			$PM_{2.5}$ (µg/m^3)			$PM_{2.5}$ absorbance (10^{-5}/m)		
		Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend
Age (years)										
<60	960 (17.9)	28.77 (24.28, 38.69)	Ref		18.31 (15.58, 24.74)	Ref		1.24 (1.01, 1.57)	Ref	
60-70	1,986 (37.1)	31.53 (25.48, 41.72)	<0.01	<0.01	20.4 (16.49, 26.7)	<0.01	<0.01	1.28 (1.03, 1.62)	0.10	<0.01
70-80	1,684 (31.4)	35.51 (29.05, 45.28)	<0.01		22.72 (18.74, 28.7)	<0.01		1.34 (1.1, 1.77)	<0.01	
≥80	726 (13.6)	34.6 (28.43, 42.68)	<0.01		22.58 (18.4, 27.7)	<0.01		1.28 (1.08, 1.65)	0.18	
Sex										
Male	2,285 (42.7)	32.8 (26.59, 42.55)	Ref		21.19 (17.04, 27.32)	Ref		1.28 (1.05, 1.64)	Ref	
Female	3,071 (57.3)	33.18 (26.62, 42.64)	0.35	-	21.44 (17.23, 27.39)	0.34	-	1.29 (1.06, 1.64)	0.5	-
Season of follow-up visit										
Winter	1,304 (24.3)	31.01 (25.49, 45.63)	Ref		19.98 (16.56, 29.59)	Ref		1.52 (1.26, 2.05)	Ref	
Spring	1,664 (31.1)	44.32 (36.28, 52.37)	<0.01	-	28.16 (23.47, 33.56)	<0.01	-	1.5 (1.23, 1.77)	0.18	-
Summer	1,083 (20.2)	29.12 (25.19, 34.59)	<0.01		18.83 (16.21, 22.43)	<0.01		0.96 (0.89, 1.06)	<0.01	
Autumn	1,305 (24.4)	29.27 (25.73, 33.97)	<0.01		18.83 (16.68, 22)	<0.01		1.23 (1.1, 1.47)	<0.01	
Income category (euros)										
<2100	555 (10.4)	33.79 (27.28, 42.61)	Ref		22.12 (17.59, 27.6)	Ref		1.28 (1.06, 1.67)	Ref	
2100-3000	772 (14.4)	33.7 (26.65, 44.6)	0.92	<0.01	21.9 (17.27, 28.59)	0.7	<0.01	1.33 (1.08, 1.67)	0.12	0.01
3000-4200	1,183 (22.1)	34.09 (28.01, 43.31)	0.73		22.18 (18.05, 27.9)	0.92		1.3 (1.07, 1.67)	0.52	
≥4200	2,325 (43.4)	31.75 (25.87, 41.75)	0.01		20.49 (16.66, 26.65)	<0.01		1.27 (1.05, 1.63)	0.65	
Missing	521 (9.7)	33.39 (26.8, 41.2)	0.69		21.39 (17.23, 26.67)	0.25		1.27 (1.05, 1.62)	0.62	
Education levels										
Lower general	2,545 (47.5)	33.42 (26.93, 42.96)	Ref		21.61 (17.38, 27.63)	Ref		1.29 (1.06, 1.65)	Ref	
Intermediate Higher	2,754 (51.4)	32.64 (26.31, 42.01)	0.06	0.01	21.05 (16.92, 27.01)	0.04	<0.01	1.28 (1.06, 1.63)	0.27	0.07
Missing	57 (1.1)	34.77 (28.79, 46.02)	0.51		22.96 (18.15, 29.08)	0.31		1.18 (1.01, 1.51)	0.13	
Community dwelling										
Yes	5,335 (99.6)	33.01 (26.59, 42.61)	Ref		21.32 (17.14, 27.39)	Ref		1.28 (1.06, 1.64)	Ref	
No	11 (0.2)	32.15 (28.92, 49.64)	0.84	0.34	20.64 (18.71, 32.84)	0.81	0.28	1.24 (1, 1.67)	0.77	0.53
Missing	10 (0.2)	32.68 (24.41, 47.11)	0.94		21.28 (15.76, 30.31)	0.99		1.3 (1.02, 1.7)	0.93	
Body mass index (kg/m^2)										
<25	1,558 (29.1)	33.29 (26.49, 43.31)	Ref		21.45 (17.16, 27.79)	Ref		1.28 (1.05, 1.64)	Ref	
25-30	2,537 (47.4)	32.57 (26.61, 42.22)	0.12	0.92	21.01 (17.14, 27.19)	0.15	0.94	1.28 (1.06, 1.64)	0.65	0.36
≥30	1,258 (23.5)	33.57 (26.64, 42.05)	0.61		21.65 (17.11, 27.24)	0.57		1.3 (1.06, 1.64)	0.27	
Missing	3 (0.1)	37.61 (28.79, 74.29)	0.61		24.87 (18.52, 45.13)	0.53		1.31 (1.2, 4.41)	0.9	
Smoking behavior										
Never	1,741 (32.5)	32.96 (26.79, 42.75)	Ref		21.38 (17.2, 27.53)	Ref		1.3 (1.06, 1.67)	Ref	
Currently	815 (15.2)	31.93 (25.78, 41.41)	0.11	0.65	20.55 (16.61, 26.56)	0.05	0.57	1.28 (1.04, 1.62)	0.46	0.14
Former	2,790 (52.1)	33.43 (26.84, 42.65)	0.31		21.49 (17.29, 27.45)	0.73		1.28 (1.06, 1.64)	0.42	

Covariate	n (%)	PM_{10} (μg/m^3)			$PM_{2.5}$ (μg/m^3)			$PM_{2.5}$ absorbance (10^5/m)		
		Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend
Missing	10 (0.2)	27.53 (24.41, 34.09)	0.26		17.89 (15.76, 21.95)	0.26		1.28 (1.02, 1.48)	0.91	
Physical activity (MET-hours/week)*										
0.12 – 15.5	1,161 (21.7)	33.69 (26.86, 42.75)	Ref		21.98 (17.34, 27.48)	Ref		1.28 (1.07, 1.65)	Ref	
15.5 – 39.3	1,143 (21.3)	33.43 (27.05, 42.93)	0.68	0.02	21.43 (17.33, 27.51)	0.18	0.01	1.3 (1.06, 1.65)	0.41	<0.01
39.4 – 76.9	1,152 (21.5)	32.22 (26.07, 41.72)	0.02		20.82 (16.83, 27)	<0.01		1.27 (1.04, 1.61)	0.5	
77.0 – 224.8	1,151 (21.5)	32.09 (26.24, 41.72)	0.01		20.89 (16.89, 27.01)	0.01		1.25 (1.03, 1.62)	0.2	
Missing	749 (14)	34.02 (27.44, 43.04)	0.66		21.99 (17.49, 27.69)	0.99		1.34 (1.09, 1.71)	0.03	
Systolic blood pressure (mmHg)*										
86 – 128	1,386 (25.9)	30.46 (24.97, 40.71)	Ref		19.78 (16.17, 26.44)	Ref		1.25 (1.01, 1.58)	Ref	
129 – 142	1,342 (25.1)	32.88 (26.17, 41.7)	<0.01	<0.01	21.2 (16.86, 26.81)	<0.01	<0.01	1.28 (1.06, 1.63)	0.11	<0.01
143 – 158	1,306 (24.4)	33.57 (27.32, 42.64)	<0.01		21.66 (17.54, 27.6)	<0.01		1.3 (1.07, 1.65)	0.01	
159 – 241	1,269 (23.7)	34.18 (28.43, 44.67)	<0.01		22.25 (18.36, 28.41)	<0.01		1.32 (1.1, 1.77)	<0.01	
Missing	53 (1)	36.67 (28.94, 47.38)	<0.01		23.02 (18.87, 30.4)	0.02		1.36 (1.09, 1.67)	0.14	
Diastolic blood pressure (mmHg)*										
50 – 76	1,470 (27.4)	32.05 (25.78, 41.62)	Ref		20.8 (16.62, 26.83)	Ref		1.24 (1.02, 1.59)	Ref	
77 – 83	1,316 (24.6)	33.06 (26.68, 42.78)	0.08	<0.01	21.45 (17.11, 27.22)	0.08	<0.01	1.3 (1.07, 1.65)	<0.01	<0.01
84 – 91	1,322 (24.7)	33.06 (26.82, 42.26)	0.08		21.37 (17.28, 27.22)	0.12		1.29 (1.07, 1.64)	0.02	
92 – 133	1,195 (22.3)	33.68 (27.41, 43.94)	0.01		21.78 (17.69, 27.97)	0.01		1.33 (1.09, 1.71)	<0.01	
Missing	53 (1)	36.67 (28.94, 47.38)	0.03		23.02 (18.87, 30.4)	0.1		1.36 (1.09, 1.67)	0.11	
Total cholesterol (mmol/L)*										
1.7 – 4.7	1,399 (26.1)	33.38 (26.9, 42.96)	Ref		21.65 (17.26, 27.87)	Ref		1.29 (1.06, 1.65)	Ref	
4.8 – 5.5	1,430 (26.7)	33.81 (26.93, 43.92)	0.48	0.01	21.9 (17.36, 27.87)	0.50	0.01	1.29 (1.07, 1.65)	0.96	0.17
5.6 – 6.2	1,248 (23.3)	32.04 (26.21, 41.5)	0.03		20.68 (16.84, 26.84)	0.01		1.27 (1.04, 1.63)	0.27	
6.3 – 12.4	1,238 (23.1)	32.33 (26.54, 41.61)	0.09		20.94 (17.05, 26.84)	0.06		1.28 (1.06, 1.64)	0.73	
Missing	41 (0.8)	30.68 (25.33, 38.64)	0.29		19.8 (16.39, 23.8)	0.24		1.17 (1.01, 1.53)	0.16	
HDL-cholesterol (mmol/L)*										
0.40 – 1.18	1,350 (25.2)	33.09 (26.35, 42.55)	Ref		21.35 (16.94, 27.44)	Ref		1.28 (1.06, 1.64)	Ref	
1.19 – 1.43	1,333 (24.9)	32.69 (26.78, 41.23)	0.49	0.19	21.08 (17.3, 26.4)	0.47	0.2	1.28 (1.05, 1.63)	0.84	0.36
1.44 – 1.74	1,326 (24.8)	32.71 (26.88, 42.75)	0.51		21.2 (17.23, 27.53)	0.69		1.28 (1.06, 1.65)	0.76	
1.75 – 4.03	1,306 (24.4)	33.57 (26.58, 43.72)	0.41		21.61 (17.21, 27.83)	0.49		1.3 (1.06, 1.65)	0.38	
Missing	41 (0.8)	30.68 (25.33, 38.64)	0.31		19.8 (16.39, 23.8)	0.31		1.17 (1.01, 1.53)	0.21	
Glucose (mmol/L) *										
3.0 – 5.1	1,432 (26.7)	33.79 (26.37, 42.61)	Ref		21.87 (16.94, 27.39)	Ref		1.29 (1.06, 1.63)	Ref	
5.2 – 5.5	1,363 (25.4)	32.55 (26.65, 42.16)	0.03	0.95	21.08 (17.24, 27.09)	0.03	0.78	1.28 (1.05, 1.64)	0.46	0.94
5.6 – 6.1	1,314 (24.5)	32.92 (26.8, 42.38)	0.12		21.25 (17.16, 27.32)	0.08		1.29 (1.06, 1.67)	0.75	
6.2 – 201	1,206 (22.5)	33.06 (26.65, 42.75)	0.20		21.09 (17.24, 27.67)	0.03		1.28 (1.06, 1.63)	0.56	
Missing	41 (0.8)	30.68 (25.33, 38.64)	0.18		19.8 (16.39, 23.8)	0.16		1.17 (1.01, 1.53)	0.14	
Insulin levels (μIU/mL) *										
0.1 – 7.5	1,381 (25.8)	32.71 (26.35, 41.78)	Ref		21.12 (16.92, 27)	Ref		1.27 (1.04, 1.64)	Ref	

Covariate	n (%)	PM_{10} (µg/m^3)			$PM_{2.5}$ (µg/m^3)			$PM_{2.5}$ absorbance (10^{-5}/m)		
		Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend
7.6 – 10.7	1,328 (24.8)	33.46 (27.07, 43.33)	0.20	0.85	21.66 (17.4, 27.58)	0.14	0.97	1.31 (1.06, 1.65)	0.08	0.14
10.8 – 15.4	1,279 (23.9)	33.03 (26.55, 44.15)	0.59		21.38 (17.11, 28.2)	0.49		1.27 (1.06, 1.64)	0.82	
15.6 – 595.4	1,322 (24.7)	32.71 (26.53, 41.41)	0.99		21.09 (17.19, 26.67)	0.93		1.3 (1.07, 1.65)	0.18	
Missing	46 (0.9)	32.96 (26.54, 42.4)	0.91		21.54 (16.84, 27.53)	0.77		1.22 (1.04, 1.62)	0.48	
HOMA-IR*										
0.02 – 1.75	1,332 (24.9)	33.18 (26.4, 42.61)	Ref		21.43 (16.95, 27.36)	Ref		1.28 (1.04, 1.64)	Ref	
1.76 – 2.62	1,321 (24.7)	32.92 (26.86, 42.12)	0.64	0.64	21.29 (17.3, 27.03)	0.7	0.46	1.29 (1.05, 1.64)	0.83	0.11
2.62 – 4.08	1,326 (24.8)	32.99 (26.53, 43.55)	0.75		21.31 (17.1, 27.69)	0.75		1.27 (1.06, 1.63)	0.59	
4.08 – 195.82	1,326 (24.8)	33.16 (26.8, 42.38)	0.97		21.33 (17.33, 27.48)	0.78		1.3 (1.07, 1.67)	0.39	
Missing	51 (1)	32.54 (26.54, 42.75)	0.76		21.54 (16.84, 27.9)	0.94		1.25 (1.05, 1.64)	0.7	
Medication intake										
Antihypertensive										
No	2,859 (53.4)	32.51 (26.3, 41.99)	Ref		20.98 (16.92, 27.01)	Ref		1.28 (1.05, 1.63)	Ref	
Yes	2,494 (46.6)	33.52 (27.02, 42.79)	0.02	-	21.69 (17.39, 27.71)	0.01	-	1.29 (1.06, 1.66)	0.75	-
Missing	3 (0.1)	26.14 (21.9, 28.94)	0.48		16.99 (13.32, 18.03)	0.48		1.3 (0.97, 1.41)	0.95	
Statin										
No	3,869 (72.2)	32.69 (26.47, 42.02)	Ref		21.12 (17.01, 27.08)	Ref		1.28 (1.05, 1.64)	Ref	
Yes	1,484 (27.7)	33.7 (26.9, 43.52)	0.03	-	21.79 (17.36, 28.2)	0.02	-	1.29 (1.06, 1.65)	0.62	-
Missing	3 (0.1)	26.14 (21.9, 28.94)	0.45		16.99 (13.32, 18.03)	0.46		1.3 (0.97, 1.41)	0.95	
Diabetic medication										
No	4,870 (90.9)	32.96 (26.59, 42.37)	Ref		21.31 (17.12, 27.28)	Ref		1.28 (1.06, 1.64)	Ref	
Yes	483 (9)	33.53 (26.71, 43.91)	0.41	-	21.7 (17.32, 28.23)	0.39	-	1.28 (1.07, 1.64)	1.00	-
Missing	3 (0.1)	26.14 (21.9, 28.94)	0.42		16.99 (13.32, 18.03)	0.44		1.3 (0.97, 1.41)	0.96	

*Stratified in quartiles

Table 24. Distribution of NO_x and NO_2 according to the characteristics of the participants of the Rotterdam Study

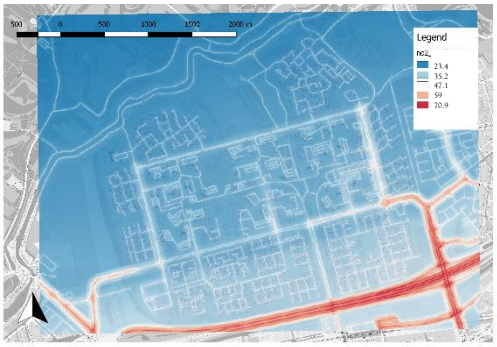
Covariate	n (%)	NO_x (µg/m^3)			NO_2 (µg/m^3)		
		Median (IQR)	p-value	p-trend	Median (IQR)	p-value	p-trend
Age (years)							
<60	960 (17.9)	58.43 (43.48, 78.82)	Ref		40.99 (33.24, 54.06)	Ref	
60-70	1,986 (37.1)	58.49 (42.72, 78.66)	0.97	0.08	42.64 (33.42, 55.12)	0.04	<0.01
70-80	1,684 (31.4)	63.44 (45.57, 86.36)	<0.01		46.18 (35.00, 56.50)	<0.01	
≥80	726 (13.6)	59.15 (43.58, 80.27)	0.69		45.12 (35.04, 54.74)	<0.01	
Sex							
Male	2,285 (42.7)	59.79 (43.18, 81.56)	Ref		43.58 (33.65, 55.17)	Ref	
Female	3,071 (57.3)	60.4 (43.83, 80.43)	0.56	-	43.86 (34.29, 55.41)	0.67	-
Season of follow-up visit							
Winter	1,304 (24.3)	81.14 (62.81, 109.48)	Ref		56.21 (46.73, 65.57)	Ref	
Spring	1,664 (31.1)	70.07 (55.97, 88.06)	<0.01	-	51.35 (43.63, 58.85)	<0.01	-
Summer	1,083 (20.2)	38.16 (33.36, 46.82)	<0.01		30.63 (27.49, 34.41)	<0.01	
Autumn	1,305 (24.4)	51.68 (41.26, 65.73)	<0.01		38.11 (32.52, 44.58)	<0.01	
Income category (euros)							
<2100	555 (10.4)	60.29 (42.74, 79.82)	Ref		45.08 (34.03, 55.92)	Ref	
2100-3000	772 (14.4)	59.84 (45.15, 79.27)	0.83	0.7	45.54 (35.59, 55.55)	0.71	<0.01
3000-4200	1,183 (22.1)	62.2 (44.01, 83.58)	0.32		44.82 (34.45, 56.15)	0.82	
≥4200	2,325 (43.4)	59.38 (43.44, 80.83)	0.61		42.06 (33.54, 54.98)	<0.01	
Missing	521 (9.7)	58.92 (42.61, 79.33)	0.55		43.94 (34.02, 53.98)	0.4	
Education levels							
Lower general	2,545 (47.5)	61.33 (43.58, 80.33)	Ref		44.79 (34.45, 55.72)	Ref	
Intermediate Higher	2,754 (51.4)	59.12 (43.63, 81.27)	0.03	0.35	42.71 (33.66, 54.9)	<0.01	0.01
Missing	57 (1.1)	58.45 (43.22, 79.34)	0.56		43.94 (33.73, 51.9)	0.78	
Community dwelling							
Yes	5,335 (99.6)	60.17 (43.62, 80.91)	Ref		43.81 (34.02, 55.31)	Ref	
No	11 (0.2)	44.19 (39.02, 67.34)	0.16	-	34.46 (28.58, 50.24)	0.19	-
Missing	10 (0.2)	54.34 (43.24, 83.02)	0.62		43.33 (36.09, 58.06)	0.95	
Body mass index (kg/m^2)							
<25	1,558 (29.1)	60.03 (42.72, 81.5)	Ref		42.82 (33.53, 55.72)	Ref	
25-30	2,537 (47.4)	59.87 (43.83, 80.64)	0.9	0.38	43.86 (34.06, 55.02)	0.16	0.25
≥30	1,258 (23.5)	61.04 (44.54, 80.83)	0.48		44.79 (34.43, 55.31)	0.03	
Missing	3 (0.1)	60.78 (59.4, 192.41)	0.97		50.68 (43.03, 92.78)	0.56	
Smoking behavior							
Never	1,741 (32.5)	61.38 (44.05, 82.82)	Ref		44.62 (34.57, 56.04)	Ref	
Currently	815 (15.2)	58.12 (42.33, 79.77)	0.04	0.16	42.34 (32.97, 55.35)	0.02	0.19
Former	2,790 (52.1)	59.84 (43.68, 79.66)	0.18		43.85 (33.96, 54.89)	0.28	
Missing	10 (0.2)	52.84 (43.24, 54.38)	0.48		39.05 (36.09, 45.28)	0.46	
Physical activity (MET-hours/week)*							
0.12 – 15.5	1,161 (21.7)	60.95 (44.43, 81.39)	Ref		44.77 (34.45, 55.68)	Ref	
15.5 – 39.3	1,143 (21.3)	61.02 (44.23, 83.58)	0.97	<0.01	43.81 (34.17, 56.21)	0.31	<0.01
39.4 – 76.9	1,152 (21.5)	58.59 (42.88, 77.55)	0.12		42.69 (33.37, 54.41)	0.03	
77.0 – 224.8	1,151 (21.5)	57.82 (42.01, 79.56)	0.04		42.04 (33.51, 54.09)	<0.01	
Missing	749 (14)	63.86 (45.15, 82.96)	0.09		46.67 (35.56, 56.43)	0.07	
Systolic blood pressure (mmHg)*							
86 – 128	1,386 (25.9)	57.46 (42.08, 78.04)	Ref		41.45 (32.97, 54.47)	Ref	
129 – 142	1,342 (25.1)	59.38 (44.05, 81.17)	0.17	<0.01	43.35 (34.13, 55.31)	0.02	<0.01
143 – 158	1,306 (24.4)	60.42 (44.18, 81.14)	0.04		44.76 (34.29, 55.28)	<0.01	

159 – 241	1,269 (23.7)	62.57 (44.39, 83.98)	<0.01		45.54 (35.04, 56.45)	<0.01	
Missing	53 (1)	62.5 (45.56, 79.91)	0.33		44.22 (35.17, 56.5)	0.35	
Diastolic blood pressure (mmHg)*							
50 – 76	1,470 (27.4)	57.24 (41.13, 77.44)	Ref		41.56 (32.96, 54.3)	Ref	
77 – 83	1,316 (24.6)	61.38 (45.48, 82.88)	<0.01	<0.01	44.81 (34.83, 55.46)	<0.01	<0.01
84 – 91	1,322 (24.7)	61.11 (43.29, 80.95)	<0.01		44.52 (34.51, 55.73)	<0.01	
92 – 133	1,195 (22.3)	61.13 (44.16, 83.32)	0.01		44.41 (34.29, 56)	<0.01	
Missing	53 (1)	62.5 (45.56, 79.91)	0.30		44.22 (35.17, 56.5)	0.40	
Total cholesterol (mmol/L)*							
1.7 – 4.7	1,399 (26.1)	60.55 (44.3, 81.27)	Ref		44.54 (34.74, 55.47)	Ref	
4.8 – 5.5	1,430 (26.7)	61 (44.71, 81.24)	0.75	0.1	44.32 (34.46, 55.68)	0.81	0.02
5.6 – 6.2	1,248 (23.3)	59.1 (42.31, 80.09)	0.32		42.69 (33.39, 54.8)	0.04	
6.3 – 12.4	1,238 (23.1)	59.82 (43.18, 81.14)	0.61		42.84 (33.59, 55.27)	0.07	
Missing	41 (0.8)	60.06 (44.71, 71.58)	0.93		40.09 (34.43, 51.98)	0.23	
HDL-cholesterol (mmol/L)*							
0.40 – 1.18	1,350 (25.2)	60.78 (44.05, 81.45)	Ref		44.24 (34.14, 55.26)	Ref	
1.19 – 1.43	1,333 (24.9)	58.75 (42.89, 79.51)	0.15	0.89	43.09 (33.37, 54.52)	0.21	0.9
1.44 – 1.74	1,326 (24.8)	60.42 (44.17, 82.46)	0.80		43.85 (34.02, 55.86)	0.67	
1.75 – 4.03	1,306 (24.4)	61.11 (43.63, 80.87)	0.81		44.12 (34.66, 55.27)	0.89	
Missing	41 (0.8)	60.06 (44.71, 71.58)	0.90		40.09 (34.43, 51.98)	0.27	
Glucose (mmol/L) *							
3.0 – 5.1	1,432 (26.7)	62.81 (44.52, 82.59)	Ref		44.92 (34.45, 55.79)	Ref	
5.2 – 5.5	1,363 (25.4)	59.68 (41.91, 80.33)	0.03	0.13	42.95 (33.41, 55.17)	0.03	0.70
5.6 – 6.1	1,314 (24.5)	58.69 (43.78, 81.16)	<0.01		43.21 (34.04, 55.56)	0.06	
6.2 – 201	1,206 (22.5)	59.68 (44.05, 79.56)	0.03		44.15 (34.21, 54.47)	0.40	
Missing	41 (0.8)	60.06 (44.71, 71.58)	0.64		40.09 (34.43, 51.98)	0.20	
Insulin levels (µIU/mL) *							
0.1 – 7.5	1,381 (25.8)	57.99 (42.12, 79.06)	Ref		41.98 (33.06, 54.62)	Ref	
7.6 – 10.7	1,328 (24.8)	62.05 (44.16, 82.33)	<0.01	0.04	44.14 (34.51, 55.86)	0.01	0.01
10.8 – 15.4	1,279 (23.9)	59.59 (43.65, 80.87)	0.26		44.02 (33.78, 55.73)	0.02	
15.6 – 595.4	1,322 (24.7)	61.39 (44.52, 81.27)	0.02		44.93 (34.59, 54.82)	<0.01	
Missing	46 (0.9)	60.06 (43.18, 84.71)	0.71		40.09 (34.45, 54.96)	0.58	
HOMA-IR*							
0.02 – 1.75	1,332 (24.9)	58.79 (42.69, 80.27)	Ref		42.34 (33.2, 55.17)	Ref	
1.76 – 2.62	1,321 (24.7)	60.21 (43.56, 81.16)	0.33	0.11	43.37 (34.4, 55.05)	0.25	0.03
2.62 – 4.08	1,326 (24.8)	59.93 (43.43, 80.87)	0.44		43.94 (33.73, 55.73)	0.07	
4.08 – 195.82	1,326 (24.8)	60.96 (44.85, 81.22)	0.14		44.93 (34.69, 55.24)	<0.01	
Missing	51 (1)	60.14 (44.71, 84.71)	0.80		43.72 (34.83, 56.05)	0.67	
Medication intake							
Antihypertensive							
No	2,859 (53.4)	59.40 (43.48, 81.50)	Ref		43.00 (33.78, 55.05)	Ref	
Yes	2,494 (46.6)	60.85 (43.63, 80.64)	0.16	-	44.66 (34.24, 55.63)	0.01	-
Missing	3 (0.1)	64.75 (37.31, 72.9)	0.81		38.38 (30.57, 41.42)	0.72	
Statin							
No	3,869 (72.2)	59.79 (43.26, 81.22)	Ref		43.31 (33.72, 55.26)	Ref	
Yes	1,484 (27.7)	60.96 (44.58, 80.46)	0.32	-	44.56 (34.51, 55.47)	0.08	-
Missing	3 (0.1)	64.75 (37.31, 72.9)	0.82		38.38 (30.57, 41.42)	0.71	
Diabetic medication							
No	4,870 (90.9)	60.06 (43.58, 80.81)	Ref		43.71 (34.02, 55.28)	Ref	
Yes	483 (9)	60.69 (43.72, 81.94)	0.72	-	44.42 (34.17, 55.86)	0.52	-
Missing	3 (0.1)	64.75 (37.31, 72.9)	0.83		38.38 (30.57, 41.42)	0.69	

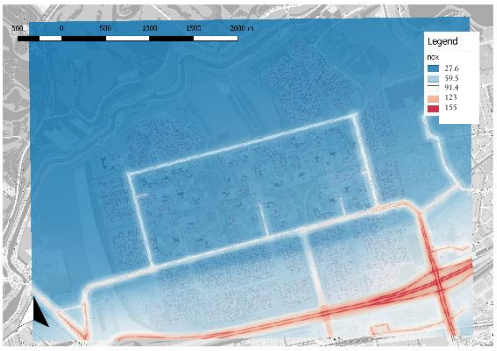
*Stratified in quartiles

Figure 18. Maps illustrating the spatial distribution of air pollutants in the Ommoord district, Rotterdam, the Netherlands

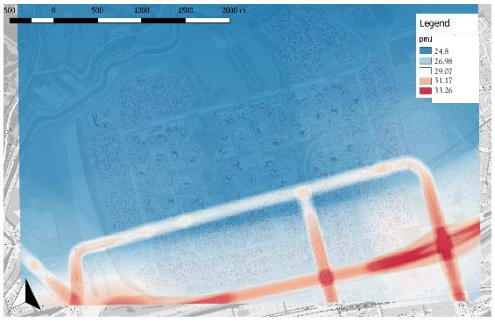
a) NO_2 concentration ($\mu g/m^3$)



b) NO_x concentration ($\mu g/m^3$)

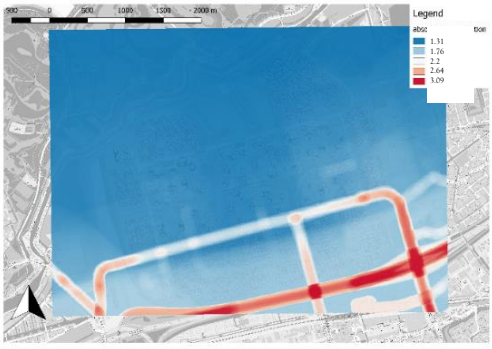


c) PM_{10} concentration ($\mu g/m^3$)



d) $PM_{2.5}$ concentration ($\mu g/m^3$)

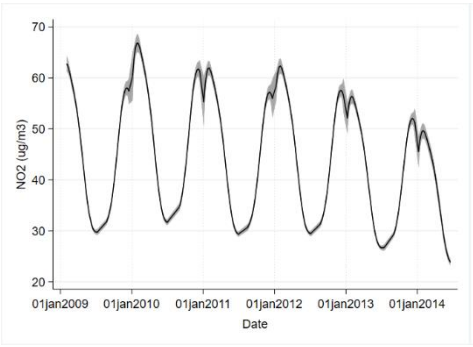
e) $PM_{2.5}$ absorbance ($10^{-5}/m$)



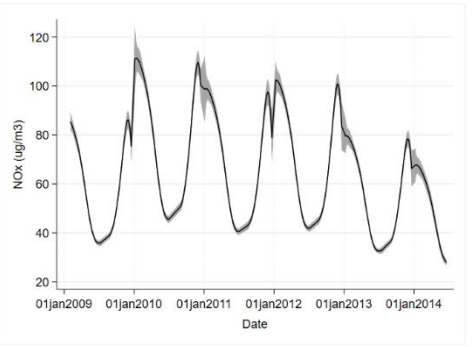
The maps show the spatial distribution of the air pollutants concentrations estimated for the Ommoord district, Rotterdam, the Netherlands. These concentrations were estimated using land use regression models validated for The Netherlands/Belgium and represent the annual concentration in 2008.

Figure 19. Annual distribution of the back-extrapolated concentrations of air pollutants among participants of the Rotterdam Study

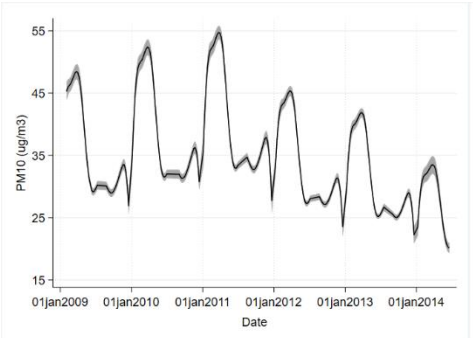
a) NO_2 concentration



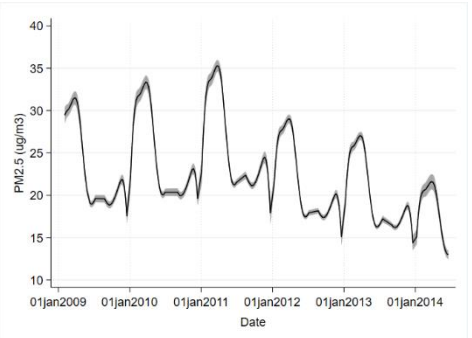
b) NO_x concentration



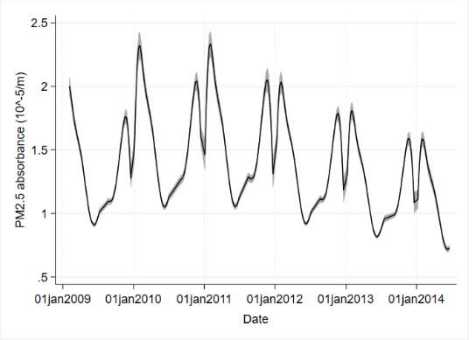
c) PM_{10} concentration



b) $PM_{2.5}$ concentration



e) $PM_{2.5}$ absorbance



Chapter 4 General discussion

Throughout this thesis, I examined the influence of environment factors in lifestyle, cardiovascular risk, cognition, and antibiotic resistance, which account for a large part of the health burden among aging population. The findings are of relevance for several challenges related to climate change. First, we showed a seasonal variation of physical activity and diet quality. For physical activity, the seasonality was larger among middle-aged than among elderly population and was larger for light intensity physical activity than for moderate-to-vigorous physical activity, sleep, and sedentary behavior. The seasonal variation of diet quality reflected that of legumes and sugar-containing beverages. Second, we showed that seasonality of cardiovascular risk factors and insulin resistance was mostly explained by ambient temperature and the influence was larger among elderly participants. Third, we showed that cognition had a larger seasonal variation among middle-aged than among elderly. A review of the literature also showed a seasonal variation of antibiotic resistance, especially of respiratory bacteria, which may be attributable to the seasonality of infectious diseases and consequently of antibiotic use. Fourth, we found that, across the evidence, active commuters inhale more pollutants than motorized commuters, although the health effects of pollutants inhalation could be offset by the physical activity health gains. Finally, we described the estimation of the air pollution exposure among the population of the Rotterdam Study. The dispersion of the pollutants concentrations was small, but there was substantial spatial variation according to the road network of the Ommoord district, where the cohort population lives. Also, the distribution of the exposure differed according to selected covariates of the population, which need to be accounted for when addressing the health effects of air pollution exposure in the Rotterdam Study.

In this chapter, I aim to put these findings in perspective, first by addressing the methodological considerations and second, by addressing the potential meaning of the observed findings within the ongoing debate regarding the challenges of environment in health of an aging population.

METHODOLOGICAL CONSIDERATIONS

Studies based on the Rotterdam Study

There are a few aspects to take into account regarding the internal validity of our results. First, we estimated the seasonality of the outcomes of interest including all the population that was relevant to the specific research question and the data obtained for all analyses were systematically collected and coded by trained interviewers and researchers from participants whom consented to participate at each wave of the cohort. Nevertheless, it is possible that the participants who remain in the cohort throughout the visits are more health conscious than those who do not attend in further visits and, in consequence, may be less likely to change their behavior on a seasonal basis. Therefore, it is possible that our estimates of seasonal variation of lifestyle factors are underestimated compared to general population. Second, our studies about seasonal variation are embedded in a prospective cohort, in which invitations to participate are sent randomly to all eligible people throughout the year. In spite of this, we found that a large part of the crude

seasonal variation observed for lifestyle factors, cognition, and cardiovascular risk was explained by factors such as age and presence of comorbidities. This finding suggests that the observed seasonal variation is partly owned to the fact that participants do not attend the research center randomly throughout the year and, in consequence, some characteristics are not evenly distributed across seasons. Although we addressed this issue by adjusting the seasonality estimates by a number of covariates that were unevenly distributed across the year and that could explain the observed seasonality, it is possible that our estimates of seasonality are affected by residual confounding. For example, at the writing of the studies about seasonality of cardiovascular risk and insulin resistance, we did not have complete information about accelerometer-based physical activity or diet. Regarding physical activity, we used questionnaire-based physical activity levels, which may not account for light physical activity or physical activity for certain domains, such as occupational.[409] However, according to our findings in chapter 2.1.1, light and occupational physical activity may make for a large part of the seasonality of physical activity levels. Therefore, it is possible that seasonality of health outcomes that can be explained by the seasonality of physical activity is underestimated. Regarding diet, we used body mass index as a proxy. However, not only body mass index does not entirely depend on diet, but also there are differences in diet patterns according to food component and diet quality (chapter 2.1.2). Therefore, also the seasonality of health outcomes that could be attributed to diet may be underestimated.

Third, an ideal scenario for seasonality estimates would be to measure the same individual several times a year, in order to reduce the variability that can be attributed to the differences of the population throughout the year. However, such approach is rather intensive and could hardly be implemented in about 14,000 individuals; such is the number of participants of the Rotterdam Study. Yet, all the studies included in average between two to three visits per participant, which helps to reduce the seasonality that could be explained by non-random participation.

In addition, we need to consider several aspects regarding the external validity. First, our population was mostly characterized by middle-aged and elderly white European participants, with a small mix of ethnicity. Second, we observed that our population reported large levels of moderate-to-vigorous physical activity and sedentary behavior; this may explain the low seasonality observed in physical activity in our population. Third, the Netherlands is a country characterized by large differences in the meteorological factors across seasons, whereas previous studies suggest that most of the seasonality of several health events is larger in warmer countries with less extreme variation in meteorological factors across seasons. Therefore, it is likely that our findings are generalizable only to settings similar to those found in the studies included in this thesis. However, our findings could still provide an indication of the potential extent of the influence of meteorological factors on the seasonality of cardiovascular risk in a population assumed to be adapted to the local conditions. Moreover, it is possible that the seasonal health effects of meteorological factors are relevant regardless the geographical region as a consequence of the variation of the meteorological factors between seasons, rather than absolute values of the meteorological factors in each season.

There are also statistical considerations to the studies included in this thesis, regarding the choice of the cosinor model to examine the seasonal variations. There are several statistical

approaches to address the seasonal variation of health outcomes. A frequent and straightforward approach is to time variable into months or seasons. However, it implies the assumption of non-biological jumps between time strata and may reduce the power by increasing the number of comparisons and increasing the number of parameters.[217] A more mathematically complex approach is based on Fourier transformations of the time variable, including the cosinor terms.[69,217] The cosinor terms are trigonometric transformations of the time variable, which are included in the model (e.g. linear, Poisson, logistic) and the coefficients are used to estimate the magnitude of the variation of the outcome.[69] A frequently mentioned disadvantage of cosinor modelling is that it only tests if there is a sinusoidal variation of the outcome of interest and assumes a regular seasonal variation across the observation time, e.g. from year to year. Nevertheless, the variation can be flexibilized with the inclusion of harmonics, i.e. cosinor terms with different periods, as shown in chapter 2.2.3. In spite of this, it is assumed that the variation does not change across the entire time of observation, which is an assumption that may not hold. A further approach to account for seasonal variation and long-term trends is the use of flexible spline functions of the time. The flexibility of this modelling may be used as a hypothesis-free approach to address the variation of outcomes for which the pattern of seasonal variation is not clear and to account for the long term and seasonal variation in time-series analyses.[217] Future studies aimed to address the seasonal variation of other outcomes may consider testing hypothesis-free variations to test the risk of type II error, if there is a clear indication of a non-sinusoidal variation.

Systematic reviews

First, we aimed to enhance the internal validity of our systematic reviews by building comparable estimates of the relevant comparisons for each study (either between modes of transport or seasons) by calculating ratios of exposure. Regarding the external validity, although we aimed for comprehensive and exhaustive reviews of the evidence aimed to include information of several settings, most of the evidence was obtained European countries, followed by North American, Asiatic and Western Pacific countries. This may have reflected in the observed evidence of publication bias. Therefore, it is possible that both the seasonal variation of antibiotic resistance (chapter 2.2.4) and the differences in the exposure to air pollution according to mode of transport (chapters 3.1.1 and 3.1.2) are underrepresented in our systematic reviews, thus potentially reducing the external validity of our findings. Nevertheless, our systematic reviews were aimed to also identify sources of heterogeneity, which can be used by stakeholders to adapt local-wise policies regarding both the exposure to air pollution according to mode of transport and the seasonality of antimicrobial resistance. Yet, further studies are granted to gather evidence from such overseen regions, to have a better understanding of the role of local environmental factors both in the variation of antimicrobial resistance and in the exposure to air pollution among commuters.

MAJOR FINDINGS AND IMPLICATIONS

Seasonal variation of lifestyle factors

Unhealthy lifestyle factors, such as low physical activity, sedentary behavior, and poor diet quality are among the most important determinants of worldwide mortality and disability.[410] In spite of the awareness of its contribution to global health, the burden attributable to these factors is increasing. In this context, the fact that these lifestyle factors have a seasonal variation implies that there are periods of the year where the population changes their physical activity levels and diet. Although it is suggested that meteorological factors have a role, societal schedules may also contribute to this seasonality. The seasonal approach is of relevance to understand the factors that can be targeted to develop public and health interventions to achieve changes in population-level lifestyle that are already occurring on a seasonal basis. On the basis of our findings, such interventions need to take into account the societal schedules that may drive the variation of these lifestyle factors throughout the year, for example working and school schedules, holidays, and festivities. Understanding the role of the societal schedules may also be of relevance in settings with a smaller variation of meteorological factors according to seasons (e.g., close to the Equator), where the societal schedules may also shape the physical activity levels and diet quality in different moments of the year. Thus, the seasonal approach may help to spot opportunities to improve the physical activity levels and diet quality.

In chapter 2.1.1 we showed that physical activity was larger for light intensity than for moderate-to-vigorous intensity, indicating that the participants may be more likely to increase or reduce the physical activity that is less structured. However, such variation was observed only among middle-aged (45-64 years) and younger-elderly (65-74 years), but not among older-elderly population (≥75 years). This difference may reflect the transition from the schedule-driven activity among the working population (middle-aged) to a less structured physical activity routine among old-elderly, with less light activity and more sedentary behavior. In addition, the small seasonality of sedentary behavior, which was not completely replaced by physical activity in the summer, suggests it is a behavior strongly ingrained in the daily routine. In chapter 2.2.2, we found that diet quality slightly increased in winter. The seasonal variation was largest among men, obese participants, and participants with high socio-economic status than in the corresponding counterparts. As these groups also had the lowest diet quality, it is possible that the seasonal variation is partly explained by a low consciousness about diet quality, which makes it more susceptible for seasonal influences. Additionally, we found that the seasonality of diet quality was mostly driven by the winter increase of the intake of legumes and nuts and the summer increase of sugar-containing beverages and dairy, which are behaviors that can be explained by the preference of Dutch people for legume soups around winter and sugar-containing beverages and ice creams in summer while outdoors. Taken together, these findings suggest that efforts to improve physical activity and diet quality would require: a) reducing the large bouts of sedentary behavior with light-intensity physical activity both indoor and outdoor; and b) enhancing the access to healthy food choices around the year, thus creating a healthy food environment tailored to the societal schedules of the population.

Seasonal variation of morbidity in an aging population

A growing body of evidence has shown that mortality is higher in winter than in summer.[6,411,412] Such seasonal variation differs according to geographical region; and a larger seasonality has been

observed in countries at mid distance from the equator than in those closer to the poles, where more extreme differences in weather conditions are present between seasons.[6] The seasonality of all-cause mortality appears to be mostly explained by the seasonality of cardiovascular mortality,[6] whereas little evidence supports the seasonality of cancer or non-cardiovascular/non-cancer outcomes. Also cardiovascular diseases, such as myocardial infarction and stroke, are more frequent in winter than in summer.[411,412] Whether this variation is explained by the seasonal variation of cardiovascular risk factors is less clear.[413]

On the basis of our findings, the seasonality of cardiovascular risk factors (Chapter 2.2.1) and insulin resistance (Chapter 2.2.2) are mostly attributable to exposure to low ambient temperature, whereas the seasonality of physical activity and diet appeared to be less relevant. The influence was larger among the elderly population. This pattern coincides with multiple studies that showed an increase of all-cause and cardiovascular mortality following the exposure to low ambient temperature, what suggests a role of the seasonality of these factors on mortality seasonality.[414,415] Nevertheless, other hypotheses suggest that the winter increase of all-cause and cardiovascular mortality could also be attributable to the increase in the incidence of influenza, the reduction of vitamin D levels due to lower exposure to sunlight, and the increase of exposure to air pollution, which is higher in winter due to production of pollutants by heating devices and thermal inversion.[412]

To further understand the underlying mechanisms driving the increase in mortality and morbidity in winter, future studies are required to disentangle the contribution of these factors. Specifically, it is necessary to address the patterns of exposure to low ambient temperature among elderly population, which would be contribute to size the burden of climate change on health. For example, if the burden is higher under sustained exposure to low ambient temperature or at cold waves; if the exposure occurs at home or while outdoors; and if the exposure is consequence of impaired sensitivity to ambient temperature, non-perceived sense of burden, or vulnerability given by energetic poverty or disability; or, most likely, a combination of these.

Climate change and health in aging population

Several projections suggest that a large part of the burden of the climate change will arise from the increase in the average temperature, the increase of the frequency of extreme weather events, and conditions that will favor the widespread of infections.[4] In all these scenarios, elderly populations are among the most susceptible and vulnerable. Therefore, the burden attributable to the climate change is magnified by the population aging. In this section, we discuss the implications of our findings within the upcoming challenges of climate change.

The seasonal approach to examine the factors underlying the variation of lifestyle factors (i.e. physical activity and diet quality) on a seasonal basis is of relevance in preparation for climate change. First, the fact that the seasonal variation of physical activity was larger in the middle-aged population than in elderly provides clues about the activity domains that vary the most across seasons, with occupation and transportation physical activity probably explaining the reduction of physical activity in winter (chapter 2.1.1). If confirmed in future studies, these findings could be used to design effective strategies to improve activity levels while addressing environmental factors contributing to climate change. For example, active transportation is an attractive endpoint that

contributes to increase physical activity levels;[416] to reduce the traffic-related pollution, such as heat islands, air pollution, and noise by reducing the amount of circulating traffic; and to intervene the build environment in order to increase the cyclists and pedestrians-friendly roads and green spaces. Second, the diet quality increased slightly in winter, with different patterns per food group. While the factors underlying this variation are less clear, we hypothesize that societal schedules partly shape these patterns, because those with lowest diet quality tended to have a larger seasonality. This suggests that the diet is most probably influenced by the offer of food products at supermarkets, which vary across seasons. Therefore, aiming for a healthy food environment may potentially contribute to improve overall diet by partnering with food markets. Additional environmental gains can be obtained by increasing the attainment to Dutch diet guidelines, as in current diet predominates the intake of red and processed meat, which production has a large impact in the environment. These guidelines and others of high income countries, with a lower reliance on animal products, have a lower environmental impact in terms of green-house emissions, eutrophication and land use compared to other from low and medium income countries[417] while reducing the risk of all-cause mortality.[418] It is less clear if aiming only for locally seasonal food reduces the environmental impact of the food industry. As there is no evidence that food transport makes a large difference in, for example, greenhouse emissions, it is suggested to prioritize the identification of cultural and individual factors that motivate diet choice, in order to maximize interventions aimed to improve the environmental and health consequences of diet behavior.[419]

We found that the winter increase of cardiovascular risk and of insulin resistance was mostly explained by exposure to low ambient temperature, especially among elderly participants. It is possible that this pattern contributes to explain the well-described seasonality of cardiovascular mortality and morbidity.[6] Whether the influence of exposure to low ambient temperature will be reduced under climate change is less clear. It has been suggested that although the burden of heat-related mortality is likely to increase by 256%, the reduction of cold-related deaths will be of only 2%, what is explained by the increasing of elderly population, what in absolute terms, keeps almost unchanged the burden of cold exposure.[159] Nevertheless, understanding the future health burden of climate change requires disentangling the contribution of two phenomena: the burden attributable to persistent exposure to low or high ambient temperature and the burden attributable to extreme weather events, such as cold and hot waves. Although both phenomena will be aggravated by climate change, there is evidence that the pathways of each are distinct. On one hand, mortality and morbidity due to persistent exposure to low or high ambient temperature may be explained by the cold-related increase of blood pressure, cholesterol, and insulin levels (chapters 2.2.1 and 2.2.2) and, arguably of insulin resistance, which may relate to the worsening of endothelial dysfunction. This burden tends to accumulate over lags of up to 27 days.[415] The age-related impairment of thermoregulation may imply a higher burden among elderly population, although poor home insulation and improper clothing may contribute to sustain the exposure. Therefore, addressing this burden implies structural efforts to reduce the risk of sustained exposure to cold or warm temperature. On the other hand, mortality and morbidity attributable to extreme weather conditions, such as heat waves, are usually observed in short time periods and lead to mortality displacement through

harvesting.[415] Harvesting means that those who die first under heat waves are the most susceptible and vulnerable populations, thus the pool of susceptible and vulnerable populations deplete after three or four days of intense heat.[415] This burden is high even in high income countries,[420] although the effect of heat waves would differ across regions, since the thresholds for temperature-related mortality depend on the adaptation of the population to local environmental weather conditions.[421] Nevertheless, unless strong systems of alarm and reaction are deployed, it may be more difficult to prevent the casualties related to these extreme events, especially among isolated and vulnerable elderly.[22] In order to enhance the adaptive capacity of the society, future studies are required to disentangle the contribution of persistent exposure to high or low ambient temperature and extreme weather events, as both the health effects and alternatives of intervention may differ.[422]

Another not largely foreseen consequence of the aging of the population under the current projections of climate change is the influence of the higher prevalence of cognition decline in the population. On the basis of our findings, elderly population have a lower cognitive function which fails to adapt to the environmental conditions as observed by a lack of seasonal variation of cognitive function, which contrasted with the larger seasonality of cognitive function among younger population, who also had a higher cognitive function. Therefore, the adaptive capacity that can be built around the prevention of exposure to sustained high or low ambient temperature and to extreme weather events would be hampered by this reduced adaptability of cognitive function, as well as the increased prevalence of population with cognitive decline. Current estimations suggest that the burden of climate change that is attributable to mental health considers mostly the acute impact of natural disasters and displacement and the physiological responses of exposure to extreme weather events.[4] Nevertheless, as a prevailing condition among an aging population, future projections should also account for the cognitive decline as a contributing factor within the burden attributable to climate change.

Finally, it is suggested that infectious diseases, especially influenza and other that increase during winter time, contribute to the seasonality of all-cause and cardiovascular mortality. Potential mechanisms involve the worsening of the underlying vascular disease, such as atherosclerosis, or acutely activating the inflammatory and pro-thrombotic states.[412] On the basis of our findings, it is also possible that the increase of these infections in winter lead to a widespread of resistant infections, increasing the burden in the population. Indeed, antimicrobial resistance rates of *S. pneumoniae* and *H. influenza* were higher in winter than in other seasons. This pattern was opposite for antimicrobial resistance rates of *E. coli*, which had a higher prevalence in summer than in other seasons, although the pattern was very heterogeneous. We did not find seasonality of antibiotic rates in *Campylobacter spp.* and *Salmonella spp.*, although available evidence suggests that seasonal variations might occur in relation of diseases outbreaks and husbandry practices in animals. These findings have several implications for upcoming challenges of climate change. First, based on the appraised evidence, the main determinant underlying the seasonality of antimicrobial resistance rates is the selective pressure exerted by antibiotic use, which follows a seasonal variation along with the seasonality of infectious diseases. Second, the reduced responsiveness of their immune system and higher disease burden increase the frequency of infectious disease and in consequence, the use of antibiotics among elderly population. Such

scenario is expected to worsen under climatic change, as the environmental conditions that will favor the spread of infections will be more frequent, such as storms and floods. Third, the fact that at least two of the most prevalent respiratory bacteria showed a winter peak of resistance to several antibiotics implies that the winter period may also become a season of multi-resistance, which is a worrisome scenario under limited effective therapeutic strategies. Therefore, our findings should increase the awareness about the potential role of antibacterial resistance in the adaptive capacity steps, to enforce the rational use of antibiotics to reduce the widespread of resistance and to enhance stewardship on active surveillance and response protocols to efficiently detect and address increasing rates of antibiotic rates on a seasonal basis.

Implications of the seasonal approach

The fact that we found large differences between the crude and adjusted seasonal patterns examined has implications for research. First, future studies need to consider that the characteristics of the participants attending research centers could differ across the year. On the basis of the participation rates in our study, we found that elderly participants were more likely to attend in winter months, thus rates of comorbidities prevalence and other age-related factors will have a higher prevalence than population recruited in summer. Therefore, there is a risk of selection bias while recruiting participants only in a given season of the year. Second, the seasonal variation of the factors addressed in this thesis should be accounted for in future studies using these variables. Especially, for diet and for physical activity information collected with questionnaires, since not only people do change their behavior across seasons, but they also are more likely to report their current levels of physical activity and diet as if these were typical levels. Therefore, there is a risk of information bias when people are asked by their typical levels of physical activity and diet since it could mostly reflect their recent behavior. Third, it is likely that accounting for this seasonal variation is even more important in settings with large meteorological variations across the year. Nevertheless, the influence of meteorological factors does not fully explain the seasonality of physical activity, sedentary behavior or sleep (chapter 2.1.1), and societal schedules are likely to play a relevant role in the variation of lifestyle factors. Therefore, because the societal schedules are rather constant across countries (e.g. holiday's season, Christmas, summer break), a) further studies may contribute to enhance our understanding about their influence in the seasonal pattern of lifestyle factors, b) their influence on potential information bias when collecting this information for research should not be ruled out.

Exposure to air pollution exposure according to mode of transport

One of the strategies suggested to increase physical activity levels is to increase active commuting, i.e. walking and cycling, instead of motorized commuting.[416] Nevertheless, the advocacy for active commuting has raised awareness of several risks that people face while commuting actively under the actual distribution of the public commuting space, including the exposure to a large amount of air pollutants that are emitted by the traffic. In urban spaces, traffic is the major source of several air pollutants. In spite the typical commuting time accounts for a brief portion of the daily time, the exposure while commuting accounts for up to 50% of the daily exposure to air pollution. Moreover, the exposure while commuting frequently exceeds air quality standards.[352]

Therefore, the actual balance in health outcomes by shifting from motorized to active commuting have been under debate.

In chapters 3.1 and 3.2 we systematically reviewed the literature that examined the exposure to air pollutants according to mode of transport. We found that car and bus commuters were more often exposed to $PM_{2.5}$, CO, BC and ultrafine particles. Nevertheless, while taking into account that active commuters have an increased breathing rate due to the increased physical activity, they would inhale much more pollutants than active commuters. Such difference would be even larger when comparing to pedestrians, since they spend more time than motorized commuters and cyclists to complete the same route. Furthermore, I examined the potential balance between the benefits of physical activity and the risks of inhalation of $PM_{2.5}$, and found that in terms of life expectancy, the gains of the physical activity due to commuting actively were larger than the burden of inhaling more $PM_{2.5}$. Nevertheless, this assessment only accounted for the risk of $PM_{2.5}$ inhalation, but not for other risks, such as noise and traffic accidents.

Therefore, policies aiming for a shift from motorized to active commuter need to account for a street configuration that not only reduces the objective and perceived barriers for actively commuting, but also reduces the risks associated to commuting actively, such as the large exposure to air pollution. Several studies have demonstrated large public health and societal gains by prioritizing the use of the street by pedestrians and cyclists.[353,379,381,423] Among them, the reduction of the traffic related emissions and the increase of pedestrians and cyclists friendly built environment, which contributes to reduce the noise and heat islands, in line with the targets of the reduction of the burden of the air pollution and climate change crisis.

Conclusions

Seasonal factors, not necessarily related to meteorological factors, contribute to explain the seasonality of lifestyle factors. Because improving the levels of physical activity and enhancing diet quality is of emergency to halt the increasing burden of cardiovascular disease, future studies are necessary to improve our understanding of the factors underlying a variation that already occur on a seasonal basis, for example to disentangle the role of societal schedules and meteorological factors in the observed variation.

Cardiovascular risk factors and insulin resistance have a seasonal variation that is mostly explained by exposure to low ambient temperature. The influence of low ambient temperature was larger among elderly population than among middle-aged, probably due to the age-related impairment of thermoregulation. These findings are likely to contribute to the well-described seasonality of cardiovascular risk. Although it has been suggested that in the face of the upcoming challenges of climate change the health burden attributable to cold exposure will be reduced, the aging trend of the population and changes in the daily temperature range are likely to keep relatively constant the burden attributable to cold exposure. Additionally, the events associated to changes of meteorological factors are likely to be different from those related to extreme weather conditions. In order to improve the adaptive capacity of the society to climate change, it is urgently needed to examine the patterns of exposure to ambient temperature, the role of the seasonal variation of cardiovascular risk factors on the seasonality of cardiovascular mortality and

morbidity and to disentangle the burden attributable to the variation of average and daily temperature range and extreme weather conditions.

Finally, it is suggested that promoting the shift from motorized to active commuting may help to increase the levels of physical activity of the population as well as to reduce the traffic-related pollution, such as air pollution and noise. Nevertheless, stakeholders need to account for the risks while commuting actively, such as higher inhalation of air pollution, in order to increase safety.

Chapter 5 Summary

Part of the increasing burden of climate change can be attributed to the increase of the population with high susceptibility to environmental challenges, due to population ageing trend worldwide. This implies that the pool of population with an age-related decline of physiological reserve capacity, deteriorated immune system response, and reduced cognitive capacity is increasing. Therefore, in order to cope with the upcoming challenges of environmental deterioration and climate change, it is necessary to enhance the adaptive capacity of the society. This involves improving our understanding of the influence of environmental factors in the health of the population, and the susceptibility of population subgroups, such as the elderly. In this thesis I examined some of the most urgent health issues that potentially will affect the susceptibility of elderly population under the upcoming challenges of climate change. This includes the seasonal variation of lifestyle factors, cardiovascular risk factors, cognition, and antibiotic resistance and exposure to air pollution.

First, I examined the seasonal variation and explored potential underlying mechanisms of seasonality of lifestyle factors, cardiovascular risk factors, and cognition in the Rotterdam Study; as well as the seasonality of antibiotic resistance using a systematic review of the literature. Second, I examined the exposure to air pollution according to mode of transport in two systematic reviews and described the estimation and preliminary findings of the exposure to air pollution among the population of the Rotterdam Study. All but the systematic reviews were based in the Rotterdam Study, a prospective Dutch cohort comprised in 1987 with middle-aged and elderly population living in the district of Ommoord, in Rotterdam. The cohort is comprised by three cohorts, the first one which started in 1987, the second one in 1996 and the third one in 2006. The age for inclusion in the cohort has moved across the cohorts, with participants being recruited at age of 55 in the first cohort and currently, being recruited at age of 45. The information from the participants is obtained through dedicated follow-up visits performed every four to five years though standardized interviews and questionnaires, physical examination, biological samples, and imaging procedures, as well as collected from medical records. In Figure 1 (page 8) we show an overview of the cohorts and the relevant visits for the studies that compose the chapters about the seasonal variation and health effects of air pollution.

In chapter 2.1.1 we examined the seasonality of the complete 24-hour spectrum, including physical activity, sedentary behavior and sleep, measured objectively with accelerometers, according to an age-specific approach, while accounting for the influence of meteorological factors in the seasonal variation. We observed that among middle-aged and elderly adults, the seasonality of physical activity was larger than that of sedentary behavior and nighttime sleep. Moreover, we observed that the seasonality of physical activity was only partly explained by meteorological factors. In chapter 2.1.2 we found a seasonal variation of diet quality, which was mostly influenced by the seasonality of the intake of legumes and sugar-containing beverages. Overall, a larger seasonality was observed among subgroups with lower diet quality, such as men and obese population. These findings highlight the role of the seasonal approach to address potential targets, in order to improve the overall population lifestyle choices. For

example, the need to reduce the large bouts of sedentary behavior with light-intensity physical activity both indoor and outdoor, and the need to provide a healthy food environment tailored to the societal schedules of the population.

In chapter 2.2 we examined the seasonal variation and potential underlying mechanisms of health outcomes in an aging population. In chapters 2.2.1 and 2.2.2 we showed an overall winter peak of the cardiovascular risk profile and of insulin resistance, according to HOMA-IR. The seasonality patterns were mostly explained by ambient temperature, especially among elderly participants. These findings suggest that elderly population may be more susceptible to ambient temperature because of the impairment of thermoregulation mechanisms, which may not only increase their risk of exposure to sustained low temperature, but also increase the burden of the exposure. Additionally, these findings may contribute to explain the winter peak of cardiovascular mortality and morbidity. In chapter 2.2.3 we showed a seasonal variation of cognitive performance, which lowered around winter time and was larger among population with higher cognitive performance. The seasonal variation of cognitive performance among population with higher cognitive performance can be interpreted as a sign of healthy aging and needs to be addressed in the future to better understand the adaptability of cognitive performance in an aging population. In chapter 2.2.4 we performed a systematic review to examine the seasonal variation of antimicrobial resistance of bacteria that pose a threat for public health. The antimicrobial resistance rate of *S. pneumoniae* and *H. influenza* was higher in winter and of *E. coli* in summer, although the pattern was very heterogeneous. No seasonal variation was observed for *Campylobacter spp.* and *Salmonella spp.* This observed seasonality may be explained by the seasonal variation of infectious diseases incidence and the consequent variation of antibiotic use. Our findings should increase the awareness about the potential role of antimicrobial resistance in the adaptive capacity of the society.

In chapter 3 we addressed the exposure to air pollution. First, we performed a systematic review to examine the exposure to NO_x, CO, BC, coarse, and fine particles (chapter 3.1.1) and to ultrafine particles (chapter 3.2.2) according to mode of transport. Overall, motorized commuters were more often exposed to $PM_{2.5}$, CO, BC and ultrafine particles. However, because pedestrians and cyclists have a higher inhalation rate, they inhaled more pollutants. Nevertheless, the gains in life expectancy of cyclists and pedestrians due to physical activity were not overcome by the burden of higher inhalation of $PM_{2.5}$. Large societal gains can be obtained by promoting the shift from motorized to active transportation.

Finally, in chapter 3.2 we describe the methodology to estimate the exposure to NO_2, NO_x, PM_{10}, $PM_{2.5}$, and $PM_{2.5}$ absorbance among the participants of the Rotterdam Study, using land use regression models developed and validated for the Netherlands/Belgium region by the ESCAPE study. We observed a lower dispersion of the pollutants in the Ommoord district compared to those observed in the Netherlands/Belgium during the ESCAPE measurement campaigns. However, there was substantial spatial variation of the pollutants, particularly of NO_2 and NO_x, in relation with the road network of the district, suggesting this is a large source of air pollution exposure among the participants of the Rotterdam Study. Additionally, we found a winter peak of air pollution exposure, and the distribution of air pollution exposure changed with certain characteristics of the participants, such as age and blood pressure. Future studies

based on this data will contribute to have a better the health effects of traffic-related air pollution exposure in an aging population.

Finally, in chapter 4 I discuss the methodological implications of our findings and discuss the major findings of our study in relation with the seasonality of mortality and morbidity, climate change, and air pollution exposure among elderly population.

In this thesis, I examined some of the most urgent health issues that may affect the susceptibility of elderly population under the upcoming challenges of the climate change. Lifestyle factors (physical activity and diet quality) had a seasonal variation. The seasonal approach may provide clues of factors already influencing the seasonal variation of lifestyle choices on a seasonal basis, such as societal schedules. Also, cardiovascular risk factors and insulin resistance had a seasonal variation and tended to worsen in winter. The pattern was mostly explained by low ambient temperature, especially among elderly. The seasonality of cardiovascular risk factors and insulin resistance may contribute to explain the winter peak of cardiovascular mortality and morbidity. Also, the seasonality of cognition and antimicrobial resistance can pose important threats for the adaptive capacity of the society to the upcoming challenges of climate change. The expected burden of these variations can increase, as the susceptibility of the population to environmental deterioration and changes in daily temperature rises, as a consequence of population aging trend. This trend will increase the pool of population with declined physiological reserve capacity, deteriorated immune system response, and reduced cognitive capacity. Therefore, to improve the adaptive capacity of the society to climate change, it is urgently needed to understand the mechanisms underlying the seasonal variation of these health outcomes. Additionally, it is suggested that promoting the shift from motorized to active commuting may help to increase the levels of physical activity of the population as well as to reduce the traffic-related pollution, such as air pollution and noise. Nevertheless, stakeholders need to account for the risks associated to commuting actively, such as higher inhalation of air pollution, in order to improve safety. Finally, I described the estimation of air pollution exposure in the population of the Rotterdam Study. Futures studies based on these estimations will help to elucidate the health effects of traffic-related air pollution exposure in an aging population.

Samenvatting

Er is bewijs dat klimaatveranderingen in toenemende mate problemen kunnen veroorzaken, deels omdat het deel van de bevolking dat hier last van kan hebben toeneemt, en deels doordat de bevolking wereldwijd steeds ouder wordt. Als een gevolg daarvan is er een toename van het aantal mensen met een verminderde fysiologische reserve, een minder goed werkend immuunsysteem en een verminderd cognitief vermogen. Om te kunnen omgaan met de komende veranderingen in het milieu en het klimaat, is het nodig om het aanpassingsvermogen van de populatie te optimaliseren. Hierbij moeten we onze kennis over de invloed van omgevingsfactoren op de gezondheid van de populatie verbreden, en moeten we meer te weten komen over de invloed van omgevingsfactoren op kwetsbare subgroepen, zoals ouderen. In dit proefschrift heb ik een aantal van de meest dringende gezondheidsproblemen onderzocht die mogelijk kunnen voortvloeien uit de klimaatveranderingen. Hierbij heb ik onder andere gekeken of leefstijlfactoren, cardiovasculaire risicofactoren, cognitie, antibioticaresistentie en blootstelling aan luchtvervuiling een seizoensgebonden patroon vertonen.

Eerst onderzocht ik het seizoensgebonden patroon, en mogelijke onderliggende mechanismen, van leefstijlfactoren, cardiovasculaire risicofactoren en cognitie binnen de Rotterdam Studie. Ook onderzocht ik het seizoensgebonden patroon van antibioticaresistentie aan de hand van een systematische literatuurstudie. Als tweede onderzocht ik in twee systematische literatuurstudies of de blootstelling aan luchtvervuiling afhankelijk was van de keuze van vervoer en beschreef ik de geschatte luchtvervuiling in de Rotterdam Studie. Met uitzondering van de literatuurstudies, is al mijn onderzoek gebaseerd op de Rotterdam Studie, een langlopend prospectief bevolkingsonderzoek in Nederland, dat in 1987 is gestart. Deelnemers van de Rotterdam Studie zijn van middelbare en oudere leeftijd en zijn woonachtig in de wijk Ommoord, in Rotterdam. De Rotterdam Studie bestaat uit drie cohorten, waarvan de eerste is gestart in 1987, de tweede in 1996 en de derde in 2006. In de eerste cohort kwamen mensen in aanmerking voor deelname als ze 55 jaar of ouder waren, en ondertussen worden mensen gerekruteerd die 45 jaar of ouder zijn. Van de deelnemers wordt informatie verzameld via bezoeken die elke 4-5 jaar herhaald worden. Hierbij worden gestandaardiseerde interviews en vragenlijsten afgenomen, wordt er lichamelijk onderzoek gedaan, wordt er bloed afgenomen en worden er verschillende beeldtechnieken gebruikt om gezondheid in kaart te brengen. Tot slot wordt er informatie verzameld via medische gegevens van de huisarts en het ziekenhuis. De bezoeken waarvan informatie is gebruikt in dit proefschrift verschillen per hoofdstuk, zoals weergegeven in Figuur 1 (pagina 8).

In hoofdstuk 2.1.1 onderzochten we of objectief gemeten fysieke activiteit, sedentair gedrag en slaap een seizoensgebonden patroon vertoonden, en of dit afhankelijk was van de leeftijd. Hierbij corrigeerden we voor de mogelijke invloed van meteorologische factoren. Bij volwassenen van middelbare leeftijd en bij ouderen vonden we dat het seizoensgebonden patroon van fysieke activiteit groter was dan het patroon van sedentair gedrag en slaap. We vonden ook dat meteorologische factoren maar een kleine invloed hadden op het seizoensgebonden patroon van fysieke activiteit.

In hoofdstuk 2.1.2 vonden we een seizoensgebonden patroon voor de kwaliteit van het eetpatroon, dat vooral verklaard kon worden door de inname van peulvruchten en

suikerhoudende dranken. Bij volwassenen met een algemeen lagere kwaliteit van het eetpatroon, zoals mannen en mensen met obesitas, vonden we een groter seizoensgebonden patroon. Deze bevindingen benadrukken dat het belangrijk is om rekening te houden met het seizoen bij het identificeren van mogelijkheden om de leefstijlkeuzes van de populatie te beïnvloeden.

In hoofdstuk 2.2 onderzochten we het seizoensgebonden patroon, en mogelijke onderliggende mechanismen, van gezondheidsuitkomsten in een verouderende populatie. In hoofdstuk 2.2.1 en 2.2.2 laten we zien dat het cardiovasculaire risicoprofiel de slechtste waarden laat zien in de winter. Ook vonden we in de winter de hoogste mate van insulineresistentie, zoals gemeten met HOMA-IR. Dit patroon kon vooral worden verklaard door de buitentemperatuur, vooral in de oudere deelnemers. Deze bevindingen suggereren dat ouderen gevoeliger zouden kunnen zijn voor de buitentemperatuur, doordat hun thermoregulatie minder goed werkt. Ook kunnen deze bevindingen bijdragen aan de verklaring waarom er in de winter meer cardiovasculaire aandoeningen ontstaan, en de mortaliteit gerelateerd aan cardiovasculaire ziekten hoger is. In hoofdstuk 2.2.3 laten we zien dat er een seizoensgebonden patroon is voor cognitief presteren, waarbij de laagste scores werden gevonden in de winter. In deelnemers met een hogere cognitieve prestatie zagen we een groter seizoensgebonden patroon. Deze laatste bevinding kan worden gezien als een teken van gezond ouder worden, en zou in de toekomst onderzocht moeten worden om het aanpassingsvermogen van cognitief presteren in een verouderende populatie beter te begrijpen. In hoofdstuk 2.2.4 laten we zien dat er in de literatuur een hogere antimicrobiële resistentie tegen S. pneumoniae en H. influenza was gevonden in de winter en een hogere resistentie tegen *E.coli* in de zomer, hoewel het patroon erg heterogeen was. Voor *Campylobacter spp.* en *Salmonella spp* was geen seizoensgebonden variatie geobserveerd. De geobserveerde seizoensgebonden patronen zouden kunnen worden verklaard door de variatie van infectieziektes gedurende het jaar, en het bijbehorende gebruik van antibiotica. Onze bevindingen kunnen het bewustzijn verbeteren met betrekking tot de mogelijke rol van antimicrobiële resistentie in het aanpassingsvermogen van de samenleving.

In hoofdstuk 3 onderzochten we de blootstelling aan luchtvervuiling. Eerst deden we een systematisch literatuuronderzoek om erachter te komen of de blootstelling aan NO_x, CO, BC, grove-, en fijnstof (hoofdstuk 3.1.1) en ultrafijnstof (hoofdstuk 3.1.2) zou verschillen tussen verschillende manieren van vervoer. We vonden dat gemotoriseerde weggebruikers vaker blootgesteld waren aan $PM_{2.5}$, CO, BC en ultrafijnstof. Echter, omdat lopende en fietsende weggebruikers een hogere ademfrequentie hebben, ademden zij meer verontreinigende stoffen in. De nadelige effecten van de hogere opname van PM2.5 tijdens fietsen en lopen zijn echter niet zo groot dat ze de positieve effecten van fysieke activiteit op de levensverwachting teniet doen. Wanneer er een transitie van gemotoriseerd naar actief transport zou plaatsvinden, zou er op grote schaal winst voor de maatschappij behaald kunnen worden.

In hoofdstuk 3.2 beschrijf ik de methoden om de blootstelling aan NO_2, NO_x, PM_{10}, $PM_{2.5}$, en $PM_{2.5}$ te schatten voor deelnemers van de Rotterdam Studie. Hierbij maak ik gebruik van landgebruik-regressiemodellen, die door de ESCAPE studie ontwikkeld en gevalideerd zijn voor Nederland en België. We vonden een lagere spreiding van luchtvervuilende stoffen in Ommoord, vergeleken met Nederlandse en Belgische wijken zoals gemeten in de ESCAPE studie. We vonden echter wel substantiële ruimtelijke variatie van de luchtvervuilende stoffen, vooral

voor NO_2 en NO_x, in relatie tot het wegnetwerk van de wijk. Dit suggereert dat NO_2 en NO_x grote bronnen van luchtvervuiling zijn voor deelnemers van de Rotterdam Studie. We vonden ook dat de distributie van blootstelling aan luchtvervuilende stoffen afhankelijk was van bepaalde karakteristieken van de deelnemers, zoals leeftijd en bloeddruk. Vervolgstudies gebaseerd op deze data kunnen bijdragen aan meer kennis over de gezondheidseffecten van verkeers-gerelateerde luchtvervuiling in een verouderende populatie.

Tot slot bespreek ik in hoofdstuk 4 de methodologische beperkingen van onze bevindingen, en bespreek ik de belangrijkste bevindingen van mijn studies in relatie tot het seizoensgebonden patroon van overlijden en morbiditeit, klimaatveranderingen, en blootstelling aan luchtvervuiling binnen de populatie van ouderen.

In dit proefschrift heb ik een aantal belangrijke gezondheidszaken onderzocht die de kwetsbaarheid van een oudere populatie kunnen beïnvloeden tijdens de opkomende uitdagingen van klimaatveranderingen. Leefstijlfactoren zoals fysieke activiteit en kwaliteit van het eetpatroon vertoonden een seizoensgebonden patroon. Een aanpak waarbij rekening wordt gehouden met het seizoen zou aanwijzingen kunnen geven voor factoren die deze leefstijlfactoren beïnvloeden gedurende de verschillende seizoenen. Ook vond ik dat cardiovasculaire risicofactoren en insulineresistentie een seizoensgebonden patroon vertoonden, en dit patroon kon met name verklaard worden door de buitentemperatuur, vooral in ouderen. Deze bevindingen zouden kunnen verklaren dat er in de winter meer mensen een cardiovasculair event doormaken, en meer mensen hieraan overlijden. Het seizoensgebonden patroon van cognitieve prestatie en antimicrobiële resistentie kan een bedreiging vormen voor het aanpassingsvermogen van de maatschappij wanneer we te maken krijgen met de uitdagingen van klimaatveranderingen. De verwachte last van de genoemde seizoensgebonden patronen kan hoger worden als de populatie verouderd, aangezien dit het aantal kwetsbare volwassenen verhoogd. Het is belangrijk om ervoor te zorgen dat de maatschappij zich goed kan aanpassen aan klimaatveranderingen, en daarom moeten we de mechanismen die ten grondslag liggen aan de patronen van deze gezondheidsuitkomsten beter begrijpen. Ook zou het helpen om een transitie te maken van gemotoriseerd vervoer naar actief vervoer, om zo verkeers-gerelateerde lucht- en geluidsvervuiling te verminderen en fysieke activiteit in de populatie te verhogen. Om actief transport veiliger te maken, moeten stakeholders zorgen dat de risico's ten gevolge van een hogere opname van luchtvervuilende deeltjes zo laag mogelijk zijn. Tot slot kunnen toekomstige studies gebaseerd op geschatte blootstelling aan luchtvervuiling in de Rotterdam Studie helpen om de gezondheidseffecten van verkeers-gerelateerde luchtvervuiling op te helderen.

Chapter 6 References

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Publication list

1. **Cepeda M**, Schoufour JD, Freak-Poli R, Koolhaas CM, Dhana K, Bramer W, Franco OH. Levels of ambient air pollution according to mode of transport: a systematic review. The Lancet Public Health 2016; 2 (1), e23-e34.
2. **Cepeda M**, Schoufour JD, Freak-Poli R, Koolhaas CM, Dhana K, Bramer W, Guxens M, Franco OH. Exposure to ultrafine particles according to mode of transport: a systematic review. Submitted.
3. **Cepeda M,** Schoufour JD, Franco OH, Guxens M. Association between traffic-related air pollution exposure and insulin resistance and diabetes incidence among participants of the Rotterdam Study. In preparation.
4. **Cepeda M**, Muka T, Ikram MA, Franco OH, Schoufour JD. Seasonality of insulin resistance, glucose and insulin levels among participants of the Rotterdam Study. J Clin Endocrinol Metab 2018;103(3):946-55.
5. **Cepeda M***, Koolhaas CM*, van Rooij FJA, Tiemeier H, Guxens M, Franco OH, Schoufour JD. Seasonality of physical activity, sedentary behavior, and sleep in a middle-aged and elderly population: The Rotterdam study. https://www.ncbi.nlm.nih.gov/pubmed/29563034 Maturitas 2018;110:41-50.
6. **Cepeda M*,** van der Toorn J*, Franco OH, Schoufour JD. Seasonality of dietary intake among participants of the Rotterdam Study. Under review in European Journal of Nutrition.
7. **Cepeda M**, Schoufour J, Erler N, Marques-Vidal P, Franco OH. Effect of meteorological factors and physical activity on the seasonality of cardiovascular risk factors: The Rotterdam Study. Under review in PLOS Medicine.
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11. Martínez P*, **Cepeda M***, Schoufour JD, Franco OH. Seasonality of antibiotic resistance. Systematic review and meta-analysis. Submitted in BMJ
12. **Cepeda M***, Lichter S*, Schoufour JD, Franco OH, Ikram A. Seasonal variation of cognitive function in The Rotterdam Study population. Submitted in Neurology.
13. Nano J, Muka T, **Cepeda M**, Voortman T, Dhana K, Brahimaj A, Dehghan A, Franco OH. Association of circulating total bilirubin with the metabolic syndrome and type 2 diabetes: A systematic review and meta-analysis of observational evidence. Diabetes & Metabolism 2016; 42 (6), 389-397
14. Ruiz AJ, Rondón Sepúlveda MA, Franco OH, **Cepeda M**, Hidalgo Martinez P, Amado Garzón SB, Salazar Ibarra ER, Otero Mendoza L. The associations between sleep disorders

and anthropometric measures in adults from three Colombian cities at different altitudes. Maturitas 2016; 94, 1-10

About the author

Magda was born in Paipa (Colombia) 1986. She graduated as a medical doctor in the Universidad Nacional de Colombia in Bogotá in 2009. During the last year as intern, Magda did a special internship in Epidemiology, where she worked as research assistant and attended lectures in biostatistics and epidemiology. During 2010, Magda worked as a medical doctor in mandatory social service in Cali, Colombia, where she started the Master of Science in Epidemiology at the Universidad del Valle. She obtained a meritorious master degree in 2012 with a thesis named "Built environment determinants of walking as a mode of transport among adults in Cali, Colombia", performed within a population-based project aimed to examine the built environment determinants of active transportation in adult population from Cali. Meanwhile, Magda collaborated in multiple projects to develop nation-wide evidence-based guidelines for several diseases of public-health relevance and worked as assistant professor of epidemiology for medical students and residents. In 2012 Magda was granted a scholarship to pursue doctoral studies outside of Colombia by COLCIENCIAS, which permitted her to start her PhD studies in Erasmus Medical Center in 2014. In 2016 Magda got the Master of Science in Health, specialty Public health by NIHES and Erasmus University of Rotterdam. Magda will continue her career in academia by working as a postdoctoral scientist with the Pontificia Universidad Javeriana, Bogota, Colombia and the University of Bern, Bern, Switzerland.

PhD portfolio

Summary of PhD training and teaching

Name PhD student: Magda Cepeda
Erasmus MC Department: Epidemiology
Research school: NIHES
PhD period: April 2014 – September 2018
Promotor: Prof. Dr. Oscar H Franco
Copromotors: Dr. Josje D. Schoufour, Dr. Mònica Guxens

PhD training and teaching	Year	Workload (ECTS)
General courses		
Integrity in science	2017	0.3
Biomedical English Writing and Communication Information	2017	4.0
Specific courses: Master of Science in Public Health (NIHES)		
Courses for the Quantitative Researcher	2016	
Study Design	2015	4.3
Biostatistical Methods I: Basic principles	2014	5.7
Repeated Measurements in Clinical Studies	2016	1.4
Bayesian Statistics	2015	1.4
Development Research Proposal	2016	2.5
Biostatistical Methods II: Classical Regression Models	2014	4.3
Principles of Research in Medicine and Epidemiology	2014	0.7
Methods of Public Health Research	2014	0.7
Introduction to Public Health	2014	0.7
Methods of Health Services Research	2014	0.7
Primary & Secondary Prevention Research	2014	0.7
Social Epidemiology	2014	0.7
Environmental Epidemiology	2015	1.1
Public Health Research Methods	2015	5.7
International Comparison of Health Care	2014	1.4
Planning and Evaluation of Screening	2015	1.4
Quality of Life Measurement	2015	0.9
Problem to Solution in Public Health	2015	1.1
Site Visit to the Municipal Health Service Rotterdam	2016	0.3
Integration Module	2016	0.3
Public Health in Low and Middle Income Countries	2015	3.0
English Language	2014	1.4
Introduction to Medical Writing	2016	1.1
Research visits		
Research visit at Instituto de Salud Global – ISGlobal, Barcelona	2018	
Attended seminars, symposia, and conferences		
ErasmusAGE research meetings	2014-2018	1.0
Seminars at the Department of Epidemiology	2014-2018	1.0
International Society of Environmental Epidemiology (ISEE), Roma, Italy	2016	1.0
11th European Congress on Menopaus and Andropause, Amsterdam, the Netherlands	2017	1.0
KNAW/DuSRA meeting, Dordrecth, the Netherlands	2017	1.0

(Inter)national conference presentations		
International Society of Environmental Epidemiology (ISEE), Roma, Italy – poster presentation	2016	1.0
11th European Congress on Menopause and Andropause – two poster presentation	2017	1.0
KNAW/DuSRA meeting, Dordrecth, the Netherlands – two poster presentation	2017	1.0
Student training		
Supervising project "Air pollution control and the occurrence of acute respiratory illness in school children of Quito, Ecuador", Bertha Magdalena Estrella Cahuenas, MSc	2017	0.5
Supervising project "Seasonality of antimicrobial resistance in critically important bacteria that pose a great threat to public health: A systematic review and meta-analysis", Evelyn Pamela Martínez, MSc	2017	0.5
Supevising Master's thesis "Seasonal variation of diet quality in a middle-aged and elderly Dutch population-based cohort", Janine E. van der Toorn, MSc	2017	0.5
Teaching activities		
Teaching assistant in "Principles of Research in Medicine and Epidemiology", Erasmus Summer Programme, the Netherlands	2016	
Journal peer-reviewer		
Environmental Science & Technology, Environmental Engineering and Management Journal, Biomédica, Public Health Nutrition, European Journal of Epidemiology, Air quality, Atmosphere & Health, European Journal of Pediatry		

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"Muchos años después, frente al pelotón de fusilamiento, el coronel Aureliano Buendía había de recordar aquella tarde remota en que su padre lo llevó a conocer el hielo"

"Many years later, as he faced the firing squad, Colonel Aureliano Buendía was to remember that distant afternoon when his father took him to discover ice."

Gabriel García Márquez, Cien Años de Soledad, 1967