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General Discussion

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DISCUSSION

In this thesis, I aimed to examine the relation between aspects of the diet, inflammation, body composition and type 2 diabetes. With regards to dietary factors, special attention was directed to the putative effects of antioxidants and dietary advanced glycation end products, as well as coffee consumption and adherence to a plant-based diet, on body composition and risk of type 2 diabetes. I also studied the role of serum uric acid, a biomarker associated with inflammation, as a risk factor for type 2 diabetes and cardiovascular disease. Furthermore, I examined whether other markers of inflammation, among them C-reactive protein, mediate the association between coffee consumption and type 2 diabetes. Specific considerations about the individual studies have been addressed in the previous chapters. In this general discussion, I will first provide a brief summary of the main findings. Afterwards, I will reflect on methodological considerations and discuss the implications of the research contained in this thesis as well as potential future research directions.

Main findings

Dietary determinants of type 2 diabetes

In chapter 2, I examined aspects of the diet as well as specific dietary patterns in relation to type 2 diabetes. In chapter 2.1, I investigated antioxidant consumption, expressed as total dietary antioxidant capacity of the diet, in relation to risk of type 2 diabetes, prediabetes and insulin resistance. I observed that among the 5,796 participants included in this study, higher dietary antioxidant consumption was associated with lower risk of type 2 diabetes. This observation applies to both the total population and the subgroup of participants who already had prediabetes at baseline. Higher dietary antioxidant consumption was also associated with lower insulin resistance, measured cross-sectionally, but not with risk of prediabetes. These findings indicate that higher antioxidant consumption may have favorable effects on risk of type 2 diabetes. In line with this, in chapter 2.2, I investigated whether a relatively more plant-based diet is associated with lower risk of type 2 diabetes and lower insulin resistance when compared to a relatively more animal-based diet. For this purpose, a plant-based diet index was constructed on which a higher score indicated a more plant-based diet. I observed that a higher plant-based diet score was associated with lower risk of type 2 diabetes, lower risk of prediabetes and lower insulin resistance, corroborating dietary guidelines that recommend preferential intake of plant-based foods compared to animal-based foods. This recommendation is also further supported by the observation that antioxidant intake is favorably associated with risk of type 2 diabetes, given that many plant-based foods are also rich in antioxidants.[1] Although the mechanisms through which the beneficial effects of a plant-based diet and higher

antioxidant consumption on risk of health outcomes occur are not precisely known, evidence suggests that diet may affect levels of subclinical inflammation.[2,3] Thus, in the following chapter, I further explored inflammation in the context of diet and type 2 diabetes.

Markers of inflammation and risk of type 2 diabetes

A large portion of the work in chapter 3 focuses on serum uric acid, the end product of purine metabolism in humans and a biomarker associated with inflammation.[4–6] High levels of serum uric acid are associated with risk of cardiometabolic disease, but the precise underlying pathways have remained largely undetermined thus far. In chapter 3.1, I further investigated serum uric acid as a risk indicator for type 2 diabetes. I provide evidence that a higher serum uric acid level is associated with risk of prediabetes, specifically among women, but not with risk of type 2 diabetes among individuals with established prediabetes. This may indicate that the strength of the association between serum uric acid and risk of type 2 diabetes differs according to the degree of which disturbances of glucose metabolism are already present. In other words, high levels of serum uric acid may play a role in early-phase mechanisms rather than late-phase mechanisms in the development of insulin resistance and eventual type 2 diabetes. However, the role of serum uric acid in disease risk prediction may not only be limited to early disease. In chapter 3.2, I demonstrate that sex and type 2 diabetes status modify the association between serum uric acid levels and both fatal and non-fatal cardiovascular events. In this study, serum uric acid was most strongly associated with all-cause mortality and cardiovascular events specifically among diabetic women, suggesting that different cardiovascular management strategies may be warranted among women and men, and individuals with and without type 2 diabetes, with regards to high serum uric acid levels. This study also highlights the potential of uric acid as a risk biomarker in advanced disease. In chapter 3.3, I further expanded upon the role of diet with regards to inflammation, and provide evidence on how inflammation may mediate the effect of coffee consumption on type 2 diabetes risk. First, I confirmed the findings of previous studies which have suggested an association between coffee consumption and type 2 diabetes risk by replicating this association among over 150,000 participants two large population-based cohorts, the Rotterdam Study and the United Kingdom (UK) Biobank. Subsequently, I provide evidence that this association is mediated by changes in C-reactive protein (CRP) levels induced by coffee. However, the proportion of the effect of coffee consumption on type 2 diabetes risk that was mediated by CRP levels was relatively small. This indicates that other factors than inflammation likely also play a prominent mediating role in the association between dietary factors and type 2 diabetes risk. One such factor, also closely related to inflammation, is adiposity.[7] Therefore, I also explored determinants of adiposity as

well as more general measures of body composition in the context of diet, inflammation and type 2 diabetes.

Determinants of body composition

This chapter is centered around body composition, an anthropometric concept which refers to the relative amounts and distribution of fat and fat-free tissue in the human body and provides a more detailed picture of adiposity compared to that which can be obtained using traditional anthropometric methods such as body mass index (BMI) or waist-to-hip ratio. In chapter 4.1, I investigated dietary antioxidant consumption in relation to repeatedly measured body composition assessed using dual X-ray absorptiometry among 4,595 participants of the Rotterdam Study. I found that higher dietary antioxidant consumption was associated with higher fat-free mass index, lower android-to-gynoid fat ratio, and lower body fat percentage. Considering the beneficial association with more fat-free mass, I additionally investigated whether dietary antioxidant consumption was associated with hand grip strength and prevalence of sarcopenia but found no association with relation to these outcomes. These findings suggest that dietary intake of antioxidants may have favorable effects on overall body composition among the middle-aged and elderly. They also underline the notion that higher antioxidant consumption potentially has diverse beneficial health effects, as I also demonstrated an inverse association between dietary antioxidant consumption and risk of type 2 diabetes in chapter 2.1. Finally, in chapter 4.2, I report that consumption of dietary advanced glycation end-products, molecular compounds that may contribute to inflammation, could have detrimental effects on body composition: higher consumption of one such compound was associated with higher fat mass index, fat-free mass index, android-to-gynoid fat ratio, BMI and body fat percentage. This provides further evidence supporting the putative role of diet-induced inflammation in the development of adiposity, considering advanced glycation end-products can induce inflammation through interacting with their shared receptor.[8]

Methodological considerations

All of the studies contained in this thesis were, at least in part, performed within the framework of the Rotterdam Study, a population-based closed cohort study involving inhabitants from the Ommoord district in the city of Rotterdam, the Netherlands. The Rotterdam Study was initiated in 1990 with the aim of studying determinants of neurologic, cardiovascular, locomotor and ophthalmologic diseases among elderly individuals.[9] In later years a wealth of information on other potential determinants of disease and mortality was collected among almost 15,000 individuals. Participants from the original cohort are still being followed up today.[10] Several of the studies presented here were also performed using data from the UK Biobank, another population-based

cohort currently under investigation in 22 research centers across England, Scotland and Wales. The UK Biobank includes over half a million individuals from a diverse age range (37-73 years) who volunteered to participate. Follow-up of these participants started in 2006. Several considerations should be taken into account when interpreting the findings from these studies. These relate to the observational nature of these studies and concomitant types of bias, potential issues regarding measurement error in the variables of interest and the representativeness of the study populations. I will address each of these factors in the following sections.

Temporality and causality in observational study design

Prospective cohort studies are generally well-suited for identifying determinants of relatively commonly occurring diseases or endpoints. Such studies also offer the advantage that they provide information on the temporal relation between exposure and outcome. In prospective cohort studies, assessment of exposure is performed before the outcome of interest occurs, and therefore systematic error stemming from selective recall or biased exposure ascertainment is largely avoided. Temporality is also one of Bradford Hill's criteria for causality.[11] While directly inferring causality from any epidemiological study is not possible, demonstrating a temporal relation between exposure and outcome (i.e. exposure preceding the outcome) still provides useful additional information in determining whether an observed association might be causal. The argument for causality becomes even stronger if it can be demonstrated that exposure not only affects risk of the outcome at one given point in time, but also throughout multiple measurements separated in time. The prospective design of the Rotterdam Study also allowed me to incorporate repeated measurements of the outcomes of interest, notably body composition, in some of the studies presented here. In this way, I was able to demonstrate that, for example, dietary consumption of antioxidants is associated with changes in body composition measured repeatedly across time rather than at a single time point (chapter 4.1). However, it should be emphasized that temporality alone does not prove causality. For instance, according to the aforementioned Bradford Hill criteria for causality, other factors should also be considered, including (but not limited to) whether there is evidence for a dose-response relationship between exposure and outcome and whether the association is reproducible.[11] Nevertheless, repeated outcome assessment provides a more robust argument to hypothesize that higher antioxidant consumption is causally associated with body composition, because the longitudinal design largely eliminates the possibility of reverse causation. Aside from this, a longitudinal design can also be used to investigate whether the strength of the association between exposure and outcome varies over time; in other words, whether interaction exists between follow-up time and levels of exposure on risk of the outcome under study. For example, I observed

this effect when investigating dietary antioxidant consumption in relation to hand grip strength, indicating that antioxidant consumption modifies the rate at which hand grip strength evolves over time (chapter 4.1). This corroborates the notion that a given association between an exposure and outcome which were both measured at a single point in time may provide incomplete information, and emphasizes the general importance of studies that consider repeated measurements. Ideally, repeated measurements of the outcome under study should also be paired with repeated assessment of exposure. In this way, it would also be possible to capture the time-varying nature of a given exposure, which seems especially pertinent with regards to diet because diets may evolve over longer periods of time within individuals. Unfortunately, repeated assessment of exposure was generally not available for the purposes of the studies presented here.

Determinants of internal validity

Arguably, the most important determinant of the accuracy of any study is the degree to which its conclusions are valid. In epidemiology, a distinction is made between internal and external validity. A discussion on external validity will be provided further below. In this section, I will focus on determinants of internal validity. A study is internally valid if the reported measures of association are free of systematic error. Such systematic error, or bias, could arise from different sources in observational studies such as the Rotterdam Study and the UK Biobank.

The first of these potential sources of systematic error is selection bias, defined as a type of bias that occurs when the relation between exposure and outcome is different for study participants compared to all those who should have been theoretically eligible to participate.[12] Selection bias should be distinguished from sampling bias, the phenomenon where participants who are enrolled into a study are in some way different from all individuals in the source population these participants are being sampled from. This occurs when certain characteristics (for instance, overall level of health) affect likelihood of participation in the study, and thus affect the representativeness of the sample compared to the source population. Such selective participation at baseline will not threaten internal validity in and of itself: participants with different levels of exposure can still be compared within those who opted to participate, even if the exposure distribution or the frequency distribution of common causes of exposure and outcome is different in the underlying source population. In this situation, the resulting effect estimates will not be biased to a large degree, as has also been demonstrated empirically for other cohorts.[13–16] However, sampling bias may limit the generalizability of a study, as will be discussed further below. In contrast, selection bias occurs when exposure and outcome are both associated with propensity

to enter the study or remain under follow-up. For instance, in our studies which relate aspects of the diet to repeated measurements of body composition (chapters 4.1-4.2), severely obese individuals could not undergo DXA measurements because they exceed the surface area limitations of the device. In addition, it is conceivable that diet quality may affect willingness or ability to undergo future DXA measurements through pathways not directly related to obesity, for instance through affecting risk of certain comorbidities. In this situation, exposure and outcome share a common effect: propensity to undergo a body composition measurement. When this common effect, or a consequence of this common effect, is conditioned upon in data analysis (that is, only individuals for whom DXA measurements were available are analyzed), the resulting measures of association will be biased, as previously outlined in the framework proposed by Hernán.[17] A generalization of this concept is presented in Figure 5.1.1. From this perspective, the concept of selection bias can be regarded as a generalization of the classic Berksonian bias described many decades ago.[18] This effect could also have occurred in our research relating to uric acid as a determinant of type 2 diabetes risk (chapters 3.1, 3.2). Very high serum uric acid levels may indicate a general level of suboptimal health. It could be hypothesized that individuals with higher serum uric acid have a higher propensity to experience mortality or withdraw from follow-up before developing any of the outcomes of interest through phenomena unrelated to the development of type 2 diabetes. Type 2 diabetes itself, or its prodromal stages, may also influence this propensity. Therefore, some degree of selection bias could have occurred in these studies.

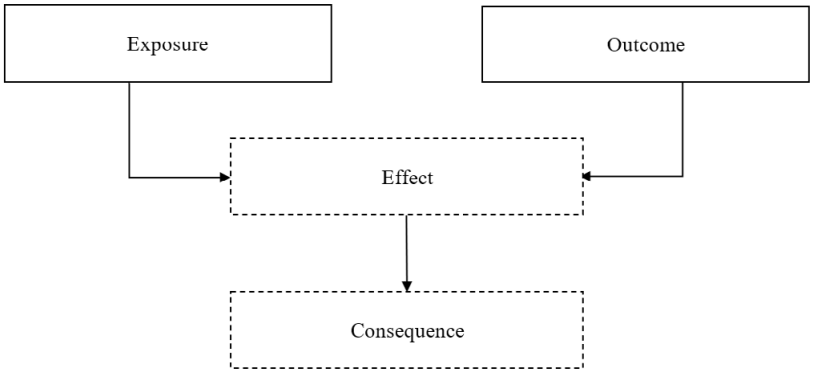

Figure 5.1.1. An exposure and outcome which share a common effect will be conditionally associated within levels of this common effect or a consequence of this common effect. Arrows indicate causal effects. Dashed lines indicate possible conditioning (adapted from Hernán et al., A Structural Approach to Selection Bias, Epidemiology, 2004; 15: 615–625; figures 3 and 4).

The second potential source of bias I will discuss here is confounding. Confounding occurs when the association between exposure and outcome is distorted by a factor which is associated with the exposure under study, is an extraneous risk factor for the outcome of interest, but is not itself affected by exposure or disease.[12] This distortion is of special concern in observational studies, where exposure assignment is not randomized. Fortunately, the wide range of covariates that were measured in both the Rotterdam Study and the UK Biobank allowed us to adjust for many confounding factors in our analyses. The relatively large number of participants in both population-based studies also allowed us to perform stratified analyses in most cases, enabling us to not only adjust for confounders but also to explore effect modification. Adjusting for confounding factors is especially relevant when investigating (aspects of) diet as an exposure, which tends to be determined by an overall level of health consciousness which is impossible to measure directly and must be approximated with multiple variables. Thus, I commonly adjusted for factors such as physical activity, level of education, adherence to dietary guidelines, smoking habits and drinking behavior in the analyses; all of which may affect both diet and the outcomes under study. Nonetheless, many other unmeasured factors may be associated with both diet and the outcomes of interest. Therefore, residual confounding of the reported measures of association in the studies presented here can never fully be ruled out.

Thirdly, inaccuracy in the measurement of any information used in a study may result in information bias.[19] Such error in measuring a variable is often referred to as misclassification, which may be further described as differential or non-differential based on whether the measurement error is dependent on the actual values of other variables.[12] Misclassification of confounding variables may also occur, making properly controlling for confounding an even more challenging task. I will relate aspects of information bias to the techniques that were used to assess diet and body composition below.

Measurement of diet

Historically, several methods have been used to measure dietary intake in epidemiological studies. Among these are the 24-hour recall and dietary record methods. Both have drawbacks; although the 24-hour recall method is easily applied, it is prone to recall and response bias.[20] Dietary records usually provide more accurate intake data, but place a considerable burden on the participant.[20] Another problem with this method is that participants tend to deviate from their normal dietary pattern knowing that their intake is being actively recorded, thus potentially introducing substantial measurement inaccuracy. In this thesis, dietary assessment was performed using food frequency questionnaires (FFQs), a refinement of the dietary history

method developed by Burke.[12,21] FFQs measure habitual intake of foods over a longer period of time, can account for seasonal variation in consumption habits and are less burdensome to participants compared to dietary records. In addition, our FFQs were semiquantitative, meaning that portion sizes were recorded as well as consumption frequencies. Although exact consumption of foods cannot be accurately recorded using this method, for the purposes of examining the effects of dietary exposures FFQs are still able to rank participants according to intake satisfactorily.[22] Furthermore, our FFQs were specifically developed for and validated among Dutch populations.[23,24] However, FFQs are not a perfect measurement instrument. The exact amount of measurement error associated with the use of FFQs is difficult to quantify because there is no gold standard available for measuring diet in the preceding year with perfect or near-perfect accuracy. However, the FFQs that were used in the studies contained in this thesis have shown reasonable correlation with dietary records, circulating biomarkers (such as fatty acids) and urea excretion samples, indicating that their overall level of measurement error is most likely moderate to low.[23–25] More importantly, since most of our studies are longitudinal and assessment of dietary intake preceded measurements of the outcome, measurement error with regards to diet is likely to be non-differential: that is, unrelated to the outcome. In this situation, measures of association will generally be biased towards the null value.[12] Although measurement of dietary intake also suffers from the phenomenon that individuals with high intake tend to underreport their true intake whereas individuals with low intake tend to overreport their intake, which would in general inflate measures of association, the effect of random measurement error usually predominates and measures of association tend to be underestimated.[26–28] However, in a number of the studies I performed using body composition as an outcome, some degree of differential misclassification cannot be ruled out. This is because it has been demonstrated that obese individuals tend to underreport intake of specific foods compared to normal-weight individuals, which by extension may have impacted our estimation of measures such as antioxidant consumption or dietary AGE intake (chapters 2.1, 4.1-4.2).[29] In addition, investigating aspects of the diet in relation to a given outcome often requires that the confounding effect of total energy intake is taken into account. Several methods can be used to do this, among which are including total energy intake in a multivariable model together with the exposure of interest (chapter 2.1) and substituting the exposure for the residuals of a statistical model where the exposure was regressed on total energy intake (chapter 4.1-4.2).[30] However, both the exposure and total energy intake may have been measured with a degree of error and the two variables may therefore adopt part of each other's effect.[28] This phenomenon has been shown to introduce a small but measurable level of additional residual confounding to measures of association, regardless of the exact method used for total energy adjustment.[28] Finally, another

potential issue is that different FFQs were used between Rotterdam Study cohorts in many of our analyses (chapters 2.1-2.2, 4.1-4.2). This may have introduced some additional between-individual variation with regards to food intake resulting from measurement inaccuracy.

Measurement of body composition

In chapter 4 of this thesis, I have studied several dietary factors as potential determinants of body composition. Traditional measures of anthropometry which are wholly or partially reliant on total body weight, notably BMI, are limited by the fact that they are unable to distinguish between different contributors to total body weight. For instance, changes in BMI over time could result from changes in either fat mass (adipose tissue), fat-free mass (musculoskeletal tissues) or both. Such changes in body composition occur especially frequently in older individuals, who comprise a large number of Rotterdam Study participants.[31] The distinction between fat mass and fat-free mass is important given that their relative quantities have differential health effects.[32] In the studies presented here, body composition was assessed by means of dual X-ray absorptiometry (DXA) which is able to quantify fat mass and fat-free mass separately. This provides important additional insights compared to if only BMI would have been used as a measure of body composition. For instance, while I observed that higher dietary antioxidant consumption was associated with higher BMI, I was also able to demonstrate that this association was driven by higher fat-free mass rather than higher fat mass (chapter 4.1). Thus, I concluded that antioxidant consumption may have favorable effects on body composition by preserving muscle tissue in the elderly, rather than detrimental effects by increasing adiposity. This further highlights the notion that BMI alone is an inadequate measure of adiposity. Fat mass can also be further compartmentalized into subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). VAT is hormonally active, and higher quantities of VAT are associated with increased risk of a wide range of health conditions.[33] Although VAT quantity can also be approximated with anthropometric measures such as waist-to-hip ratio, these traditional measures generally provide inaccurate estimations of VAT.[33] Unfortunately, it is also not possible to directly distinguish between SAT and VAT using DXA, because DXA only provides two-dimensional images while subcutaneous and visceral fat tissue overlap each other in three-dimensional space.[34] In theory, VAT can be approximated from DXA measurements algorithmically, but this technique was not available for the purposes of the studies presented in this thesis.[35] However, I did incorporate information on the distribution of fat mass in the present studies in the form of android-to-gynoid fat ratio. Android fat is located around the truncal region whereas gynoid fat is located around the hips, and these two types of fat have differing effects on metabolic parameters.[36,37] It has also been demonstrated that

android fat mass as estimated by DXA is reasonably correlated with estimations of VAT obtained through computed tomography.[38] I demonstrated that higher consumption of antioxidants and lower dietary AGE consumption were associated with lower android-to-gynoid fat ratio (chapter 4.1, 4.3). This may provide some indication that these dietary parameters could affect visceral fat mass.

While DXA provides accurate assessment of body composition as has been demonstrated in validation studies, it is not a perfect instrument.[34] DXA measurements are inaccurate in individuals with very high BMI due to inherent surface area limitations of the device. In an attempt to mitigate the effects of this measurement error, I preemptively excluded morbidly obese individuals from analysis as discussed previously. Nevertheless, some systematic measurement inaccuracy will have persisted, especially among the more obese participants in our remaining study population, and could have introduced bias into the reported measures of association.[39] However, it is unlikely that the accuracy of DXA as a measurement instrument is affected by the dietary exposures that were studied. Therefore, in the context of our studies, this systematic measurement error will likely have resulted in a relatively limited degree of differential misclassification.

On generalization

Having discussed potential threats to the internal validity of the studies in this thesis, I will here provide some considerations on external validity, also referred to as generalizability. It could be argued that since nearly all of the findings in this thesis stem from analyses in population-based cohorts consisting of individuals from a delineated geographical location who share certain sociodemographic characteristics, generalizing our findings to other populations is not straightforward. For example, the vast majority of the participants in the Rotterdam Study is ethnically Dutch and elderly, characteristics which may not accurately describe any given population an investigator might want to generalize their findings to. In particular, in our research relating to antioxidant consumption and risk of type 2 diabetes (chapter 2.1), I report that participants included in the statistical analysis were significantly different from those participating in the Rotterdam Study cohort but excluded from analysis: those analyzed were generally older, less healthy and lower educated. A similar concern has also been raised in objection to previous findings from the UK Biobank (chapter 3.1-3.2), because individuals from this cohort self-selected into participation at a very low response rate (approximately 6%) and do therefore not represent an accurate probability sample of any particular source population.[16,40,41] For instance, it has been demonstrated that UK Biobank participants are generally less socio-economically deprived and have fewer self-reported health conditions compared to non-participants.[42]

As discussed previously, the most important prerequisite of the external validity of a study is its internal validity. In this regard, it must be acknowledged that the design of both the Rotterdam Study and the UK Biobank is prospective and that participant recruitment happened before the exposures and outcomes of interest were assessed. In this situation, selective non-participation at baseline will generally not threaten internal validity to a large extent. Moreover, especially with regards to the Rotterdam Study, the relative homogeneity of a population-based cohort should be regarded as a strength rather than a weakness. This is because the internal validity of the conclusions drawn from such samples is higher compared to when our study population would be a truly random sample of, for example, Dutch citizens. In such a less homogeneous sample, the degree of unmeasured confounding would generally be higher and attaining universally accurate measurements would be more challenging.[12] Comparing the relative generalizability of the Rotterdam Study and the UK Biobank, it should be noted that the study population of the Rotterdam Study is far more narrowly defined, including only individuals from one suburb in the Netherlands whereas the UK Biobank includes participants with a diverse age range from all over the United Kingdom. This makes findings from the Rotterdam Study less generalizable to other populations at first glance, but the circumscribed nature of the study population and high participation rate (generally about 70% from those invited agreed to participate) do ensure that this population is highly similar to the source population it aims to represent. Thus, measures of association derived from the Rotterdam Study are likely close to the true population values. In contrast, the study population of the UK Biobank is more diverse and thus arguably more representative of the general population of the entire United Kingdom, notwithstanding the issues with selective participation as described above. However, the higher amount of unmeasured confounding and between-individual variability in this study population, as previously mentioned, will make generalization of findings from the UK Biobank less straightforward.

While external validity of study results is clearly of great importance, rigorous internal comparisons should precede generalization, and concerns about sample representativeness should take priority only after it has been established that the reported measures of association are valid. Indeed, the process of generalization should not principally be informed by the degree to which two populations are spatially, temporally or demographically comparable, but by how strongly, if at all, these differences are expected to affect the associations that were observed in the source population. The latter consideration also largely depends on prior knowledge about biological processes and is not solely dependent on previous findings from epidemiology.[12] Population-based studies with a large sample size, such as the Rotterdam Study and especially the UK Biobank, also enable investigators to perform well-powered stratified

analyses and allow for analyses in predefined subpopulations. While such secondary analyses do not directly improve generalizability, they can provide clues as to whether differences between subpopulations exist and may thus, at least partially, inform the process of generalization. Finally, in clinical epidemiology, decisions on treatment regimens are often informed by randomized controlled trials, which generally consist of highly selected populations; yet findings from such studies are often widely applied in populations that do not reflect the trial populations closely. Assuming high internal validity, compelling evidence for an association may be widely generalizable and does not require high representativeness of a given study population.[43,44] However, no study is universally generalizable without any further consideration and thus multiple studies, themselves internally valid and ideally performed in different homogeneous populations, are generally needed to confirm whether a given association applies without exception. Still, for any individual study, it is imperative that efforts to reduce potential bias prevail over concerns regarding representativeness. Studies should strive to generalize highly internally valid estimates rather than potentially compromise internal validity for the sake of sample representativeness.

Implications and future directions

Despite the large amount of research that has been performed on the topic in the past decades, the prevalence of obesity and type 2 diabetes has continued to rise and is projected to increase even further in the coming years.[45–48] This emphasizes the need for an even better understanding of the determinants of these metabolic disorders and how these could be acted upon from a public health perspective. In the following section, I will reflect on the potential implications of the findings from this thesis and provide directions for future research.

Oxidative stress, defined as an imbalance between the production of reactive oxygen species and the capacity of antioxidant systems, is an important mechanism contributing to the pathophysiology of insulin resistance as well as eventual type 2 diabetes and its complications. [49] Obesity, which is in itself a major contributing factor to insulin resistance, is also closely interwoven with oxidative stress and may, in fact, be a consequence of increased oxidative stress levels.[50–52] Thus, from a disease prevention standpoint, lowering oxidative stress levels across populations may have favorable effects. This notion has led to the study of antioxidative compounds contained in the diet in relation to health.[53] The results from this thesis indicate that higher antioxidant consumption is associated with a more favorable body composition profile, lower insulin resistance and lower risk of type 2 diabetes (chapters 2.1, 4.1). Conversely, our results also indicate that consumption of AGEs, which may contribute to inflammation, is associated with a more unfavorable body composition profile and

higher probability of type 2 diabetes (chapter 4.2). The observed associations were independent of overall diet healthiness, indicating that the putative health effects of antioxidant and AGE consumption may occur regardless of established diet prudency. With regards to antioxidant consumption, our findings stand in apparent contrast a number of randomized trials that have investigated the health effects of antioxidant supplementation, and reported no clear benefits.[54–56] It could be that the beneficial effects of antioxidant supplementation only occur in those who are already deficient, or that these effects only become apparent after prolonged periods of supplementation. Antioxidant supplements generally also contain only several antioxidants in high doses and may thus not accurately replicate the antioxidant composition of the diet as a whole, in which individual antioxidants may synergize or interact with each other.[57] Future research into the potential benefits of antioxidant supplementation should attempt to address these methodological shortcomings. In this context, more research is also needed to increase our understanding of the effects of dietary antioxidants in tissue and how they interact with each other as well as with the body's innate antioxidant systems. With regards to dietary AGEs, our findings are in line with previous studies suggesting that lower dietary AGE consumption is associated with lower inflammation, lower insulin resistance and lower oxidative stress, suggesting that the potential health benefits of dietary AGE restriction might extend beyond the prevention of obesity alone.[58] However, most of these studies had small sample sizes available for analysis and follow-up, if available, was generally limited to short periods of time.[58] More high-quality research is needed into the health effects of dietary AGEs. Ideally, future studies should include direct measurements of food AGE contents as opposed to estimations based on database linkage, explicitly account for cooking methods in their analyses, investigate hard endpoints as opposed to biomarkers and allow for sufficient follow-up or duration of intervention. Nevertheless, the results presented here, coupled with those from previous studies, still provide an argument to place more emphasis on the role of dietary antioxidant and AGE consumption in health policy making. In line with the classic prevention paradigm devised by Rose in 1985, if diet could be ameliorated even by a small amount across an entire population, this could make a significant contribution to the prevention of obesity and type 2 diabetes on a population level even though the benefits on the individual level would be comparatively small.[59] Increased attention for the role of foods rich in antioxidants and low in AGEs in the design of dietary guidelines could provide an important first step towards this goal.

The need to improve diet quality in the general population remains pressing considering that overall adherence to dietary guidelines in the general population is far from optimal. Indeed, in the Rotterdam Study, average adherence to dietary guidelines

was previously shown to be only around seven on a fourteen-point scale – a number very similar to the average adherence I report in the studies presented in this thesis (chapters 2.1, 4.1, 4.2).[60] Especially striking with regards to these guidelines is the fact that only 12.8% of participants met the recommendation to consume less than 300 grams of red and processed meat per week.[60] Our research into plant versus animal based diets indicated that a relatively more plant-based and less animal-based diet has substantial health benefits with relation to insulin resistance and type 2 diabetes. These health benefits can occur not only by increasing consumption of plant-based foods, but also by decreasing consumption of meat and other animal-based foods. The low adherence to the Dutch meat consumption guideline indicates that there is still substantial room for improvement from a public health perspective in this regard. Substituting animal-based with plant-based foods will likely also contribute to higher antioxidant consumption, considering that fruits, vegetables and nuts are generally rich in antioxidants.[1] Similarly, it is plausible that this substitution would also lead to lower AGE consumption, considering that animal-based products high in fat and protein (notably beef, cheese, poultry, pork, fish and eggs) are generally rich in AGEs and especially prone to additional AGE formation when broiled, fried or roasted.[61,62] This provides additional rationale for modifying the diet to contain a higher proportion of plant-based foods. Substantial reductions in dietary AGE content can also be achieved by heating food for shorter periods of time, heating food at lower temperatures, using moist heat (boiling, poaching, steaming) instead of dry heat when preparing food and using margarine or oil as cooking fat as opposed to butter.[62] This implies that informing the public not only about consuming a more healthy plant-based, antioxidant-rich diet, but also about the detrimental effects of dry heating foods and encouraging the use of alternative cooking procedures, may potentially contribute to improving diet quality and the prevention of obesity and type 2 diabetes in the general population.

Given that composition of the diet may affect the aforementioned health outcomes through modulating systemic inflammation, an important subsequent step is to identify which inflammatory compounds are involved in this process. This might not only increase our understanding of the link between diet and health outcomes, but could also aid risk stratification in clinical practice and potentially inform clinical management decisions with regards to cardiometabolic disease. In this thesis, one of the inflammatory biomarkers I focused on was serum uric acid. It has been demonstrated that elevated serum uric acid is associated with higher levels of a large number of inflammatory markers, notably CRP, interleukin 6 and tumor necrosis factor alpha.[4–6] In this thesis, I demonstrated that higher serum uric acid is associated with higher risk of a wide range of cardiovascular events, as well as increased risk of all-cause mortality (chapter 3.3). These associations were particularly pronounced among women

and among those with established type 2 diabetes. This indicates that serum uric acid may have potential as a clinical biomarker which may inform cardiovascular risk assessment. I also demonstrated that higher serum acid is associated with prediabetes among healthy individuals, but not with risk of type 2 diabetes among those with prediabetes (chapter 3.1). Especially striking in light of the previously discussed findings is that this association was also found to be strongest among women. Likewise, previous literature has also reported marked differences in the strength of the association between serum uric acid and cardiovascular outcomes as well as insulin resistance and type 2 diabetes.[63–66] However, the biological etiology of these sex differences remains unclear. This highlights the need for future studies to investigate what causes the excess cardiometabolic disease risk imposed by hyperuricemia, or by the metabolic state that hyperuricemia represents, among women specifically. No consensus has been reached on whether high serum uric acid merely reflects an inflammatory state or acts as a causative agent for inflammation.[4] However, increasing evidence supports the hypothesis that uric acid may play a role in inducing inflammation by activating pro-inflammatory pathways, and that uric acid itself exhibits pro-oxidative properties under certain circumstances.[5,67,68] This notion is supported by previous studies which have suggested that administration of xanthine oxidase inhibitors, a class of pharmacologic agents that lower uric acid levels, could potentially play a role in lowering cardiovascular risk.[69–71] A recent study also provided evidence that low-dose colchicine, traditionally used to mitigate inflammation resulting from deposition of uric acid crystals, may have a place in the prevention of cardiovascular events because of its anti-inflammatory properties.[72] However, these previous studies generally did not focus on sex differences and did not examine type 2 diabetes patients, specifically. Considering our findings, further studies are warranted to investigate whether women and individuals with type 2 diabetes would indeed benefit particularly strongly from such pharmacologic prevention approaches.

Aside from serum uric acid, a marker which may promote inflammation, I have also investigated other biomarkers more directly involved in the inflammatory response, for example CRP, in relation to diet and health outcomes. This work expand upon previous studies which have reported associations between higher coffee consumption and lower risk of type 2 diabetes by demonstrating that this association is partly mediated by coffee-induced changes in biomarkers related to inflammation, notably C-reactive protein and adiponectin (chapter 3.2).[73–75] However, it remains unclear whether this mediation occurs due to a direct effect of these biomarkers or secondarily to more complex underlying pathways that are only partially reflected by the studied biomarkers. The latter appears more plausible, given the large amount of metabolic and inflammatory factors coffee is known to be associated with.[76] More-

over, the fraction of the effect that was mediated by the biomarkers, although statistically significant, was relatively small. This indicates that the beneficial effects of coffee consumption on the pathophysiology of type 2 diabetes are likely to be highly multifactorial, and that coffee-induced changes in the comparatively few markers of inflammation that I investigated might only represent part of a putative causal effect of coffee consumption on risk of type 2 diabetes. Further research is needed to unravel what other factors play a role in this association.

In summary, the findings from this thesis provide further insights into the complex relationship between diet, inflammation, body composition and type 2 diabetes. I provide evidence that consumption of antioxidants, preferably in the context of a diet relatively rich in plant-based foods, may have beneficial effects on risk of obesity and type 2 diabetes. I also explored the role of inflammation in the context of diet and adverse health outcomes, and suggest that serum levels of uric acid are associated with cardiovascular disease and type 2 diabetes. Future research may provide further grounds to adapt these findings to dietary guidelines and recommendations for clinical practice.

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