Early Life Determinants, Cognition, and Survival in Population-based Studies

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Determinanten in het vroege leven, cognitie en overleving in de algemene bevolking

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For my mother Zaib and my daughter Ariel

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Chapter 1

Introduction

Changes in global demographics have resulted in an increase in life expectancy, both in western populations and in populations in low and middle-income countries.[1] As people age, most will develop some degree of cognitive deterioration, probably due to the accumulation of diseases and subclinical biological changes and damage.[2] Indeed, with increasing life expectancy, cognitive decline became a major public health problem. The degree of age-related cognitive decline partly depends upon early cognitive development.[3] Despite the recognition that cognitive decline may be determined by early childhood cognitive development itself or by common risk or vulnerability factors, clinical research rarely focuses on how to determine and optimize the factors that constitute cognitive development in early life. Rather clinicians focus on preventing or, most commonly, try to treat risk factors that affect loss of cognitive function in late life. Against this background, further studies of environmental and pathophysiologic risk factors that are associated with cognition in young age as well as with cognition and survival in old age on a population level are needed.[4,5]

One way of approaching healthy cognitive ageing is to determine developmental factors in early life that can affect cognition later in life. We know that the development of cognitive function is influenced by many modifiable environmental factors.[6] A particular focus of infant research has been on the primary mode of nutrition i.e. breastfeeding. Breastfeeding has been linked to influence cognitive outcomes later in life.[7] From a biological point of view, several aspects of breastfeeding such as duration, exclusivity, and constitutional elements have been hypothesized to have influence on cognitive development of children.[8] Many other factors such as maternal IQ and socio-demographic status, however, have also been linked to both breastfeeding during infancy and cognitive function in childhood.[9] Previous lines of literature have reported mixed findings regarding the associations between breastfeeding with child cognitive outcomes.[7-9] Therefore, we aimed to investigate the associations between breastfeeding and child cognition in a large 21st century cohort study that generated prospectively collected breastfeeding data along with a variety of confounding variables that would reduce potential bias among these associations.

It is also hypothesized and demonstrated that the origins of cardiovascular diseases start early in life.[10] The accumulation of risk factors throughout the life course most probably predisposes to disease processes that lead to cognitive functional decline in old age.[11] Therefore, we investigated the associations between cardiovascular risk factors and cognition early in life as well as in old age. In two population-based cohorts, we tried to decipher whether the onset of cognitive decline may have early childhood origins.

A second radical approach to healthy cognitive function in old age is to determine the effect of different subjective measures on diseases that affect cognition. In clinical research, the use of subjectively assessed measures that may be predictive of health, onset of disease and survival remains largely unexplored.[12] Against the background of

the many acclaimed biomarkers studied in blood, tissue, imaging or genetic material, we studied subjective measures that may predict health outcomes. Subjective well-being may help disentangle the factors that affect health across the lifespan. In this context, we aimed to determine the association of self-reported variables on stroke and mortality .

A third approach to decipher factors related to healthy cognitive ageing is to systematically review existing body of literature. Such reviews can inform both practice and future research and may help identify gaps in literature and address areas where further research is needed. Throughout the available literature, there is a lack of consensus on certain epidemiological aspects of cognition in old age that makes it difficult to generalize findings of individual studies. One example is the concept of "cognitive reserve". Cognitive reserve is a phenomenon that determines the extent of pre-morbid intelligence. This reserve makes the cognitive functioning of some persons more resilient than others to disease.[13] Thus, we aimed to explore previous lines of literature on the assessment or measurement of cognitive reserve to reach a consensus on its operationalization in the general population. Another area of research that lacks consensus is the methodology used across published studies to estimate the burden of stroke in low- and middle-income countries.[14] Thus there is a need to accurately estimate the burden of stroke and establish standardized disease surveillance systems in order to implement preventive and rehabilitative strategies in these resource-poor settings.

AIMS OF THIS THESIS

The aims of this thesis are two-fold: 1) to extend existing knowledge on determinants of cognition in early life; 2) to determine factors that affect cognition and survival in late-life.

The studies presented in this thesis are imbedded within two population-based prospective studies in Rotterdam, the Netherlands. For research questions regarding the first aim, we used data from the Generation R Study,[15] a child cohort from foetal life onwards. To meet the second aim, we used data from the Rotterdam Study,[16] a cohort study among people of 45 years and older in the district of Ommoord.

OUTLINE OF THIS THESIS

In ***chapter 2.1***, we investigate the association of breastfeeding duration and exclusivity with non-verbal IQ in children. In ***chapter 2.2***, we explore whether arterial stiffness measured by carotid-femoral pulse wave velocity can affect child IQ or general cognition in an elderly population. In ***chapter 3.1*** we study subjective indicators of health and well-being as predictors of all-cause mortality among the elderly. In ***chapter 3.2*** we investigate the relation between subjective complaints about memory and incident stroke and whether these associations depend on the level of education. In ***chapter 4.1*** we systematically review reviews published on the concept of cognitive reserve and its operationalisation in the general population. In ***chapter 4.2*** we review and meta-analyse previous literature determining the incidence rate of stroke in low-and middle-income countries. In ***chapter 5***, the main findings of these studies are discussed, together with their methodological considerations, clinical implications and future directions for research.

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Chapter 2

Early life determinants and cognition


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Chapter 2.1

Breastfeeding duration and non-verbal IQ in children

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Journal of Epidemiology and Community Health. 2015 Aug;69(8):775-81.

ABSTRACT

Background Breastfeeding has been related to better cognitive development in children. However, due to methodological challenges such as confounding, recall bias or insufficient power, the mechanism and nature of the relation remains subject to debate.

Methods We included 3761 participants of a population-based cohort study from fetal life onwards and examined the association of breastfeeding duration (exclusive and partial continuously and in categorical-two month bands) with non-verbal intelligence in children of age 6 years. Maternal and paternal lifestyle, sociodemographic factors, child factors, and maternal IQ were tested for their confounding effects on the association.

Results We observed an initial association between breastfeeding duration and child IQ conferring an advantage of 0.32 (95% CI 0.20 - 0.44) points for each additional month of breastfeeding. This association strongly attenuated to 0.09 (95% CI -0.03 - 0.21) points after adjustment for child factors, socio-demographic factors, parental lifestyle factors, and maternal IQ. Similarly, the associations with breastfeeding duration as a categorical variable largely disappeared after confounding factors were added to the models.

Conclusions The association between breastfeeding and child IQ can be largely explained by sociodemographic factors, parental lifestyle and maternal IQ. Our results cannot confirm beneficial effects of breastfeeding on child intelligence.

INTRODUCTION

The World Health Organization (WHO) recommends six months of exclusive breastfeeding followed by partial breastfeeding until age two years for overall healthy growth and development of children.[6] The scientific evidence on the long-term benefits of breastfeeding for child cognitive development, however, offers mixed support and is still a subject of debate.

Several observational studies showed positive effects of breastfeeding on child IQ,[7,18-26] whereas others concluded that breastfeeding is a proxy for parenting and other cognitive factors in the home environment instead of a causal factor in itself.[27-32] However, the majority of published articles have important methodological issues, such as retrospective data collection of breastfeeding, risk of misclassification bias by measuring breastfeeding as a dichotomous variable (ever vs. never), small sample sizes or insufficient adjustment for critical potential confounders, of which maternal IQ is most important. The large cluster-randomized trial on breastfeeding promotion (PROBIT), which by design minimized effects of confounding, also found significant differences between the intervention group and the control group.[33] However, information bias due to non-blinding of clinicians assessing the cognitive outcomes is plausible and exclusion of mothers who decided not to initiate breastfeeding prior to trial inclusion limit generalizability to Western countries. Moreover, due to ethical constraints, this trial could only study the effect of a promoting intervention instead of measuring the effects of breastfeeding itself. Therefore, cognitive effects between groups could be driven by other factors, such as increased self-confidence and better sensitivity to infant cues in young mothers who received additional information in the early postpartum period.[34,35]

The hypothesized underlying mechanism to explain the association between breastfeeding and child intelligence centers around the biological components in breast milk, such as essential long-chain polyunsaturated fatty acids (LCPUFAs).[36] These LCPUFAs have been linked to optimal neurodevelopment in infancy due to accumulation in the cell membranes of the neurons.[37] Following this research, formula feeding is increasingly being fortified with LCPUFAs since the late 90s. One could hypothesize that the benefits for child cognition are less clear in studies performed afterwards, although randomized controlled trials did not find clear effects of supplementation on neurodevelopment in term infants.[38,39]

To fully address the subtle effects of breastfeeding on child IQ, there is a continuing need of large studies with sufficient power, prospective collection of breastfeeding data, including quantification of breastfeeding duration and exclusivity to allow detection of dose-response relationships, as well as data collection on important confounding variables including maternal IQ. Within the Generation R Study, a prospective population-based study from fetal life onwards, we aimed to further investigate whether

breastfeeding duration (either exclusive or partial) was associated with child cognitive development. This study was performed in a transition period of LCPUFAs fortification, in which about 50% of formulas were fortified. Participants in our study show high variability in ethnic background, which is why we focused on non-verbal intelligence. We hypothesized significant attenuation of the effect of breastfeeding on child non-verbal intelligence by confounding factors, most notably by maternal IQ.

METHODS

Study population

This study used data from the Generation R Study, a population-based cohort in Rotterdam, the Netherlands, designed to identify early determinants of growth, development and health.[16] In short, children were born between April 2002 and January 2006 from mothers who were enrolled in early pregnancy. For this study, we selected all mother-child dyads with available data on breastfeeding duration (N = 6,205). Valid information on child IQ was available in 3,761 (61%) children (Supplementary figure 1). Some mothers in our study participated with two (n = 470) or three (n = 12) children. Underestimation of standard errors could have resulted due to the clustered nature of data among siblings. However, since the number of surplus participants was small (2 siblings=235; 3 siblings=8) and the associations did not materially change after random exclusion of these siblings, they were included in the analysis. The study was conducted in accordance with the guidelines as proposed in the World Medical Association Declaration of Helsinki and was approved by the local Medical Ethics Committee. Written informed consent was obtained from all adult participants.

Breastfeeding

Information about initiation of breastfeeding was collected from delivery reports; data on continuation of breastfeeding was obtained from postal maternal self-report questionnaires at 2, 6 and 12 months postpartum.[40] Breastfeeding duration was defined as the age of the child (in months) at which breastfeeding was stopped completely. We used duration of any breastfeeding as a continuous variable. Also, we categorized breastfeeding duration as previously described in the literature into <2 months, 2-6 months, 6-10 months, and >10 months.[41] An approximation of exclusiveness of breastfeeding (i.e. the infant received no other milk or solids) was defined using information about the age at which other types of milk and/or solids were introduced. The information about duration and exclusiveness of breastfeeding was combined and grouped into the following 4 breastfeeding categories: (1) never; (2) partial for less than 4 months, not thereafter; (3) exclusive for 4 months, and partial thereafter. Since only 37 mothers in our cohort were

breastfeeding exclusively at 6 months we included them in group 3 (exclusive breastfeeding at 4 months). In order to differentiate between the determinants of breastfeeding initiation and duration, we also compared breastfeeding initiation to never breastfeeding and early weaning (<3months)[42] to extended breastfeeding (>3months). At age 6 months, most children were fed by LCPUFA-fortified brand milk products (in those children for which we had accurate brand name data, 61% were fed by fortified brands).

Nonverbal Intelligence

At age 6 years (mean age = 6.0 ± 0.4 years), children were invited at the Generation R research center. Non-verbal IQ was assessed using two subtests of a Dutch non-verbal IQ test: Snijders-Oomen Non-verbal Intelligence Test–Revised (SON-R 2½-7).[43] The two subsets we used were 'Mosaics', which taps into spatial visualization abilities, and 'Categories', which assesses abstract reasoning abilities (Mosaics: 15 items, Cronbach's α = 0.90; Categories: 15 items, Cronbach's α = 0.95). The raw test scores were converted into non-verbal IQ using norms tailored to exact age and sex.[43] The correlation of the IQ score derived by the mosaics and categories subsets and the IQ scores derived by the total test was high (r = 0.86).[44] Mean IQ was 102.6 (SD 14.7, range 50–150), which corresponds to the general population.[43] The study focused only on non-verbal IQ, due to the high heterogeneity on ethnic origin in the Generation R study sample. Verbal IQ-measures in children with different exposure to the Dutch language would have led to high variability in findings, which cannot be attributed to the main determinant in focus, but to their ethnic background.

Confounding variables

Confounders were selected on the basis of earlier studies on breastfeeding and child IQ.[45] We included information obtained from midwives and hospital registries on child gender, birth weight (in grams), gestational age at birth, and mode of delivery. Time spent in day care (< 8 or ≥8 hours/day) was obtained by questionnaires at infant's age of 6 and 12 months. Parity, maternal age, maternal BMI, parental ethnicity, maternal smoking during pregnancy, maternal alcohol intake during pregnancy, marital status, family income and parental education were assessed by various questionnaires during pregnancy and when children were aged 6 years. We classified children of different national origin on the basis of country of birth of the parents and grandparents into 'Dutch', 'Moroccan', 'Turkish', 'Antillean', 'Surinamese', 'Cape-Verdean', 'other western' and 'other non-western'. Both maternal and paternal educational levels were defined by the highest completed educational level and were classified into three categories: low, intermediate, and high. At 20 weeks of gestation, we used the Brief Symptom Inventory (BSI) to measure maternal psychopathology during pregnancy, from which a Global Severity Index (GSI) was derived.[46] Maternal employment was dichotomized as 'paid jobs' versus 'no jobs'.

Maternal intelligence was assessed during the accompanying child's visit to the research center at the age of 6 years using a computerized Ravens Advanced Progressive Matrices Test, set I. This set consists of 12 items and has been shown to be a reliable and valid short form of the Raven's Progressive Matrices to assess nonverbal cognitive ability parallel to child non-verbal IQ.[47] Family functioning was measured at 30 weeks of pregnancy using the Family Assessment Device, a 12-item self-report questionnaire.[48]

In mothers of Dutch origin, we ran additional analyses with further adjustment for maternal diet in early pregnancy, which is highly related to maternal diet in the postpartum period. Maternal nutritional intake in early pregnancy was assessed by using a validated semi-quantitative food frequency questionnaire (FFQ). Two dietary patterns "Mediterranean" and "Traditionally Dutch" have been extracted previously based on the FFQ consisting of 293 food items.[49] Presented scores represent the adherence to a particular dietary pattern corrected for total daily caloric intake by the mother.

Statistical analysis

We constructed multivariable linear regression models to assess the associations between duration of breastfeeding and child IQ and to examine the effect of confounders. We also added quadratic terms for breastfeeding duration to test for non-linear associations. Model 1 included child gender, birth weight, birth order, gestational age at birth, age of the child at testing and child's ethnicity. A series of three models were run adding a set of variables sequentially to model 1 after another. Model 2 included sociodemographic variables, i.e. time spent in childcare, ethnicity, maternal age, maternal BMI, household income, maternal employment, family functioning, maternal education and paternal education. To determine and demonstrate the specific change in effect size when adjusting for maternal intelligence, we added maternal IQ as a single variable in Model 3. Finally, maternal lifestyle factors including maternal smoking and alcohol intake during pregnancy and maternal psychopathology were added in Model 4. In additional analyses, we varied the order of covariate entry to evaluate whether the determined effect size changed.

We reran analyses comparing breastfeeding initiation to never breastfeeding and early weaning (<3months) to extended breastfeeding (>3months).To explore whether maternal diet affects the association between breastfeeding and child IQ, we ran additional analyses in children of mothers of Dutch national origin. Missing values for the confounding variables (0.1 – 32.9% missing data) were imputed by multiple imputation using chained equations in which 40 completed data sets were generated and analyzed by using the standard combination rules for multiple imputation. All levels of associations are presented with their 95% confidence intervals (CIs). Statistical analyses were conducted by using Stata13 (Stata Corp, College Station, TX).

Table 1. Study Population Characteristics of 3,761 Children with Data on Breastfeeding Duration and Available non-verbal IQ Scores in The Generation R Study, Rotterdam, The Netherlands.

	Any Breastfeeding					
	Never n=369 *9.8%*	<2 months n=965 *25.7%*	2-6 months n=1242 *33.0%*	6-10 months n=626 *16.6%*	>10 months n=559 *14.9%*	Group difference
Child Characteristics						
Gender (% girls)	52	51	51	51	53	
Cesarean section (%)	18	16	12	11	10	**
Mean birth weight (*g*)†	3374 (583)	3384 (565)	3420 (572)	3500 (549)	3493 (517)	**
Gestational age at birth (*weeks*)†	39.5 (1.8)	39.8 (1.8)	39.8 (1.9)	40.0 (1.7)	40.1 (1.5)	**
Prematurity (% preterm)	7	6	6	5	4	
Child daycare						
≥8 hours/week (%)	86	91	92	88	78	**
Child's age at testing in years †	6.1 (0.4)	6.1 (0.4)	6.0 (0.3)	6.0 (0.4)	6.0 (0.4)	*
Child IQ †	100 (13)	100 (15)	104 (15)	105 (15)	104 (15)	**
Child Ethnicity						
Dutch (%)	76	70	66	72	55	**
Maternal characteristics						
Maternal age at enrollment †	31 (4.8)	30 (5.0)	31 (4.5)	32 (4.4)	32 (4.7)	**
Parity (primipara) (%)	50	64	62	55	53	**
Maternal BMI (kg/m^2) †	26 (5.4)	25 (4.8)	24 (3.9)	24 (3.7)	24 (3.9)	**
Maternal smoking during pregnancy (%)	26	20	12	9	8	**
Maternal alcohol during pregnancy (%)	37	38	51	51	39	**
Maternal dietary pattern score						
Mediterranean †	-0.4 (1.0)	-0.2 (0.9)	0.1 (0.9)	0.2 (0.9)	0.4 (1.1)	**
Traditional Dutch†	0.1 (1.0)	-0.1 (1.0)	-0.1 (0.9)	-0.1 (1.0)	-0.3 (1.2)	**
Maternal psychopathology during pregnancy (GSI)‡	0.1 (0.1, 0.3)	0.2 (0.1, 0.4)	0.1 (0.1, 0.3)	0.1 (0.1, 0.3)	0.2 (0.1, 0.3)	*
Maternal IQ†	92 (13)	95 (14)	99 (14)	101 (14)	101 (15)	**
Socio-demographic characteristics						
Family Income in euro/month						
(% high)	68	65	79	77	64	**
Maternal employment (%)	76	80	83	81	74	**
Maternal educational (% high)	30	40	60	70	63	**
Mean family functioning (FAD score)‡	1.4 (1.2, 1.8)	1.5 (1.2, 1.9)	1.4 (1.1, 1.8)	1.3 (1.1, 1.8)	1.4 (1.2, 1.8)	**

Abbreviations: BMI-Body Mass Index; FAD-Family Assessment Device; GSI-Global Severity Index.

***Note*:** Values are percentages for categorical variables, †mean (*SD*) for continuous normally distributed variables and ‡ median (interquartile range) for continuous, non-normally distributed variables. Differences in maternal and child characteristics for the breastfeeding duration groups were evaluated using χ^2 tests for categorical variables, ANOVA for normally distributed continuous variables, and Kruskal-Wallis test for non-normally distributed continuous variables: *$P<0.05$, ** $P<0.01$

RESULTS

Non-response analyses

Missing data analyses were conducted to examine whether non-response was selective in our study population. Children with available data on the determinant but no IQ testing (n = 2,007) were compared to children included in the analysis (n = 3,761) (supplementary figure 1). The group of children with missing IQ-data were more often of non-Dutch origin (54% compared to 65%, $P < 0.001$). Their mothers were less likely to be primiparous (54.3% compared to 58.6%, $P < 0.05$), had lower family income (21.6% compared to 11.9%; $P < 0.001$), and lower education (11.1% compared to 5.8%, $P < 0.001$). No differences were found on maternal age, gender, mean birth weight and gestational age at birth.

Sample characteristics

Characteristics of the study population are presented in Table 1. The mean child age at which SON non-verbal tests were performed was 6 years. The mean duration of breastfeeding was 4.5 months (±3.9 months). Children who were never breastfed (9.8%), and their mothers, differed on almost every tested covariate from children who received any breastfeeding. Most mothers breastfed their children for 2-6 months (non-exclusively). Generally, mothers with the most advantageous psychosocial factors were in the group who breastfed their children for 6 - 10 months. Longer duration of breastfeeding, i.e. > 10 months, was associated with lower maternal IQ, lower family income and more psychopathology during pregnancy. Mothers in this category were less often of Dutch national origin and used less day care facilities than the majority of the sample. In total, 23.5% of all children were breastfed exclusively for 4 months.

Non-exclusive breastfeeding and child IQ

Table 2 shows the associations of breastfeeding duration with non-verbal IQ in 6 year old children. In the initial model, longer duration of breastfeeding was associated with 0.32 (95% CI 0.20 - 0.44) points higher IQ per month of breastfeeding. The results obtained from the analyses using breastfeeding duration as a categorical measure were in line with those obtained from the analyses using breastfeeding as a continuous variable. The largest effect of breastfeeding was seen for children breastfed for 6-10 months that corresponded to an advantage of 4.79 (95% CI 2.96 - 6.62) IQ points compared to children who were never breastfed. Each model conferred a substantial reduction in effect sizes. Adjustment for socio-demographic factors including parental education, family functioning and maternal employment reduced the advantage substantially, both on the categorical and the continuous scale. Further strong attenuation of associations was seen after adjustment for maternal IQ for all breastfeeding durations and breastfeeding

as a continuous variable. To test the full confounding effect of maternal intelligence, we re-ran model 1 with inclusion of maternal IQ. In this model, child IQ was 0.19 (95% CI 0.08 - 0.32) points higher per month extra duration of breastfeeding. Maternal intelligence reduced the effect by 41%, yet maternal age, maternal BMI, family functioning, and parental education were also important confounders (Supplementary Table 1).

Introducing a quadratic term for duration of breastfeeding in months did not significantly confirm a non-linear association between of breastfeeding duration and child IQ (*P*=0.46).

When we compared children whose mothers initiated breastfeeding (n = 3,392) to children that were never breastfed (n=369), the observed 3.10 (95% CI 1.56 – 4.63) IQ point advantage in unadjusted analyses reduced to 0.57 (95% CI -0.95 – 2.10) IQ points in model 4. Similarly, when comparing children with extended breastfeeding (n = 2,025) to early weaning (n = 1,367), the initially observed advantage of 2.50 (95% CI 1.51 – 3.49) IQ-points reduced to 0.77 (95% CI -0.24 – 1.78) IQ-points after full adjustment. Again, inclusion of sociodemographic characteristics and maternal IQ attenuated the effects to non-significant differences between groups.

Table 2. Associations of Breastfeeding Duration with IQ in Six Years Old Children in The Generation R Study, Rotterdam, The Netherlands.†

	Child IQ SON (*n* = 3761)								
Breastfeeding duration		Model 1		Model 2		Model 3		Model 4	
	N	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Categorical model									
Never	369	Reference		Reference		Reference		Reference	
<2 months	965	0.99 (-0.71, 2.70)	0.26	0.47 (-1.21, 2.16)	0.58	-0.01 (-1.69, 1.66)	0.99	-0.11 (-1.79, 1.56)	0.89
2-6 months	1242	3.63 (1.97, 5.28)	<0.001	1.92 (0.27, 3.58)	0.02	1.12 (-0.53, 2.77)	0.19	0.86 (-0.80, 2.51)	0.31
6-10 months	626	4.79 (2.96, 6.62)	<0.001	2.72 (0.88, 4.57)	0.004	1.82 (-0.02, 3.65)	0.05	1.52 (-0.32, 3.36)	0.10
>10 months	559	3.74 (1.85, 5.62)	<0.001	1.94 (0.03, 3.83)	0.04	0.82 (-1.08, 2.72)	0.40	0.58 (-1.32, 2.48)	0.55
Continuous model									
Breastfeeding duration (months)	3761	0.32 (0.20, 0.44)	<0.001	0.17 (0.05, 0.29)	0.004	0.11 (-0.01, 0.23)	0.08	0.09 (-0.03, 0.21)	0.14

***Note*: Using multiple linear regression models, values given as regression coefficients and 95% CI and *P* values**
† Models are adjusted for confounders as listed. Each variable is added in the following order:
Model 1: Adjusted for gender, birth weight, birth order, gestational age, child ethnicity, age of child at testing
Model 2: Model 1 + time spent in day care + age of mother at intake, BMI of mother at intake, family income, maternal employment, family functioning , maternal education, and paternal education
Model 3: Model 2 + maternal IQ
Model 4: Model 3 + maternal smoking during pregnancy + maternal alcohol during pregnancy + maternal psychopathology during pregnancy

Exclusive breastfeeding and child IQ

Table 3 shows the results of the analysis of children with data on exclusive breastfeeding. Children who were exclusively breastfed until 4 months had 4.21 (95% CI 2.47 - 5.95) IQ points advantage over children who were never breastfed. Adjusting for socio-demographic factors reduced this advantage to half; 2.07 (95% CI 0.31 - 3.82) points. Again, we observed that the association further attenuated after adjustment for maternal IQ. The effect of partial breastfeeding until age 4 months also reduced four-fold after adjustment for maternal IQ.

Table 3. Association of Exclusive Breastfeeding Duration with IQ in Six Years Old Children in The Generation R Study, Rotterdam, The Netherlands.†

	Child IQ SON (*n* = 3469)								
Exclusive breastfeeding duration		Model 1		Model 2		Model 3		Model 4	
	n	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Never	369	Reference		Reference		Reference		Reference	
Partial until 4 months	2247	2.53 (0.96, 4.09)	0.002	1.34 (-0.21, 2.90)	0.09	0.62 (-0.92, 2.17)	0.43	0.43 (-1.12, 1.97)	0.59
Exclusive for at least 4 months	853	4.21 (2.47, 5.95)	<0.001	2.07 (0.31, 3.82)	0.02	1.14 (-0.61, 2.89)	0.20	0.94 (-0.81, 2.70)	0.29
P for trend		<0.001		0.03		0.19		0.25	

***Note*: Using multiple linear regression models, values given as regression coefficients and 95% CI and *P* values**
† Models are adjusted for confounders as listed. Each variable is added in the following order:
Model 1: Adjusted for gender, birth weight, birth order, gestational age, child ethnicity, age of child at testing
Model 2: Model 1 + time spent in day care + age of mother at intake, BMI of mother at intake, family income, maternal employment, family functioning, maternal education and paternal education
Model 3: Model 2 + maternal IQ
Model 4: Model 3 + maternal smoking during pregnancy + maternal alcohol during pregnancy + maternal psychopathology during pregnancy

Confounding by maternal diet

We conducted additional analyses in children of mothers who were of Dutch national origin. The largest effect size of 6.20 (95% CI 3.98 - 8.41) IQ points was seen in children breastfed for 6-10 months which reduced to 2.43 (95% CI 0.18 - 4.69) points after combined adjustment for socio-demographic and maternal IQ. After further adjustment for maternal lifestyle and dietary factors, this effect changed to 2.24 (95% CI -0.01 - 4.50) points for children breastfed 6 – 10 months compared to children who were never breastfed (supplementary table 2). In the continuous models, we observed no evidence for an association between breastfeeding duration and child IQ after adjustment for socio-demographic confounders in exclusively breastfed children of mothers of Dutch national origin (supplementary table 3).

DISCUSSION

Overall, we found no evidence that longer duration of breastfeeding (non-exclusive, exclusive or partial) is associated with higher non-verbal IQ at age 6 years when confounding variables are appropriately taken into account. The most important confounders were maternal age, maternal BMI, parental education, family functioning, and maternal intelligence.

Our study adds knowledge to the existing body of literature on effects of breastfeeding practices on child cognitive development. In the last decade, many observational studies described significant associations between breastfeeding and child IQ,[18-26] but many other observational studies found full attenuation of the effect when adjusting for confounding variables.[27-32] Most studies with positive results, however, did not have maternal intelligence available[19-21,23-26] or chose to exclude maternal IQ because of high correlation with maternal education,[22] whereas studies that did include maternal IQ generally reported no association.[27,29,30] The only recent study that did account for maternal IQ and reported still significant effects of breastfeeding is the one from Belfort et al.[18] Our study in a nearly three times larger sample did not confirm these findings. The sufficiently large sample size could permit us to identify small and subtle effects of breastfeeding on child IQ and the role of confounders on the change of these small effect sizes.

Another methodological limitation of earlier studies is the potential recall bias due to retrospective assessment of breast feeding. Only a few studies, including ours, prospectively assessed breastfeeding at different time-points during the first year of life.[18,20-22,32] By doing this, we were able to look at breastfeeding from different perspectives (any, exclusive, partial, duration, and initiation). Results, however, were very similar, suggesting that breastfeeding initiation and breastfeeding duration share similar determinants and that the effect on cognitive outcome is attenuated by a similar set of potential confounding factors.

Whereas observational studies are hampered by the possibility of residual confounding, this bias is minimized in the single largest trial on human lactation (PROBIT).[33] In this study, despite showing an overall advantage in IQ, the cluster-adjusted mean differences in *non-verbal* IQ (block designs and matrices) were not significant, which is in line with our results. However, the PROBIT trial studies the beneficial effects of a promotion intervention rather than the beneficial (nutritional) effects of breastfeeding. Furthermore, the recruitment ended before formula milk was fortified with essential fatty acids.

In the present study, we also attempted to assess the role of maternal nutrition in the form of dietary patterns. The content of nutrients, including LCPUFAs, in breast milk is variable and depends on sources in the maternal diet.[50] Previous studies have looked at fish intake and maternal folate levels as markers of a "healthy diet" reporting beneficial

effects on child IQ and overall child cognitive and neurodevelopment.[18,51] We observed a small attenuation of 0.02 IQ points per month of breastfeeding after adjusting for maternal dietary patterns. However, interpretation of these results is limited by 1) the availability of maternal dietary patterns in mothers of Dutch origin only, 2) information collected in pregnancy and not during lactation and 3) lack of biological measures of nutrient contents in breast milk.

We observed that mothers with advantageous socio-demographical and psychosocial characteristics tend to breastfeed for longer periods, which is likely because these mothers are more aware of the potential beneficial effects of breastfeeding. Nevertheless, in the Netherlands, most mothers quit breastfeeding before their child reaches the age of 6 months. This is hypothesized to be due to the return to work of young mothers – the timing of which influences the duration of breastfeeding practices and which varies in Western countries.[52] This return to work, in the Netherlands on average 3 months after giving birth, is also associated to social class and leads to a reduction in breastfeeding frequency and duration.[53] In our sample, the group of mothers who continued (partial) breastfeeding for >10 months is indeed unique as they were the least likely to send their children to day care, were less often employed, had lower family income and were less likely to be of Dutch origin.

Limitations of the present study include selective attrition of families with low income and low educational level. This would lead to potential selection bias when the association between breastfeeding and child IQ in nonparticipating children differed, e.g. with respect to breastfeeding practices, IQ and effects of specific confounding variables. Furthermore, since these factors were more homogenous in children included in the study, this may have resulted in different effect sizes and different confounding effects. The use of self-report of breastfeeding without information on the exact number of weeks of duration is a second limitation. This could lead to misclassification of the exposure. Third, although our study included available information on a large number of confounders, the association of interest may further be influenced by other unmeasured factors, such as paternal intelligence. On the other hand, we may have overadjusted our associations by controlling for several interrelated socioeconomic and health-promoting factors.

In conclusion, the observed relationship between cumulative effect of longer duration of breastfeeding and child IQ is the result of confounding. Our results suggest that, in addition to socio-demographic factors, maternal IQ is the main confounder accounting for most of the associations observed between breastfeeding and child IQ regardless of the duration of breastfeeding and whether breastfeeding was conducted exclusively. Despite the proven benefits of breastfeeding for other child outcomes, it is unlikely that breastfeeding, by itself, directly effects child IQ.

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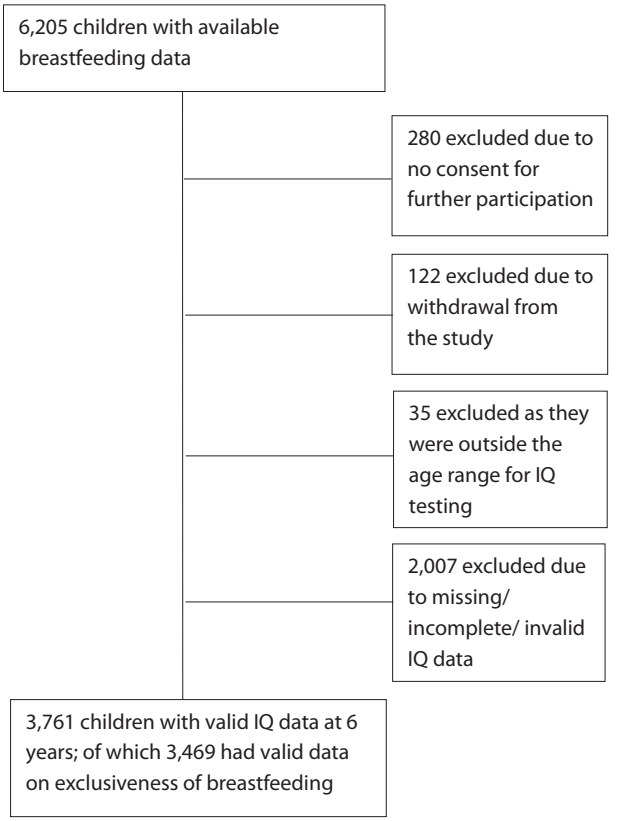
SUPPLEMENTARY MATERIAL

Supplementary figure 1. Flow chart of study population.



Supplementary table 1. The Effect of Confounders on the Associations of Any Breastfeeding Duration with IQ in Six Years Old Children of Dutch Mothers in The Generation R Study, Rotterdam, The Netherlands.†

	Child IQ SON (*n* = 3761)							
Breastfeeding duration	Model 1		Model 2		Model 3		Model 4	
	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Categorical model								
Never	Reference		Reference		Reference		Reference	
<2 months	0.99 (-0.71, 2.70)	0.26	0.47 (-1.21, 2.16)	0.58	-0.01 (-1.69, 1.66)	0.99	-0.11 (-1.79, 1.56)	0.89
2-6 months	3.63 (1.97, 5.28)	<0.001	1.92 (0.27, 3.58)	0.02	1.12 (-0.53, 2.77)	0.19	0.86 (-0.80, 2.51)	0.31
6-10 months	4.79 (2.96, 6.62)	<0.001	2.72 (0.88, 4.57)	0.004	1.82 (-0.02, 3.65)	0.05	1.52 (-0.32, 3.36)	0.10
>10 months	3.74 (1.85, 5.62)	<0.001	1.94 (0.03, 3.83)	0.04	0.82 (-1.08, 2.72)	0.40	0.58 (-1.32, 2.48)	0.55
Gender	-0.19 (-0.72, 0.73)	0.69	-0.06 (-0.97, 0.84)	0.89	-0.12 (-1.02, 0.77)	0.79	-0.19 (-1.08, 0.70)	0.68
Weight	0.002 (0.001, 0.003)	<0.001	0.002 (0.001, 0.003)	<0.001	0.002 (0.001, 0.003)	<0.001	0.002 (0.00, 0.003)	<0.001
Birth order								
First	Reference		Reference		Reference		Reference	
Second	-0.23 (-1.79, 1.33)	0.77	-1.04 (-2.59, 0.50)	0.18	-1.40 (-2.93, 0.13)	0.07	-1.39 (-2.92, 0.14)	0.08
Third	-7.11 (-16.40, 2.19)	0.13	-7.82 (-16.96, 1.31)	0.09	-8.02 (-17.12, 0.98)	0.08	-8.09 (-17.13, 0.94)	0.08
Gestational age at birth	0.05 (-0.28, 0.37)	0.77	0.003 (-0.32, 0.33)	0.99	-0.02 (-0.34, 0.30)	0.91	0.005 (-0.32, 0.33)	0.97
Child ethnicity								
Dutch	Reference		Reference		Reference		Reference	
Other Western	-1.84 (-3.43, -0.25)	0.02	-1.49 (-3.07, 0.09)	0.07	-1.40 (-2.97, 0.17)	0.08	-1.40 (-2.97, 0.16)	0.08
Turkish	-7.29 (-9.35, -5.23)	<0.001	-3.25 (-5.43, -1.06)	0.004	-2.14 (-4.32, 0.03)	0.05	-1.46 (-3.68, 0.76)	0.20
Moroccan	-10.61 (-12.95, -8.27)	<0.001	-7.05 (-9.48, -4.61)	<0.001	-5.15 (-7.61, -2.69)	<0.001	-4.93 (-7.42, -2.45)	<0.001
Antillean	-6.87 (-9.96, -3.78)	<0.001	-3.99 (-7.09, -0.89)	0.01	-3.70 (-6.77, -0.63)	0.02	-3.43 (-6.51, -0.36)	0.03
Surinamese	-7.49 (-9.42, -5.56)	<0.001	-4.91 (-6.89, -2.94)	<0.001	-3.98(-5.94, -2.02)	<0.001	-3.86 (-5.83, -1.89)	<0.001
Cape Verdean	-8.24 (-11.33, -5.14)	<0.001	-5.02 (-8.18, -1.86)	0.002	-3.19 (-6.34, -0.03)	0.04	-3.19 (-6.34, -0.03)	0.04

Supplementary table 1. (continued)

	Child IQ SON (*n* = 3761)							
Breastfeeding duration	Model 1		Model 2		Model 3		Model 4	
	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Other Non-western	-4.91 (-6.85, -2.97)	<0.001	-3.54(-5.50, -1.59)	<0.001	-2.81 (-4.76, -0.87)	0.005	-2.65 (-4.60, -0.71)	0.01
Age of child at testing	-2.16 (-3.38, -0.93)	0.001	-1.67 (-2.88, -0.46)	0.01	-1.49 (-2.69, -0.29)	0.02	-1.49 (-1.69, -0.30)	0.01
Time spent in daycare								
None			Reference		Reference		Reference	
<8 hours/week			2.30(-0.99, 5.60)	0.17	2.30 (-0.97, 5.56)	0.17	2.20 (-1.06, 5.46)	0.19
≥8 hours/week			2.58 (-0.06, 5.22)	0.06	2.39 (-0.23, 5.01)	0.07	2.36 (-0.25, 4.97)	0.08
Age of mother at intake			0.18 (0.07, 0.28)	0.001	0.18 (0.07, 0.28)	0.001	0.15 (0.05, 0.26)	0.004
Maternal BMI			-0.23 (-0.34, -0.12)	<0.001	-0.21 (-0.32, -0.09)	<0.001	-0.19 (-0.30, -0.08)	0.001
Family income								
Low income			Reference		Reference		Reference	
Middle income			1.66 (-0.24, 3.55)	0.09	1.28 (-0.61, 3.15)	0.19	1.29 (-0.59, 3.18)	0.18
High income			2.02 (0.29, 3.75)	0.02	1.55 (-0.17, 3.27)	0.08	1.42 (-0.32, 3.15)	0.11
Maternal employment			-0.90 (-1.95, 0.15)	0.09	-0.64 (-1.68, 0.40)	0.23	-0.51 (-1.55, 0.54)	0.34
Family functioning			-1.66 (-2.79, -0.53)	0.004	-1.40 (-2.53, -0.28)	0.02	-1.22 (-2.38, -0.06)	0.04
Maternal education								
Low education			Reference		Reference		Reference	
Intermediate education			2.64 (0.51, 4.77)	0.02	1.53 (-0.60, 3.65)	0.16	1.63 (-0.49, 3.76)	0.13
High education			4.73 (2.52, 6.94)	<0.001	2.83 (0.59, 5.07)	0.01	2.73 (0.49, 4.98)	0.02
Paternal education								
Low education			Reference		Reference		Reference	
Intermediate education			2.03 (-0.54, 4.59)	0.12	1.87 (-0.68, 4.41)	0.16	1.80 (-0.74, 4.34)	0.16
High education			3.68 (1.17, 6.18)	0.004	3.35 (0.87, 5.83)	0.01	3.07 (0.59, 5.56)	0.02

Supplementary table 1. (continued)

	Child IQ SON (*n* = 3761)							
Breastfeeding duration	Model 1		Model 2		Model 3		Model 4	
	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Maternal IQ					0.15 (0.12, 0.19)	<0.001	1.15 (0.11, 0.19)	<0.001
Maternal smoking during pregnancy							-1.00 (-1.57, -0.43)	0.001
Maternal alcohol during pregnancy							0.74 (0.18, 1.29)	0.01
Maternal psychopathology							-0.39 (-2.08, 1.30)	0.65

***Note*: Using multiple linear regression models, values given as regression coefficients and 95% CI and *P* values**

† Models are adjusted for confounders as listed. Each variable is added in the following order:

Model 1: Adjusted for gender, birth weight, birth order, gestational age, child ethnicity, age of child at testing

Model 2: Model 1 + time spent in day care + age of mother at intake, BMI of mother at intake, family income, maternal employment, family functioning , maternal education, and paternal education

Model 3: Model 2 + maternal IQ

Model 4: Model 3 + maternal smoking during pregnancy + maternal alcohol during pregnancy + maternal psychopathology during pregnancy

Supplementary table 2. Associations of Any Breastfeeding Duration with IQ in Six Years Old Children of Dutch Mothers in The Generation R Study, Rotterdam, The Netherlands.†

	Child IQ SON (n=2332)										
Breastfeeding duration		Model 1		Model 2		Model 3		Model 4		Model 5	
	n	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Never	256	Reference		Reference		Reference		Reference		Reference	
<2 months	544	1.30 (-0.82, 3.42)	0.23	0.30 (-1.79, 2.39)	0.78	-0.19 (-2.27, 1.89)	0.86	-0.13 (-2.20, -1.95)	0.91	-0.20 (-2.28, 1.87)	0.85
2-6 months	788	4.63 (2.61, 6.64)	<0.001	2.13 (0.10, 4.17)	0.04	1.33 (-0.70, 3.37)	0.20	1.34 (-0.70, 3.38)	0.20	1.18 (-0.85, 3.21)	0.26
6-10 months	420	6.20 (3.98, 8.41)	<0.001	3.40 (1.15, 5.66)	0.001	2.43 (0.18, 4.69)	0.04	2.47 (0.21, 4.73)	0.03	2.24 (-0.01, 4.50)	0.05
>10 months	324	3.77 (1.41, 6.12)	0.002	1.05 (-1.34, 3.44)	0.39	-0.21 (-2.61, 2.19)	0.86	-0.14 (-2.54, 2.26)	0.91	-0.37 (-2.77, 2.02)	0.76
Continuous breastfeeding duration (months)	3761	0.35 (0.20, 0.50)	<0.001	0.14 (-0.01, 0.30)	0.07	0.06 (-0.10, 0.22)	0.45	0.05 (-0.11, 0.20)	0.57	0.03 (-0.13, 0.18)	0.75

***Note*: Using multiple linear regression models values given as regression coefficients and 95% CI and *P* values**

† Models are adjusted for confounders as listed. Each variable is added in the following order:

Model 1: Adjusted for gender, birth weight, birth order, gestational age, child ethnicity, age of child at testing

Model 2: Model 1 + time spent in day care + age of mother at intake, BMI of mother at intake, family income, maternal employment, family functioning, maternal education, and paternal education

Model 3: Model 2 + maternal IQ

Model 4: Model 3 + maternal smoking during pregnancy + maternal alcohol during pregnancy + maternal psychopathology during pregnancy

Model 5: Model 4 + maternal dietary patterns

Supplementary table 3. Sensitivity Analysis of Associations of Exclusive Breastfeeding with IQ in Six Years Old Children of Dutch Mothers in The Generation R Study, Rotterdam, The Netherlands.†

		Child IQ SON (n=2147)								
Exclusive breastfeeding duration		Model 1		Model 2		Model 3		Model 4		Model 5	
		b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*	b (95% CI)	*P*
Never	256	Reference		Reference		Reference		Reference		Reference	
Partial until 4 months	1312	3.32 (1.40, 5.24)	0.001	1.35 (-0.57, 3.27)	0.17	0.62 (-1.29, 2.53)	0.52	0.52 (-1.39, 2.43)	0.59	0.43 (-1.49, 2.34)	0.66
Exclusive until 4 months	579	4.78 (2.67, 6.90)	<0.001	1.84 (-0.32, 4.01)	0.10	0.85 (-1.31, 3.00)	0.44	0.71 (-1.44, 2.86)	0.52	0.49 (-1.68, 2.65)	0.66
P for trend		<0.001		0.12		0.48		0.55		0.71	

***Note*: Using multiple linear regression models values given as regression coefficients and 95% CI and *P* values**

† Models are adjusted for confounders as listed. Each variable is added in the following order:

Model 1: Adjusted for gender, birth weight, birth order, gestational age, child ethnicity, age of child at testing

Model 2: Basic Model + time spent in day care + age of mother at intake, BMI of mother at intake, family income, maternal employment, family functioning, maternal education, and paternal education

Model 3: Model 1 + maternal IQ

Model 4: Model 2 + maternal smoking during pregnancy + maternal alcohol during pregnancy + maternal psychopathology during pregnancy

Model 5: Model 3 + maternal dietary patterns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Chapter 2.2

The association of arterial stiffness with cognition in young and old

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ABSTRACT

Background Several markers of subclinical vascular damage affect cognitive function in elderly. Arterial stiffness is one such marker. However, it remains unknown whether arterial stiffness affects cognition already in early life.

Methods We examined the relation of carotid-femoral pulse-wave velocity (a measure of arterial stiffness), systolic and diastolic blood pressures with cognitive function in 4634 children from the Generation R Study (mean age 6.2 years, 50.7% female) as well as 5230 elderly from the Rotterdam Study (mean age 61.8 years, 57.2% female). All participants underwent pulse-wave velocity measurement using ultrasound. In children, we assessed non-verbal IQ, while in the elderly, a cognitive test battery was employed that yielded the g-factor, a standardized measure of global cognitive functioning. Linear regression analysis was used to assess the associations of pulse-wave velocity, systolic and diastolic blood pressures with non-verbal IQ in children and g-factor in elderly.

Results In children pulse-wave velocity, systolic and diastolic blood pressures were not significantly associated with child IQ after adjustment for child, maternal and sociodemographic characteristics. In elderly, each standard deviation increase in pulse-wave velocity and systolic blood pressure were significantly associated with 0.04 (95% CI -0.072 - -0.008) decrease in g-factor after adjustment for age, gender, education, BMI, smoking, diabetes, and blood pressure lowering medication. These associations were primarily driven by participants younger than 75 years. Diastolic blood pressure was not related to g-factor in the elderly.

Conclusions The association of arterial stiffness with cognitive function is not evident in early childhood. Its accumulation throughout the ageing process may be responsible for cognitive changes in old age.

INTRODUCTION

Vascular damage plays a role in cognitive decline in old age. Previous studies have identified several vascular risk factors that are related to poor cognitive function in adults and elderly.[1] From the various vascular risk factors, arterial stiffness is of particular interest because it has been linked to cognitive impairment independently of traditional vascular risk factors.[2,3] This could be due to structural and functional changes of the vessel walls in the brain that are a result of increased arterial stiffness.[3] Arterial stiffness is also related to increased blood pressure that adversely affects the brain microcirculation.[4,5] A previous study has shown that increased blood pressure is inversely related to cognitive function in old age but these associations differed across age groups.[6]

There is also evidence that vascular damage actually starts accumulating very early in life.[7] Whether early life vascular influences affect late-life health, particularly cognitive health has been understudied. Thus, we aimed to investigate the relation of subclinical vascular risk factors with cognitive function in early life that can give a clue about vascular pathology that may be associated with cognitive decline in late life. To this aim, we studied the associations of arterial stiffness, systolic and diastolic blood pressures with cognition in children and in the elderly.

METHODS

Study population

Generation R Study

The Generation R Study is a population-based cohort in Rotterdam, the Netherlands, designed to identify early determinants of growth, development and health.[8] In short, children were born between April 2002 and January 2006 from mothers who were enrolled in early pregnancy. For this study, we selected 4634 children with available data on pulse wave velocity, systolic blood pressure, diastolic blood pressure and child IQ that were included in the analysis. The study was conducted in accordance with the guidelines as proposed in the World Medical Association Declaration of Helsinki and was approved by the local Medical Ethics Committee. Written informed consent was obtained from all adult guardians of the participants.

Rotterdam Study

The Rotterdam Study is a large prospective population-based cohort that started in 1990 among inhabitants aged ≥55 years residing in a district of Rotterdam, the Netherlands (N=7,983). An expansion of the cohort took place in year 2000, consisting of 3,011 par-

ticipants who had become 55 years of age or moved into the study district since the start of the study. In 2006, a further extension of the cohort was initiated in which 3,932 subjects were included, aged 45–54 years, out of 6,057 invited, living in the Ommoord district.[9] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants. For the present study, 5235 participants with complete data on pulse wave velocity, systolic blood pressure, diastolic blood pressure, and g-factor were included in this analysis. Aortic and common carotid artery stiffness were measured during the third examination phase of the first cohort (RS I-3); first examination phase of the second cohort (RS II-1) and the first examination phase of the third cohort (RS III-1);g-factor was measured during the fourth examination phase of the first cohort (RS I-4); second examination phase of the second cohort (RS II-2) and the first examination phase of the third cohort (RS III-1).[9] Blood pressure measurements were taken prior to the assessment of carotid-femoral PWV during the same visit. Participants with prevalent dementia (n=527) were excluded at the time of arterial stiffness measurement from the analyses.

Measurement of blood pressure and carotid femoral pulse-wave velocity

Generation R Study

We measured blood pressure with the child in supine position quietly awake. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the right brachial artery, 4 times with 1-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus (Paramus, NJ).[10] A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference. More than 90% of the children who visited the research center had 4 successful blood pressure measurements available. SBP and DBP were determined by excluding the first measurement and averaging the other measurements. Carotid–femoral pulse wave velocity, the reference method to assess aortic stiffness,[11] was assessed using the automatic Complior device (Artech Medical, Pantin, France) with participants in supine position. The distance between the recording sites at the carotid (proximal) and femoral (distal) artery was measured over the surface of the body to the nearest centimeter. Through piezoelectric sensors placed on the skin, the device collected signals to assess the time delay between the pressure upstrokes in the carotid artery and the femoral artery. Carotid–femoral pulse wave velocity was calculated as the ratio of the distance travelled by the pulse wave and the time delay between the feet of the carotid and femoral pressure waveforms, as expressed in meters per second.[12] To cover a complete respiratory cycle, the mean of at least 10 consecutive pressure waveforms was

used in the analyses. Recently, it has been shown that pulse wave velocity can be measured reliably with good reproducibility in a large pediatric population-based cohort.[13]

Rotterdam Study

Aortic and common carotid artery stiffness were measured with participants in supine position. Before measurement of PWV, blood pressure was measured twice with a sphygmomanometer after 5 minutes of rest, and the mean was taken as the subject's reading. Carotid-femoral PWV was assessed with an automatic device (Complior, Colson)[12] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery. The distance traveled by the pulse wave between the carotid artery and the femoral artery was measured over the surface of the body with a tape measure. PWV was calculated as the ratio between the distance traveled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses.

Intelligence

Nonverbal IQ in children

At age 6 years (mean age = 6.0 ± 0.4 years), children were invited at the Generation R research center. Non-verbal IQ was assessed using two subtests of a Dutch non-verbal IQ test: Snijders-Oomen Non-verbal Intelligence Test–Revised (SON-R 2½-7).[14] The two subsets we used were 'Mosaics', which taps into spatial visualization abilities, and 'Categories', which assesses abstract reasoning abilities (Mosaics: 15 items, Cronbach's $\alpha = 0.90$; Categories: 15 items, Cronbach's $\alpha = 0.95$). The raw test scores were converted into non-verbal IQ using norms tailored to exact age and sex.[14] The correlation of the IQ score derived by the mosaics and categories subsets and the IQ scores derived by the total test was high ($r = 0.86$).[15] Mean IQ was 101.0 (SD 15.0, range 50–150), which corresponds to the general population.[14] The study focused only on non-verbal IQ, due to the high heterogeneity on ethnic origin in the Generation R study sample. Verbal IQ-measures in children with different exposure to the Dutch language would have led to high variability in findings, which cannot be attributed to the main determinant in focus, but to their ethnic background.

g-factor in elderly[16]

Cognition can be studied by utilizing tests that determine cognitive functioning in a variety of cognitive domains. However, it is equally important to study global cognition that consists of a general underlying construct which is not dependent on any specific domain and reflects an individual's general cognitive function. This construct is linked to

intelligence and can be quantified as a general cognitive factor, or g-factor.[16] To calculate a g-factor, we performed a principal component analysis incorporating color-word interference subtask of the Stroop test, LDST, verbal fluency test, delayed recall score of the 15-WLT, and Purdue pegboard test. For tests with multiple subtasks we chose only one subtask in order to prevent highly correlated tasks distorting the factor loadings. Principal component analysis was performed on complete case data of 5,235 persons. The g-factor was identified as the first unrotated component of the principal component analysis and explained 49.2% of all variance in the cognitive tests. This is a typical amount of variance accounted for by the g-factor. The g-factor is thus a stable concept, comprising the shared variance between cognitive tests, and can be interpreted as a common underlying factor to a variety of cognitive domains.

Measurement of Covariates

Generation R Study

We included information obtained from midwives and hospital registries on child gender, birth weight (in grams) and complications during delivery. Weight of the child was measured while the child was wearing lightweight clothes and without shoes by using a mechanical personal scale (SECA, Birmingham, United Kingdom), and height was measured by a Harpenden stadiometer (Holtain Limited, Dyfed, United Kingdom) in standing position; both the scale and stadiometer were calibrated on a regular basis. BMI was calculated using the formula; weight (Kg)/height $(m)^2$. Maternal age, maternal smoking during pregnancy and maternal education were assessed by various questionnaires during pregnancy. Maternal height was measured during visits at our research center. On the basis of height and pre-pregnancy weight, we calculated pre-pregnancy BMI (weight (Kg)/height $(m)^2$). Maternal educational level was defined by the highest completed educational level and was classified into three categories: low, intermediate, and high. Mean arterial pressure (MAP) was calculated by the following formula: diastolic blood pressure+1/3×(systolic blood pressure – diastolic blood pressure).

Rotterdam Study

We used covariates measured at baseline of each examination round. Smoking status and information on the number of cigarettes smoked per day in each decade of life was obtained by a self-administered questionnaire during the home interview. Smoking status was characterized as never, past and current smoking. Participants were also invited to visit the research centre for clinical examinations and laboratory assessments. Body mass index (BMI) was measured using weight in kilograms divided by the square of height in meters. Diabetes mellitus type-2 was defined as having a fasting glucose level of ≥7.0 mmol/L or using blood glucose–lowering medication. Data on indication

for use of blood pressure-lowering medication were based on information collected by a physician at the research centre. Mean arterial pressure (MAP) was calculated by the following formula: diastolic blood pressure+1/3×(systolic blood pressure—diastolic blood pressure).

Statistical analysis

We constructed multivariable linear regression models to assess the associations between arterial stiffness and IQ in children and g-factor in elderly. In children, Model 1 included child age, gender, birth weight, and BMI. Model 2 included the following maternal characteristics: education, BMI, smoking during pregnancy, diabetes and use of blood pressure lowering medication. For the models with arterial stiffness as a determinant, additional adjustments were made for mean arterial pressure, heart rate and ultrasound device in Model 2.

In the elderly population within the Rotterdam Study, all models were adjusted for age, gender and cohort. Since the exposure and outcome were measured on two separate examination rounds in the first (PWV in RS I-3 and RS II-1) and second (g-factor in RS I-4 and RS II-2) cohorts, we additionally adjusted for the time interval time between exposure and outcome measurements in Model 1. We subsequently adjusted for educational level, BMI, Smoking status, diabetes mellitus type-2, and, blood pressure lowering medication in Model 2. For the models with arterial stiffness as a determinant, additional adjustments were made for mean arterial pressure, and heart rate in Model 2.

To investigate whether very old age affected the association between arterial stiffness and blood pressure with g-factor, we performed a subgroup analysis in strata of age (cut-off of 75 years).

Missing values for the confounding variables (0.1–16.2% missing data in Generation R Study and 0.6–1.9% missing data in the Rotterdam Study) were imputed by multiple imputation using chained equations in which 40 completed data sets were generated and analyzed by using the standard combination rules for multiple imputation. All levels of associations are presented with their 95% confidence intervals (CIs). Statistical analyses were conducted by using Stata13 (Stata Corp, College Station, TX).

Results

Generation R study

Characteristics of the study populations are presented in Table 1. The mean child age at which SON non-verbal tests were performed was 6.2 (0.5) years. The mean carotid-femoral pulse wave velocity was 5.5 (0.9) m/s, the mean systolic blood pressure was 102.7 (8.3) mm Hg and the mean diastolic pressure was 60.8 (6.8) mmHg. He average child IQ was 101.0 (15.0) points. More than half of the children's mothers were Dutch in

origin. The average maternal age at the time of delivery was 30.6 (5.1) years. 47% of the maternal population was highly educated and their average IQ was 95.7 (15.3) points.

Table 1: Baseline characteristics of the Generation R study population

Study population characteristics	**Participants**
Generation R Study	**N=4634**
Child characteristics	
Gestational age at birth, weeks	39.8 (1.9)
Birth weight, g	3399.6 (573.3)
Gender, n (% female)	2348 (50.7%)
Age at outcome, years	6.2 (0.5)
BMI, kg/m^2	16.2 (1.8)
IQ child	101.0 (15.0)
Systolic blood pressure, mmHg	102.7 (8.3)
Diastolic blood pressure, mmHg	60.8 (6.8)
Carotid-femoral pulse wave velocity, m/s	5.5 (0.9)
Heart rate, beats/min	82.2 (11.9)
Mean arterial pressure, mmHg	88.7 (7.1)
Maternal characteristics	
Ethnicity, n (%)Dutch	2459 (54.4)
Maternal age, years	30.6 (5.1)
Maternal BMI, kg/m^2	24.8 (4.4)
Maternal smoking during pregnancy, n (%)	650 (16.3%)
Maternal education	
Low, n (%)	384 (9.1%)
Intermediate, n (%)	1849 (43.9%)
High, n (%)	1981 (47.0%)
Preeclampsia, n (%)	83 (2.1%)
Maternal IQ	95.7 (15.3)

Values are means (standard deviation) or number of participants (percentage).

Table 2 shows the associations of carotid-femoral PWV, systolic and diastolic blood pressures with non-verbal IQ in 6 year old children. In the initial model, adjusted for child characteristics that included child age, gender, birth weight, and BMI, each standard deviation increased in diastolic blood pressure was associated with -0.43 (95% CI -0.85 - -0.02) point decrease child IQ. A strong attenuation of effect sizes to non-significant results were observed for all determinants after adjusting for maternal characteristics in the fully-adjusted model. Ethnicity, maternal BMI, maternal education and continuation of smoking during pregnancy were important covariates.

Table 2: Association between z-scores of blood pressure, pulse wave velocity and child IQ at age 6 years (Age range 4.8 – 9.1 years)

	N	β (95% CI)	*P* value	β (95% CI)	*P* value
		Model 1		Model 2	
Carotid-femoral pulse wave velocity†	4634	-0.182 (-0.600, 0.236)	0.393	-0.167 (-0.578, 0.245)	0.427
Systolic blood pressure	4634	-0.371 (-0.799, 0.057)	0.089	-0.225 (-0.647, 0.197)	0.296
Diastolic blood pressure	4634	-0.434 (-0.850, -0.018)	0.041	-0.295 (-0.706, 0.116)	0.159

N, number of persons in the total population; CI, confidence interval
Model 1: Adjusted for child characteristics: child age at outcome, gender, birth weight, and child BMI at outcome
Model 2: Model 1 + maternal characteristics: ethnicity, maternal BMI, maternal smoking, maternal education, and pre-eclampsia
†Additionally adjusted for heart rate and mean arterial pressure and ultrasound device

Rotterdam Study

Characteristics of the study population are presented in Table 3. The mean age was 61.8 (7.0) years, with 18.5% who had attained a high level of education. The mean carotid-

Table 3: Baseline characteristics of the Rotterdam Study population

Study population characteristics	**Participants**
Rotterdam Study (RS-I-3, II-1& III-1)	**N=5230**
Elderly characteristics	
Age, years	61.8 (7.0)
Gender, n (% female)	2991 (57.2%)
BMI, kg/m^2	27.1 (4.0)
Smoking	
Never, n (%)	1611 (31.1%)
Current, n (%)	1013 (19.5%)
Past, n (%)	2560 (49.4%)
Education	
Low, n (%)	499 (9.7%)
Intermediate, n (%)	3702 (71.8%)
High, n (%)	955 (18.5%)
Diabetes, n (%)	503 (9.7%)
Systolic blood pressure, mmHg	143.7 (21.5)
Diastolic blood pressure, mmHg	83.5 (10.4)
Blood pressure lowering medication, n (% yes)	1544 (30.1%)
Carotid-femoral pulse wave velocity, m/s	11.6 (3.0)
Heart rate, beats/min	71.5 (12.9)
Mean arterial pressure, mmHg	103.5 (13.0)

Values are means (standard deviation) or number of participants (percentage).

femoral pulse wave velocity was 11.6 (3.0) m/s, the mean systolic blood pressure was 143.7 (21.5) mm Hg and the mean diastolic pressure was 83.5 (10.4) mmHg.

Table 4 shows the associations of z-scores of carotid-femoral PWV, systolic and diastolic blood pressures with g-factor in the elderly population. In the initial model, adjusted for age, gender, cohort and time difference between exposure and outcome measurements, one standard deviation increase in carotid–femoral PWV was associated with -0.07 (95% CI -0.10 - -0.04) point decrease in g-factor and one standard deviation increase in systolic blood pressure was associated with -0.06 (95% CI -0.09 - 0.04) point decrease in g-factor. Diastolic blood pressure was not significantly associated with g-factor. The association between PWV and systolic blood pressure with g-factor did not change significantly after adjustment for educational status, BMI, smoking status, prevalent diabetes and blood pressure lowering medication (β -0.04 (95% CI -0.07 - -0.01) and β -0.04 (95% CI -0.06 - -0.02) respectively) in the fully-adjusted model.

After we stratified our study sample by age (cut-off 75 years), we observed significant associations between PWV (β 0.04 (95% CI -0.08 - -0.01)) and systolic blood pressure (β 0.04 (95% CI -0.07 - -0.02)) with g-factor in participants aged less than 75 years in the fully-adjusted model. No significant associations were observed in persons >75 years.

Table 4: Association between z-scores of blood pressure, pulse wave velocity and g-factor in the Rotterdam study (I-3, II-1& III-1) (Age range: 45.7-93.9)

	N	β (95% CI)	*P* value	β (95% CI)	*P* value
		Model 1		Model 2	
Carotid-femoral pulse wave velocity†	5230	-0.068 (-0.098, -0.038)	<0.001	-0.040 (-0.072, -0.008)	0.013
<75 years	4955	-0.074 (-0.106, -0.043)	<0.001	-0.044 (-0.077, -0.010)	0.011
≥75 years	275	-0.006 (-0.107, 0.096)	0.912	-0.002 (-0.109, 0.105)	0.972
Systolic blood pressure	5230	-0.061 (-0.086, -0.037)	<0.001	-0.039 (-0.064, -0.015)	0.002
<75 years	4955	-0.063 (-0.088, -0.038)	<0.001	-0.042 (-0.067, -0.017)	0.001
≥75 years	275	-0.033 (-0.147, 0.081)	0.568	0.001 (-0.113, 0.115)	0.987
Diastolic blood pressure	5230	-0.022 (-0.045, -0.001)	0.059	-0.007 (-0.030, 0.016)	0.557
<75 years	4955	-0.025 (-0.048, -0.001)	0.041	-0.009 (-0.033, 0.014)	0.437
≥75 years	275	0.005 (-1.104, 0.095)	0.926	0.016 (-0.083, 0.116)	0.744

N, number of persons in the total population; CI, confidence interval
Model 1: Adjusted for age, gender, cohort and time difference between exposure and outcome measurements
Model 2: Model 1 + education, BMI, smoking, diabetes, and blood pressure lowering medication
†additionally adjusted for heart rate and mean arterial pressure in Model 2

DISCUSSION

In this large prospective cohort study comprising of two populations at both extremes of the life-span, we found that arterial stiffness, systolic and diastolic blood pressures are not related to non-verbal IQ in children of age 6 years. While in the elderly, we found that increase in arterial stiffness and systolic blood pressure was associated with decline in g-factor. Persons aged less than 75 years primarily governed these findings. Diastolic blood pressure was not associated with g-factor in the elderly.

Arterial stiffness is considered one of the earliest detectable marker of structural and functional changes in the vessel wall that may precede cardiovascular injury.[17] Previous studies have shown that arterial stiffness is not only an independent marker of cardiovascular disease but also a strong predictor of cognitive impairment.[18-20] Previous studies have also found significant inverse associations between arterial stiffness and cognitive function in the elderly.[18,21,22] The possible mechanism in the explanation of the association between arterial stiffness and cognitive impairment is direct insult to distal vasculature in the brain due to decreased wave-reflection between aorta and carotids and increased transmission of excessive pulsatility into the brain.[23] There is also evidence that increased arterial stiffness also affects the integrity of the brain microvasculature that may cause insufficiency in cerebral perfusion, accumulation of which combined with reduced flow to the brain parenchyma may result in microvascular ischemia that may lead to cognitive impairment.[24] In the present study, the association between arterial stiffness and g-factor differs across age groups with a cut-off of 75 years, above which there is no association between arterial stiffness and g-factor. One explanation of these findings could be that stiffening of arteries reaches a maximum in very old age in order to maintain adequate cerebral perfusion to sustain life. As a result, the lack of further decline in perfusion may just be enough to preserve cognitive function at a standstill without any further deterioration.

We also observed that systolic blood pressure was associated with decline in g-factor in the elderly. This could be because arterial stiffness may result in lower vascular distensibility that may be a predecessor of systolic hypertension.[25] Another explanation is that the relation between arterial stiffness and blood pressure that affects cognition may be bidirectional; Long term elevated blood pressure that start in young adults can lead to vascular remodeling with structural changes in the vessel walls that result in their stiffening.[25] One study also showed that the relationship between blood pressure and cognitive function is highly dependent on age.[6] In this study hypertensive persons <75 years old showed cognitive decline later in life. On the contrary, persons >75 years who had elevated blood pressure had better cognitive outcomes. Therefore, slightly elevated blood pressure, maintained in the pre-hypertensive range has been shown to be beneficial for cognition in persons older than 75 years.[26] Although in our study, the effect

size of the association between systolic blood pressure and g-factor among ≥75 year old persons is very small and not significant in the fully-adjusted model; the inverse nature of it may indicate that higher systolic blood pressure is required to maintain perfusion pressure in very old persons in order to preserve cognitive function.

A major strength of our study is the large sample size in a population-based setting of both child and adult populations. In this study we combined several executive function tests that accounts for a global measure of cognition in various cognitive domains instead of using cognitive tests separately.[3] A limitation of our study is that very old persons with advanced cognitive impairment were not capable of participation in the study. Therefore, selective attrition may have influenced our results. In children, we measured non-verbal IQ owing to the high diversity in ethnic background. However, in elderly, the g-factor included verbal fluency test. Another limitation is that there is no data available on participants of age ranging from 6 years to 45 years. Future studies should explore the relation between arterial stiffness, blood pressure and cognitive function through the life course by including participants of all ages.

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Chapter 3

Subjective health and survival


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Chapter 3.1

Subjective well-being and all-cause mortality.

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ABSTRACT

Background Subjective indicators of various aspects of well-being have been related to mortality, but their independent relation remains unknown. We aimed to determine which aspect of subjective well-being is associated with mortality. To this aim, we tested the independent relation of various subjective well-being measures with all-cause mortality.

Methods We studied the relation of subjective well-being with all-cause mortality. Subjective well-being was measured by various instruments that included basic activities of daily living (BADL), instrumental activities of daily living (IADL), quality of life (QoL), somatic symptoms, positive affect, and negative affect. Participants were evaluated for each measure of subjective well-being and followed for mortality for a mean of 12.2 years (SE=0.09). Cox regression analysis was conducted in the total sample.

Results In this cohort, 2021 persons died during 48,534 person-years of follow-up. All measures of subjective well-being were initially related to mortality after adjusting for age, gender, education, cognition, prevalent chronic diseases, and cardiovascular risk factors (BADL hazard ratio (HR)=1.35; 95% confidence interval (CI) 1.29-1.41)); (IADL (HR=1.27; 95% CI 1.22 - 1.32)); (QoL HR=0.84; 95% CI 0.81 - 0.88)); somatic symptoms (HR=1.11; 95% CI 1.06 - 1.16)); negative affect (HR=1.05; 95% CI 1.01 - 1.10)); positive affect (HR=0.92; 95% CI 0.88 - 0.96)). In the mutually adjusted model, only basic activities of daily living (HR=1.24; 95% CI 1.16 - 1.32) and instrumental activities of daily living (HR=1.10; 95% CI 1.04 - 1.17) remained independently associated with mortality.

Conclusions Measures of subjectively assessed well-being are important indicators of mortality. The best independent subjective well-being measure predicting mortality in older individuals is self-rated physical functioning.

INTRODUCTION

Subjective well-being is defined as a summary measure of how people perceive their functioning across multiple domains.[1] Previous research on the assessment of subjective well-being was mostly conducted within the realm of psychological research focused on life satisfaction and mental aspects of well-being such as positive affect and lack of negative affect. Perception of physical functioning is, however, understudied. Against this background, we conceptualised subjective well-being as a continuum consisting of physical functioning at one end and mental health at the other (Figure 1 and Table 1). Within the possible parameters of this continuum, we emphasized the various measures of subjective well-being in the present study. Each subjective well-being measure serves as a specific combination of items or indicators, which cover a broad aspect of physical functioning and mental health. A large aspect of overall subjective physical functioning is assessed by basic activities of daily living (BADL) that directly measure locomotive health in daily functioning such as eating, bathing, and grooming; and instrumental activities of daily living (IADL), which cover the cognitive attributes of performing self-reliant daily tasks such as meal preparation, shopping, and managing finances.[2] Similarly, the broad aspect of mental health is evaluated by somatic symptoms (i.e. the physical manifestations of depression), negative affect (i.e. symptoms of depressive mood) and positive affect (i.e. the level of happiness). Finally, we studied quality of life that assesses perceived general well-being of daily life and aims to bridge the physical and mental attributes of overall well-being (Figure 1 and Table 1). Importantly, any of these indicators is affected strongly by both physical and mental aspects of health, even if the instruments directly measure only one of these specific aspects.

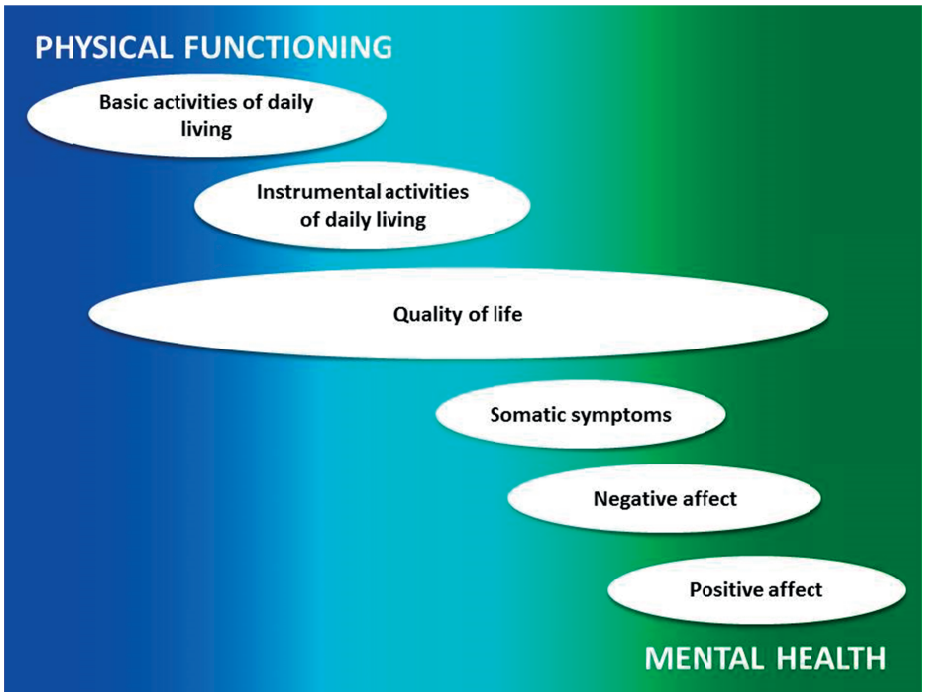
Previous literature suggests that lower ratings on subjective well-being questionnaires are strongly associated with mortality in population studies.[3-5] Therefore, measures of subjective well-being can be used for screening of health, health outcomes and mortality on a population level. It is also suggested that each measure of subjective well-being shows some degree of independence.[6] Therefore, in order to distinguish the associations

Table 1. Guidance for conceptual terminology used throughout the text.

Concept/aspect	Measure/indicator	Scale
Physical functioning	BADL	Stanford health assessment questionnaire
	IADL	IADL scale by Lawton and Brody
Quality of life	Quality of life (QoL)	FLZ
Mental health	Somatic symptoms*	Somatic scale of CES-D
	Negative affect	Negative effect scale of CES-D
	Positive affect	Positive affect scale of CES-D

*Somatic symptoms of depression

Figure 1. A conceptual framework of overall subjective well-being.*



*For conceptual terminology and instrument see Table

between specific aspects of subjective well-being and mortality, it becomes important to determine the independent effect of different measures of subjective well-being on mortality that largely remains unclear in previous literature.

Our study aims to investigate the associations of six different measures of subjective well-being with all-cause mortality. Furthermore, we aimed to determine the independent associations of these subjective well-being measures with all-cause mortality if mutually adjusted.

METHODS

Study Population

This study was embedded in the Rotterdam Study, a prospective population-based cohort that started in 1990 among inhabitants aged ≥55 years residing in a suburb of Rotterdam, the Netherlands.[7] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants. For the present study, the completed measurements

in 6055 persons taken between 2002 and 2005 constituted the baseline. After exclusion of participants with missing interview dates (n=5), with baseline missing data on one of the determinants (IADL (n=169); BADL (n=58); QoL (n=114); negative affect (n=9); positive affect (n=12)), and prevalent dementia (n=150), information on each determinant was available from 5538 participants for analyses. The total number of person-years in the analysis of total all-cause mortality was 48,534 person-years (mean follow-up 12.2 years (SE=0.09).

Measures and Scales

Assessment of Basic Activities of Daily Living (BADL)

BADL was measured using the Dutch version of the disability index from the Stanford Health Assessment Questionnaire.[8] The disability index consists of 20 items constituting eight components: dressing and grooming, arising, eating, walking, hygiene, grip, reach, and activities. Two of the three items belonging to the eating component (ability to cut meat and ability to life a glass) were combined into one item. Each item was scored from 0 to 3, with higher scores indicating worse ability: 0 = without any difficulty, 1 = with some difficulty, 2 = with much difficulty, 3 = unable to. The component score was calculated as the value of the highest scored item available within that component. The overall BADL score was calculated by summing all component scores, thus obtaining a score between 0 and 24.[8]

Assessment of Instrumental Activities of Daily Living (IADL)

IADL was measured using the IADL scale by Lawton and Brody.[9] The IADL scale consists of eight complex tasks that require higher cognitive abilities to successfully live independently: telephone use, medication maintenance, shopping, traveling on your own, management of finances, laundry, housekeeping, and meal preparation. Consistent with the disability index, we scored items from the IADL scale from 0 to 3. For telephone use, participants using an adapted phone were categorized as 2 (with much difficulty). The IADL score was subsequently calculated by summing the scores on all individual items, obtaining a score between 0 and 24.

Assessment of Quality of Life (QoL)

A short questionnaire for measuring QoL adapted from Questions on Life Satisfaction measure (in the original German: Fragen zur Lebenszufriedenheit- FLZ^{M}) was used.[10] This module contains eight dimensions of health (including physical condition/fitness, ability to relax, energy/vitality, mobility, vision and hearing, freedom from anxiety, freedom from aches and pains, and independence from help/care) which the subject must rate twice on a four-point scale; once for the subjective importance of the component

and once for the subjective degree of satisfaction with this area. The importance and satisfaction ratings are then combined to yield the weighted satisfaction score, which varies from -12 and +20 for each dimension.[10] The sum of eight individual weighted satisfaction scores represents overall quality of life.

Somatic Symptoms, Negative Affect and Positive Affect

We present data for somatic symptoms, negative affect and positive affect that indicate mental health. These measures are included in the Center for Epidemiological Studies Depression Scale (CES-D). The CES-D is a validated, widely used standardized self-report instrument that broadly assesses current depressive symptoms.[11] The CES-D scale consists of 20 items, reported on a four-point scale indicating mood and feelings experienced in the past week: 0 = "Rarely or none of the time (0-1 day)", 1 = "Some or a little of the time (1-2 days)", 2 = "Occasionally or a moderate amount of the time (3-4 days)", and 3 = "Most or all of time (5-7 days)". The CES-D has four underlying factors: interpersonal relations, somatic symptoms, negative affect and positive affect.[11 12] We selected the somatic scale, which emphasizes the experience of physical symptoms related to depression. Seven scale items relate to somatic symptoms that are summed to produce a score range from 0 to 21. Also, we tested the negative affect scale with seven items related to depressed affect. These are summed to produce a score range from 0 to 21. Further, we tested positive affect. Four scale items relate to positive affect, which are reversed coded and summed producing a score range from 0 to 12. Weighted scores were calculated if 25% or less of questions were missing.

Assessment of Mortality

Municipal records were checked bimonthly for information on vital status. In addition, deaths were continuously reported through automatic linkage of general practitioner files. Research physicians reviewed all available information and coded the events according to the International Classification of Diseases, 10th edition (ICD-10). The follow-up was complete until October 1, 2015.

Other measures

Smoking habits were assessed during home interview asking participants whether they were currently smoking cigarettes, cigars, or pipe. Information on alcohol consumption was also assessed using home interview. The attained level of education was measured and educational levels were defined by highest completed educational level and classified into three categories: low, intermediate, and high. Participants were invited to visit the research centre for clinical examinations and laboratory measurements. Body mass index was determined using weight divided by the square of height. Total cholesterol and HDL-cholesterol levels were acquired by an automated enzymatic procedure. Blood

pressure was measured at the research center twice in the sitting position on the right arm with a random zero sphygmomanometer. The average of the 2 measurements was used in the analyses. Diabetes mellitus was defined as a fasting glucose level of ≥7.0 mmol/L or use of blood glucose–lowering medication. Prevalent heart failure at baseline was measured using a validated score, that was based on the heart failure definition of the European Society of Cardiology.[13] Joint problems were obtained from home interview and a physician also measured the participants for joint complaints. Radiographic assessment for the diagnosis of osteoarthritis and rheumatoid arthritis were made at the research centre. Cognitive function was tested by trained investigators with the 30-point Mini-Mental State Examination (MMSE). A history of stroke was determined by interview or proxy informant interview. Confirmation of stroke diagnosis by a treating physician was required. A previous myocardial infarction was determined using an electrocardiogram. Diagnosis of COPD was confirmed by spirometry.

STATISTICAL ANALYSIS

We first examined the Spearman rank-order correlations between BADL, IADL, QoL, somatic symptoms, negative affect and positive affect. Next, we investigated the associations of each measure of subjective well-being with all-cause mortality using Cox proportional hazards models adjusting for confounders. The proportional hazard assumption was evaluated using Schoenfeld residuals.[14] The underlying time-scale in these models was the follow-up time in years, which was complete until October 1, 2015. Participants were censored within this follow-up period at date of death, date of loss to follow-up, or October 1, 2015, whichever date came first.

We presented the results per Z-score for a uniform representation of data. All models were adjusted for age and sex. Next, we adjusted for education level, MMSE, joint problems, prevalent stroke, prevalent myocardial infarction (MI), prevalent diabetes mellitus, prevalent chronic obstructive pulmonary disease (COPD), prevalent heart failure, BMI, smoking, and alcohol consumption. In order to test the independent association of each subjective well-being measure with mortality, we further mutually adjusted for the other measures in a third model.

To investigate whether the association between subjective well-being and mortality remained after the exclusion of participants with a psychiatric diagnosis, we excluded participants with major depressive disorder (n=109) at baseline in a sensitivity analysis. A trained psychiatrist, geriatrician or psychologist conducted a semi-structured psychiatric interview in persons with depressive symptoms as defined by a CES-D score of 16 or above, which is considered indicative of a depressive disorder.[15] This interview was performed with the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).[16]

Major depressive disorder (MDD) was classified according to the DSM-IV criteria, with an algorithm based on the item-scores.[15]

Missing values for the confounding variables (0.1 – 13.2% missing data) were imputed by multiple imputation using chained equations in which 40 completed data sets were generated and analysed by using the standard combination rules for multiple imputation. All analyses were done using Stata13 (Stata Corp, College Station, TX).

RESULTS

Baseline characteristics of the study population are presented in Table 2. The mean age of participants was 64.2 years (SD = 6.6), 13.1% of the study population was highly educated and 14.1% were current smokers. The average BMI of the participants was 27.6 (4.1). 32.3% (n=1790) had prevalent cardiovascular disease (including stroke, MI, diabetes, heart failure), 8.2% (n=455) had COPD, 38.4% (n=2126) had joint problems, and the average MMSE score was 27.6 (2.2). The total number of person-years in the analysis of total mortality was 48,534 person-years (mean follow-up 12.2 years (SE 0.09)), during which a total of 2021 participants died.

The spearman's correlations (correlation coefficient (ρ)) between all subjective well-being measures are presented in Supplementary table 1. Table 3 shows the hazard ratios of all-cause mortality for each determinant of mortality. The proportional hazards assumption was met for all associations tested. In the initial model, adjusted for age and sex, all subjective well-being indicators were related to mortality. After further adjustment for education status, MMSE, blood pressure, total and HDL cholesterol, joint problems, cardiovascular factors, prevalent COPD, smoking and alcohol consumption (Model 2), higher impairment in BADL (HR per SD = 1.35 (95% CI 1.29 - 1.41)) and IADL (HR per SD = 1.27 (95% CI 1.22 - 1.32)) and higher scores on somatic symptoms (HR per SD = 1.11 (95% CI 1.06 - 1.16)) and negative affect (HR per SD = 1.05 (95% CI 1.01 - 1.10)) were associated with higher all-cause mortality. Correspondingly, higher scores on QoL (HR per SD = 0.84 (95% CI 0.81 - 0.88)) and positive affect (HR per SD = 0.92 (95% CI 0.88 - 0.96)) were associated with lower all-cause mortality.

Further mutual adjustment for each determinant showed that only BADL (HR per SD increase in BADL impairment, 1.24 (95% CI 1.16 - 1.32)) and IADL (HR per SD increase in IADL impairment, 1.10 (95% CI 1.04 - 1.17)) remained associated with all-cause mortality in the mutually-adjusted model (Model 3). The associations of QoL, somatic symptoms, negative affect, and positive affect with mortality were strongly attenuated after mutual adjustment for each measure of subjective well-being.

Additional analyses (after exclusion of persons with diagnosed major depressive disorder at baseline) showed that the association between negative affect and mortal-

ity were attenuated substantially (supplementary table 2) in the fully adjusted model (model 2). The associations of all other subjective well-being measures with mortality remained virtually unchanged.

Table 2. Characteristics of the study population.

Characteristics	**Participants** (N = 5538)
Age, years	64.2 (6.6)
Gender (Female), n (%)	3232 (58.4%)
QoL, wS- score	83.4 (42.6)
Education, n (%)	
Low	1387 (25.6%)
Intermediate	3326 (61.3%)
High	713 (13.1%)
CES-D Somatic symptoms (scale 0-21)	2.7 (3.1)
CES-D Positive affect (scale 0-12)	9.8 (2.8)
CES-D Negative affect (scale 0-21)	1.4 (2.6)
MMSE, score	27.6 (2.2)
IADL impairment (scale 0-24)	3.0 (3.7)
BADL impairment (scale 0-24)	4.0 (4.3)
Prevalent major depressive disorder	109 (1.9%)
Prevalent stroke	256 (4.6%)
Prevalent MI	388 (7.1%)
Prevalence diabetes	868 (15.7%)
Prevalence COPD	455 (8.2%)
Prevalence heart failure	278 (5.1%)
Alcohol (ever)	4691 (84.7%)
Smoking (current)	778 (14.1%)
Joint problems	
Osteoarthritis	1025 (18.9%)
Rheumatoid arthritis	74 (1.4%)
Other joint problems[a]	1027 (19.0%)
BMI, kg/m^2	27.6 (4.1)
Systolic blood pressure, mmHg	149.6 (21.2)
Diastolic blood pressure, mmHg	79.8 (10.9)
Total cholesterol, mmol/L	5.7 (1.0)
HDL cholesterol, mmol/L	1.4 (0.4)

Values are means (standard deviation) or number of participants (percentage). Abbreviations: n – number of participants; QoL– quality of life; wS – weighted satisfaction; CES-D – Center for Epidemiologic Studies Depression scale; MMSE –Mini-Mental State Examination; IADL – Instrumental Activities of Daily Living scale; BADL – Basic Activities of Daily Living scale; MI – Myocardial Infarction; COPD – Chronic Obstructive Pulmonary Disease; BMI – Basal metabolic Rate; HDL – High Density Lipoprotein [a]Other joint problems included gout, back pain and ankylosing spondylitis

Table 3. The relationship of subjective well-being with all-cause mortality.

		All-cause mortality	
Measures of subjective well-being per 1-SD*		HR per z-score (95% CI) n/N = 1893/5538	P
Basic activities of daily living	Model 1	1.41 (1.35, 1.47)	<0.001
	Model 2	1.35 (1.29, 1.41)	<0.001
	Model 3	1.24 (1.16, 1.32)	<0.001
Instrumental activities of daily living	Model 1	1.35 (1.30, 1.40)	<0.001
	Model 2	1.27 (1.22, 1.32)	<0.001
	Model 3	1.10 (1.04, 1.17)	0.001
Quality of life	Model 1	0.79 (0.76, 0.83)	<0.001
	Model 2	0.84 (0.81, 0.88)	<0.001
	Model 3	0.97 (0.91, 1.03)	0.280
Somatic symptoms	Model 1	1.18 (1.13, 1.23)	<0.001
	Model 2	1.11 (1.06, 1.16)	<0.001
	Model 3	0.99 (0.93, 1.05)	0.664
Negative affect	Model 1	1.10 (1.05, 1.14)	<0.001
	Model 2	1.05 (1.01, 1.10)	0.010
	Model 3	1.01 (0.95, 1.07)	0.801
Positive affect	Model 1	0.87 (0.84, 0.91)	<0.001
	Model 2	0.92 (0.88, 0.96)	<0.001
	Model 3	0.99 (0.94, 1.05)	0.819

SD standard deviation; HR indicates hazard ratio; CI, confidence interval; n, number of persons who died; N, number of persons in the total population.
Model 1: Adjusted for age and gender;
Model 2: additionally adjusted for education level, MMSE, joint problems, prevalent stroke, prevalent MI, prevalent diabetes mellitus, prevalent COPD, prevalent heart failure, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, BMI, smoking, alcohol
Model 3: Model 2 + mutual adjustment for each predictor
*Basic activities of daily living, Instrumental activities of daily living, Somatic symptoms and Negative affect: Higher scores indicate greater impairment; quality of life and positive affect: Lower scores indicate greater impairment.

DISCUSSION

In this large population-based cohort study of 5538 persons (aged on average 64 years), 2021 persons died during 48,534 follow-up person-years (mean follow-up 12.2 years). In this study, all six measures of subjective well-being that include basic and instrumental activities of daily living, quality of life, somatic symptoms, negative affect, and positive

affect each were related to mortality after adjustment for age, gender, education, cognition, prevalent chronic diseases, cardiovascular risk factors, and lifestyle factors such as BMI, smoking and drinking. After mutual adjustment for each measure of subjective well-being were carried out to determine their independent effect on mortality, only impairment in physical functioning assessed by either self-report of basic or instrumental activities of daily living was related to mortality. Further, the association of negative affect with mortality was primarily explained by persons with diagnosed major depressive disorder.

In the present study, we observed strong associations of BADL and IADL with mortality after controlling for educational status, cognition as assessed by MMSE, numerous prevalent diseases and several lifestyle factors. Several previous studies have reported higher mortality in older people with impairments in basic and instrumental activities of daily living.[17 18] Higher impairment in BADL may decrease the ability to maintain minimal level of physical activity. The loss of independence may ultimately lead to shortened survival.[19] Since impairments in IADL may be due to progressive cognitive decline, this loss of independence may be more pronounced in already cognitively impaired persons.[20] There is evidence that neuropathological changes leading to executive and frontal lobe dysfunction are related to deterioration in performing BADL and IADL tasks.[21 22] There is also evidence that limitations in physical functioning have a profound impact on mood.[23] In particular depression and some somatic diseases exhibit similar symptoms.[24] In one study, the risk of depression on mortality was more pronounced by somatic symptoms of depression, rather than cognitive affective symptoms of depression.[25] As somatic symptoms related to depression are a component of the CES-D questionnaire, it can be difficult to assess whether persons with physical dysfunction exhibit depression or have somatic comorbidity only. Therefore, we simultaneously tested the association of negative affect with mortality. It is well known that depressive symptoms may lead to early mortality.[26] It is also previously known that patients with major depressive disorder have a two-fold increased risk of death than their non-psychotic depressed counterparts.[27] The mechanisms include physiological changes that adversely affect endocrine, neurologic and immune processes.[28 29] Conversely, disorders that inhibit daily physical function may be responsible for the onset of depressive symptoms, depressive disorders, and ultimately mortality. In one study by Blazer and Hybels,[30] somatic symptoms and negative affect were not associated with mortality; while positive affect was the only subscale predicting mortality after controlling for potential confounders. To exemplify, in the present cohort another study by Krijthe et al.,[31] showed that positive affect was associated with survival but the associations were no longer significant once controlled for health status. Concurrently, we found that subjective quality of life was related to mortality after adjustment for relevant confounding variables in model 2. Quality of life combines assessments of perceived physical and mental life satisfaction that contribute

towards overall health into one scale. Ford et al.[32] reported higher levels of quality of life to be inversely associated with mortality. However, survival was reported without controlling for any prevalent diseases. It follows that the association of mental health with mortality is strongly attenuated once perception of physical functioning are carefully accounted for. In this context, we may have over-adjusted our associations in the final model by controlling for other measures of subjective well-being tested in this study that may be inter-related. We argue that this was deliberate; it was our goal to determine the independent relation between each subjective well-being measure and mortality while other measures of well-being are accounted for. However, if the interest lies in the understanding of the association between subjective well-being and mortality in general, as in most previous studies, the second model should be considered primarily.

This is the first study to determine the independent association of various measures of subjective well-being with mortality. In this study we showed that IADL and BADL have an independent relation with mortality after other measures of subjective well-being are accounted for. Previously, only few studies have showed an effect of physical functioning independent of other subjective measures.[33 34] These findings may have clinical implications on risk factor reduction for debilitating diseases that hamper physical functioning. Older individuals living in the community, who have impairments in daily living tasks, are more vulnerable to health and safety risks within their environment.[35] Thus, these older individuals may be at a higher risk of neglect, mistreatment, exploitation, comorbidity, and decreased survival.[36 37] Furthermore, ageing may accelerate the inability to compensate functional loss referred to as frailty. A previous study imbedded within the Rotterdam Study found an increased risk of death in frail persons compared to non-frail elderly persons.[38]

The strengths of our study include a large sample size and a population-based longitudinal approach. Most importantly, several instruments were tested. This enabled us to investigate the specific associations of somatic symptoms, negative affect, and positive affect with mortality above that of other measures of subjective well-being. Nearly all participants included in our study had a complete follow-up; there was less than 1% loss of person-years. A potential limitation of the current study was the absence of evaluation of nutritional state of participants. Malnutrition in older adults has been linked to mortality, especially the oldest-old with disabilities who are hindered in regular functioning of preparing their own meals and are dependent on others for their nutritional provisions.[39 40] We used BMI as a proxy for nutritional status in order to account for malnutrition as previously undertaken in other studies.[41] Additionally, incident comorbidities occurring in the time period between baseline and death were not assessed and could lead to underestimation of our results.

In conclusion, the level of basic activities of daily living and instrumental aspects of physical functioning are a prominent independent determinant of mortality in older

individuals. Our study suggests that this aspect largely explains the association of subjective well-being and mortality. These findings may have clinical implications for risk factor reduction in debilitating diseases that hamper physical functioning. Quality of life, somatic symptoms, negative affect and positive affect did not predict mortality once self-rated physical functioning is accounted for and may be less optimal screening goals. Therefore, interventions aiming at improving survival could focus on subjective indicators of physical well-being namely basic and instrumental activities of daily living.

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SUPPLEMENTARY MATERIAL

Supplementary table 1. Spearman's correlations (ρ) between study variables.

	BADL impairment	IADL impairment	Quality of life	Somatic symptoms	Negative affect	Positive affect
BADL impairment	1.000					
IADL impairment	0.539	1.000				
Quality of life	-0.509	-0.381	1.000			
Somatic symptoms	0.400	0.243	-0.497	1.000		
Negative affect	0.272	0.125	-0.395	0.530	1.000	
Positive affect	-0.318	-0.232	0.497	-0.516	-0.575	1.000

BADL: Basic activities of daily living; IADL: Instrumental activities of daily living; *P for all* < 0.0001

Supplementary table 2. The relationship of subjective well-being with all-cause mortality after exclusion of persons with major depressive disorder.

		All-cause mortality	
Measures of subjective well-being, per 1-SD*		HR per z-score (95% CI) n/N = 1968/5429	P
Basic activities of daily living	Model 1	1.39 (1.33, 1.45)	<0.001
	Model 2	1.33 (1.27, 1.39)	<0.001
	Model 3	1.23 (1.15, 1.31)	<0.001
Instrumental activities of daily living	Model 1	1.33 (1.28, 1.38)	<0.001
	Model 2	1.25 (1.20, 1.30)	<0.001
	Model 3	1.10 (1.04, 1.16)	0.001
Quality of life	Model 1	0.80 (0.77, 0.84)	<0.001
	Model 2	0.86 (0.82, 0.90)	<0.001
	Model 3	0.97 (0.91, 1.03)	0.294
Somatic symptoms	Model 1	1.16 (1.12, 1.21)	<0.001
	Model 2	1.09 (1.05, 1.14)	<0.001
	Model 3	0.99 (0.93, 1.05)	0.722
Negative affect	Model 1	1.07 (1.03, 1.12)	<0.001
	Model 2	1.03 (0.99, 1.07)	0.155
	Model 3	0.99 (0.94, 1.04)	0.669
Positive affect	Model 1	0.88 (0.85, 0.92)	<0.001
	Model 2	0.94 (0.90, 0.98)	0.002
	Model 3	0.99 (0.94, 1.05)	0.868

SD standard deviation; HR indicates hazard ratio; CI, confidence interval; n, number of persons who died; N, number of persons in the total population.
Model 1: Adjusted for age and gender;
Model 2: additionally adjusted for education level, MMSE, joint problems, prevalent stroke, prevalent MI, prevalent diabetes mellitus, prevalent COPD, prevalent heart failure, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, BMI, smoking, alcohol
Model 3: Model 2 + mutual adjustment for each predictor
*Basic activities of daily living, Instrumental activities of daily living, Somatic symptoms and Negative affect: Higher scores indicate greater impairment; quality of life and positive affect: Lower scores indicate greater impairment.


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Chapter 3.2

Subjective memory complaints and the risk of stroke

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ABSTRACT

Background Persons with cognitive impairment, as assessed by cognitive tests, are at a higher risk of stroke. Subjective memory complaints might be an earlier marker for stroke, especially in persons with higher education. Their cognitive reserve might mask their cognitive impairment during cognitive testing. In a population-based setting, we investigated the association between subjective memory complaints and stroke. We simultaneously investigated the association between Mini-Mental State Examination (MMSE) and stroke. We also assessed whether these associations varied with educational level.

Methods 9,152 participants from the Rotterdam Study (baseline 1990-1993 or 2000-2001) completed the subjective memory complaints questionnaire and underwent MMSE assessment. Subsequently, the entire cohort was followed for incident stroke until 2012. We used Cox proportional hazard models to estimate the associations between subjective memory complaints and MMSE, with stroke.

Results During a follow-up of 111,593 person years, 1,134 strokes were identified, of which 663 were ischemic and 99 hemorrhagic. In the fully adjusted model, presence of subjective memory complaints was independently associated with a higher risk of stroke (hazard ratio [HR] 1.20 (95% confidence interval (CI) 1.04-1.39), but a higher MMSE was not (HR per point increase 0.99 (95% CI 0.95 - 1.02)). The association between subjective memory complaints and risk of stroke was modified by educational level, with a higher risk of stroke in persons with a higher level of education (HR 1.39 95% CI 1.07 - 1.81).

Conclusions Subjective memory complaints might be an early indicator of stroke risk especially in highly educated individuals.

INTRODUCTION

Cognitive impairment and dementia are very often long-term sequelae of stroke.[1] This could be due to direct loss of brain parenchyma during stroke, especially when such damage is located at strategic sites in the brain, for instance in the thalamus.[2] However, cognitive impairment and stroke might also be linked through a shared etiology as vascular risk factors for stroke are also determinants of cognitive impairment and dementia.[3, 4] To test this hypothesis, several studies that investigated how cognitive impairment relates to incident stroke were reviewed, and found an increased risk of stroke in persons with lower cognitive performance.[5]

Most studies have used objective cognitive tests, such as the Mini-Mental State Examination (MMSE), to determine the presence of cognitive impairment.[6-9] However, subjective memory complaints may appear earlier and might therefore be an earlier marker of vascular damage that could also lead to stroke.[10] This may apply especially to persons with higher education, who perform well on cognitive testing, probably due to a higher cognitive reserve,[11] which can mask subtle changes in cognition. As a result, these persons may continue to harbor subclinical vascular insults to the brain. Previous studies have found an association between subjective memory complaints and risk of dementia,[12, 13] mostly in persons with higher education.[11] Still, the clinical importance of subjective memory complaints for the prediction of stroke remains unclear.

The aim of the present study was to evaluate the independent association between subjective memory complaints and the risk of stroke. In addition, we used the MMSE as an objective measure to relate with incident stroke. Furthermore, we also sought to determine whether these associations vary with educational level.

METHODS

Study Population

This study was embedded in the Rotterdam Study, a large prospective population-based cohort that started in 1990 among inhabitants aged ≥55 years residing in a district of Rotterdam, the Netherlands (N=7,983). An expansion of the cohort took place in year 2000, consisting of 3,011 participants who had become 55 years of age or moved into the study district since the start of the study.[14] The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants. For the present study, after exclusion of participants at baseline with prevalent stroke (n=291), prevalent dementia (n=437), prevalent dementia and stroke (n=74) and no informed consent for data linkage

(n=151), 9152 participants with data on subjective memory complaints were eligible for analysis (Supplementary figure 1).

Assessment of Subjective Memory Complaints and Objective Cognition

Trained investigators interviewed all participants at home. The presence of subjective memory complaints was assessed by the question, "Do you have memory complaints?"[11]

Cognitive function on an objective scale was tested with the 30-point Mini-Mental State Examination (MMSE).[15] The MMSE contains 20 items covering orientation, memory, attention, language, and visuospatial construction.

Assessment and Follow-up of Stroke

At study entry, history of stroke was assessed using home interviews and confirmed by reviewing medical records. Once participants entered the Rotterdam Study, they were continuously followed up for stroke through automatic linkage of general practitioner files with the study database. Also, nursing home physicians' files and files from general practitioners of participants who moved out of the district were checked on a regular basis. Of the potential strokes, additional hospital and general practitioner information was collected. Research physicians reviewed the stroke information, and an experienced neurologist adjudicated the strokes using standardized definitions, as described in detail previously.[16] The follow-up was complete until January 1, 2012, for 97% of potential person-years.

Assessment and follow-up of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[17, 18] Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[19] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuro-imaging was required and available, it was used for decision making on the diagnosis. For all suspected cases of dementia, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R) and Alzheimer's disease (NINCDS-ADRDA).[20, 21]Follow-up for incident dementia was virtually complete until September 2, 2011.

Measurement of covariates

We used covariates measured at baseline. Smoking status and information on the number of cigarettes smoked per day in each decade of life was obtained by a self-administered questionnaire during the home interview. Smoking status was characterized as never, past and current smoking. Participants were also invited to visit the research centre for clinical examinations and laboratory assessments. Body mass index (BMI) was measured using weight in kilograms divided by the square of height in meters. Total cholesterol and HDL-cholesterol levels were acquired by an automated enzymatic procedure. Diabetes mellitus type-2 was defined as having a fasting glucose level of ≥7.0 mmol/L or using blood glucose–lowering medication. Blood pressure was measured at the research center twice in the sitting position on the right arm with a random zero sphygmomanometer. The average of the two measurements was used in the analyses. Data on indication for use of blood pressure-lowering medication were based on information collected by a physician at the research centre. In the case of missing information, data from the home interview was taken. Information on *APOE*-ε4 genotype (at least one *APOE*-ε4 allele vs. no APOE-ε4 alleles) was determined from blood samples. Basic Activities of Daily Living (BADL) was assessed using the Dutch version of the disability index from the Stanford Health Assessment Questionnaire.[22] The disability index consists of 20 items constituting eight components: dressing and grooming, arising, eating, walking, hygiene, grip, reach, and activities. Two of the three items belonging to the eating component were combined into one item. Each item was scored from 0 to 3, with higher scores indicating worse ability: 0 = without any difficulty, 1 = with some difficulty, 2 = with much difficulty, 3 = unable to.[23]

Statistical Analysis

We investigated the associations of subjective memory complaints and MMSE, as a continuous measure, with stroke incidence using Cox proportional hazards models. The proportional hazard assumption was evaluated using Schoenfeld residuals.[24] The underlying time-scale in these models was the follow-up time in years. Participants who did not suffer from stroke were censored at date of death, date of loss to follow-up, or January 1, 2012, whichever occurred first. All models were adjusted for age, sex, and cohort. We subsequently adjusted for BMI, diabetes mellitus type-2, smoking status, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, total serum cholesterol, HDL-cholesterol, lipid lowering medication, BADL, APOE-ε4 allele carrier status, and MMSE or subjective memory complaints (where applicable). To examine whether subclinical subjective memory complaints are associated with stroke, two sensitivity analyses were performed: first, we censored for incident dementia during follow-up. Second, we additionally excluded participants with a MMSE score of <26 at baseline.[25]

To investigate whether education level affected the association between subjective memory complaints and MMSE with stroke, we constructed interaction terms with education and performed stratified analyses. We categorized level of education into three groups: low education (primary education only), intermediate education (primary education plus a not completed higher education, lower vocational education, intermediate vocational education, or general secondary education), and high education (higher vocational education or university training).

Missing values on blood measurements were due to failure of blood draw or the blood sample was inadequate to run all tests. Blood pressure measurements and anthropometry measures were missing due to physical inability of persons. Respective questions on smoking status, education level, use of medication were missing due to time constraints in administering the questions at the time of home interview. Missing values for the confounding variables (0.1 – 5.8% missing data) (supplementary figure 1) were imputed by multiple imputation using chained equations in which 40 completed data sets were generated and analyzed by using the standard combination rules for multiple imputation. Each variable was used as a predictor in the imputation model. All continuous variables in our data had normal distribution. No interactions were included in the final analysis thus no interactions were included in the imputation models. The data was imputed assuming that the data was missing at random. All analyses were performed using Stata13 (Stata Corp, College Station, TX).

RESULTS

Baseline characteristics of the study population are presented in Table 1. Individuals with any missing data on covariates (n=999) were compared with persons with complete data (n=8153); Persons with incomplete data were older in age (72.2 years (10.2) compared to 67.1 years (8.4), P<0.001), were more often female (63.8% compared to 57.6%, P<0.001), had more complaints about their memory (23.7% compared to 16.1%, P<0.001), and had 0.5 point lower score on MMSE (27.3 (2.2) compared to 27.7 (1.8)).

During 111,593 person-years (mean follow-up 12.2 years), 1,134 incident strokes were reported; 663 of these were ischemic and 99 were hemorrhagic. A total of 372 cases remained unspecified, mostly because they were not referred to the hospital and hence did not undergo brain imaging. 2668 persons had incomplete amount of person-years until 2012; whose last date of contact was before the potential end-date if there was no loss to-follow-up. In addition, 3793 persons had died during follow-up.

Table 2 shows hazard ratios for the associations of subjective memory complaints and MMSE, with total, ischemic, and hemorrhagic stroke. The proportional hazards assumption was met for all associations tested. After adjusting for age, sex, and education,

Table 1: Baseline characteristics of the study population

	Participants (N=9152)*	
	SMC=no (N=7600)	SMC=yes (N=1552)
Age (years)	67.1 (8.5)	70.3 (9.4)
Female	4368 (82.0%)	962 (18.1%)
Education		
High	2315 (30.9%)	428 (28.3%)
Intermediate	3852 (51.3%)	699 (46.2%)
Low	1338 (17.8%)	386 (25.5%)
Systolic blood pressure (mmHg)	140.4 (22.0)	139.8 (22.7)
Diastolic blood pressure (mmHg)	75.5 (11.5)	74.3 (11.9)
Use of blood pressure lowering medication	2246 (29.6%)	511 (33.0%)
Total cholesterol (mmol/L)	6.4 (1.2)	6.4 (1.3)
HDL-cholesterol (mmol/L)	1.4 (0.4)	1.4 (0.4)
Use of lipid lowering medication	394 (5.2%)	72 (4.7%)
Diabetes mellitus	760 (10.0%)	165 (10.7%)
Smoking	1721 (22.8%)	285(18.7%)
Never	2558 (33.7%)	592 (38.1%)
Past	3321 (43.7%)	675 (43.5%)
Current	1721 (22.6%)	285 (18.4%)
Body mass index (kg/m2)	26.6 (3.8)	26.4 (3.8)
Basic Activities of Daily Living (impairment score)	0.3 (0.5)	0.5 (0.6)
MMSE score	27.8 (1.8)	27.3 (2.1)
APOE-ε4 allele carriers	1948 (27.2%)	440 (30.4%)

Values are means (standard deviation) or number of participants (percentage).
*291 participants with prevalent stroke excluded at baseline; 437 participants with prevalent dementia excluded at baseline; 74 participants with both prevalent stroke and prevalent dementia excluded at baseline; 151 excluded with no informed consent for data linkage.

presence of subjective memory complaints was associated with a higher risk of stroke, hazard ratio (HR) 1.19 (95% CI 1.03 - 1.38). The association between subjective memory complaints and stroke did not change significantly after adjustment for lifestyle factors, vascular risk factors, and APOE-ε4 and MMSE, HR 1.20 (95% CI 1.04 - 1.39). The association between subjective memory complaints and ischemic stroke was similar in strength, HR 1.22 (95% CI 1.01 - 1.49) in the fully-adjusted model.

An increase in MMSE score was not significantly associated with the risk of stroke: fully adjusted HR per point higher MMSE score 0.99 (95% CI 0.95 - 1.02). The effect sizes for the association between MMSE with ischemic and hemorrhagic stroke were also non-significant. Mutual adjustment (model 2) showed that subjective memory complaints was associated with stroke independently of MMSE. Additional analyses (censoring at

Table 2. Subjective memory complaints, Mini-Mental State Examination (MMSE) and risk of incident stroke.

		Total strokes	Ischemic strokes	Hemorrhagic strokes
		HR (95% CI)	HR (95% CI)	HR (95% CI)
Total population		n/N=1134/9152	n/N=663/9152	n/N=99/9152
Subjective memory complaints (yes vs no)	Model 1	1.19 (1.03, 1.38)	1.17 (0.96, 1.42)	1.32 (0.81, 2.15)
	Model 2	1.20 (1.04, 1.39)	1.22 (1.01, 1.49)	1.39 (0.85, 2.26
MMSE (per point increase)	Model 1	0.97 (0.94, 1.00)	1.02 (0.98, 1.07)	0.96 (0.86, 1.07)
	Model 2	0.99 (0.95, 1.02)	1.04 (0.99, 1.09)	0.97 (0.87, 1.09)
Censoring for dementia		n/N=1000/9152	n/N=630/9152	n/N=91/9152
Subjective memory complaints (yes vs no)	Model 1	1.17 (1.00, 1.37)	1.15 (0.94, 1.41)	1.35 (0.81, 2.25)
	Model 2	1.19 (1.01, 1.39)	1.20 (0.98, 1.47)	1.42 (0.85, 2.37)
MMSE (per point increase)	Model 1	0.98 (0.95, 1.02)	1.01 (0.97, 1.06)	0.95 (0.85, 1.07)
	Model 2	0.99 (0.96, 1.03)	1.02 (0.98, 1.07)	0.97 (0.86, 1.09)
Censoring for dementia and excluding MMSE<26		n/N=891/8200	n/N=577/8200	n/N=82/8200
Subjective memory complaints (yes vs no)	Model 1	1.17 (0.99, 1.39)	1.14 (0.92, 1.41)	1.33 (0.77, 2.31)
	Model 2	1.20 (1.02, 1.42)	1.19 (0.96, 1.48)	1.39 (0.80, 2.41)
MMSE (per point increase)	Model 1	0.99 (0.93, 1.04)	1.01 (0.94, 1.08)	1.00 (0.83, 1.20)
	Model 2	1.00 (0.95, 1.06)	1.02 (0.95, 1.10)	1.02 (0.85, 1.23)

CI indicates confidence interval; HR, hazard ratio; n, number of stroke events; and N, number of persons in the total population.

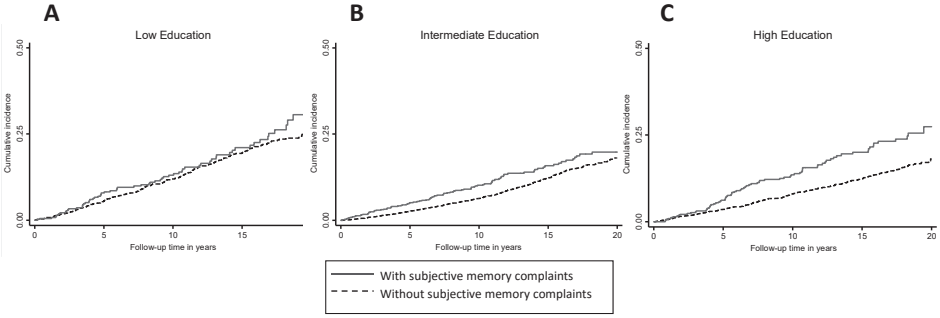
Model 1: Adjusted for age, sex, education, and cohort

Model 2: Model 1 + BMI, diabetes, smoking, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, total serum cholesterol, HDL-cholesterol, lipid lowering medication, and APOE-ε4, basic activities of daily living, and MMSE or subjective memory complaints

the date of diagnosis of incident dementia, and excluding persons with MMSE < 26 at baseline) showed virtually unchanged results.

We found a significant interaction between the level of education and subjective memory complaints in relation to stroke (P=0.007). The education-stratified analyses showed that subjective memory complaints were significantly associated with stroke only in persons with high education, HR 1.39 ((95% CI 1.07 - 1.81) fully adjusted); Table 3 and Figure 1). Time to incident stroke was also shorter in persons with high education level who complained about their memory (Figure 1). The effect sizes of MMSE with stroke were similar across the strata of education.

Figure 1. Kaplan-Meier cumulative incidence curves of total strokes in (A) low educated, (B) intermediate educated and, (C) highly educated persons with or without subjective memory complaints.



DISCUSSION

We found that individuals with subjective memory complaints, especially those with a high level of education, had an increased risk of stroke compared with those who did not complain about their memory. The association between subjective memory complaints and stroke was independent of the MMSE score. All associations remained similar after censoring for incident dementia and further excluding persons with an MMSE score <26 at baseline.

Subjective memory complaints are common in the elderly with prevalence rates reported from 11% in persons above 65 years to 88% in those aged above 85 years.[26, 27] In our population of community-dwelling individuals of on average age 67 years, the prevalence was 17% and is very comparable to numbers in the literature.

The associations between subjective memory complaints and the risk of stroke are probably mainly explained by shared vascular risk factors. Subjective memory complaints may be a marker of cerebral microvascular injuries which may ultimately lead to clinical stroke.[5] Previous studies have found associations between subjective memory complaints and cerebral microbleeds or white matter lesions.[28, 29] This supports our hypothesis that early vascular damage presenting as memory complaints, which are not yet evident in cognitive tests, may in future lead to clinical stroke. Similarly, it has been shown among hypertensive patients that those with subjective memory complaints have more arterial stiffness and white matter lesions than patients without subjective memory complaints.[30] One study showed the presence of amyloid-β protein deposition in people with subjective memory complaints, who were otherwise cognitively unimpaired (MMSE ≥28). Yet in the same study, no association was found between objective memory measures and amyloid burden.[31] Thus, the deposition of amyloid-β proteins in the vessels of the brain can compromise their integrity, which may lead to leakage and subsequently contribute to clinical stroke.[32]

We found that the association between subjective memory complaints and stroke was strongest in highly educated persons. This is comparable to a previous finding that the association between subjective memory complaints and Alzheimer's disease is strongest in highly educated persons.[11] An explanation may be that persons who are highly educated are more likely to notice subtle changes in their cognitive performance than the less educated. This makes the perception of memory changes of highly educated persons a suitable measure to assess subtle cerebrovascular degeneration. This is evident in our data after adjustment for age, sex and MMSE at baseline, we found that the odds of having subjective memory complaints is 1.56 times in the high education group compared to low education group. A counter-argument against this reasoning is that in our population, memory complaints were more frequent in the low educated group compared to the highly educated group. Another explanation is that education reflects cognitive reserve.[33] Higher cognitive reserve allows persons to cope better with accumulating vascular injury in the brain, thereby maintaining their performance on cognitive testing. Subjective memory complaints in these highly educated persons might therefore be a better marker than cognitive testing to assess vascular brain injury.

The strengths of our study are its population-based prospective design and availability of data on more than 9000 participants at baseline with a long follow-up. The main novelty of our study is that we describe subjective memory complaints in addition to objective cognitive testing as an independent predictor of stroke. Furthermore, we also assess the association between subjective memory complaints and stroke in a population without stroke and dementia at baseline.[5] Our study is limited by the use of MMSE as the only comparative objective measure of cognitive impairment because the severity of cognitive impairment cannot be reliably assessed by MMSE alone. Future studies with more extensive cognitive testing batteries are needed to determine the associations between cognitive impairment and stroke. Since different cognitive tests target different cognitive domains, it would be mandatory to apply a variety of objective measurements to determine which cognitive domains are particularly associated with a higher risk of stroke. Our study is limited by the unavailability of neuro-imaging data. Future studies should include MRI findings to explore the evidence of small vessel disease as an underlying mechanism of the associations between subjective memory complaints and stroke. Among the 372 unspecified strokes, silent strokes were not diagnosed since all strokes being clinical strokes were reported at the hospital. Furthermore, although we adjusted for a variety of vascular risk factors, we cannot exclude residual confounding by measurement error or unmeasured factors. Moreover, the covariates in our list may not be exhaustive of all vascular risk factors and there may also be some risk factors that do not go through the vascular risk factor pathways. The unavailability of data on depression and/or depressive symptoms as an unmeasured confounder is also a major limitation of this study since it has been suggested that the associations with

subjective indicators of health especially memory may be confounded by prevalence of depression.[34]

In conclusion, subjective memory complaints are associated with a higher risk of incident stroke, especially in persons with a high level of education. In these persons, cognitive tests are not of incremental value, since they might perform well despite their subjective memory dysfunction. This suggests the importance of a single self-rated question about memory complaints that can prompt clinicians to consider screening for and treatment of vascular risk factors. People with high level of education who complain about changes in their memory should be a primary target for further risk factor screening and prevention of stroke.

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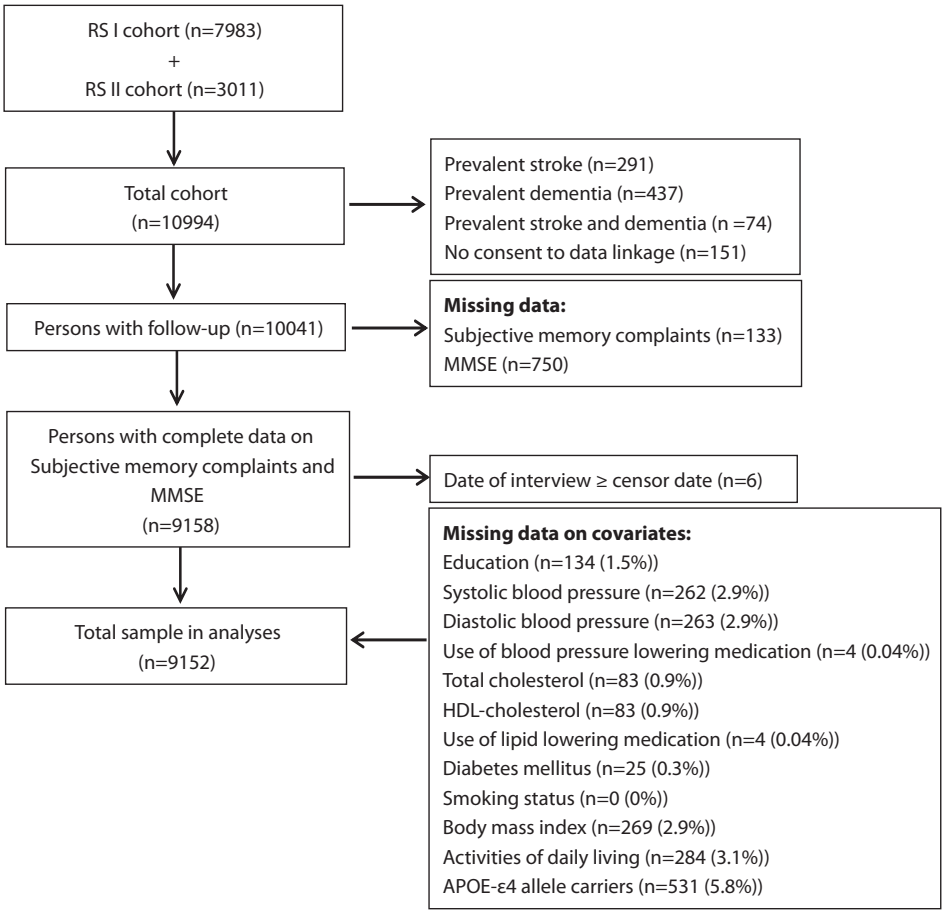
SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Flow of participants included in the study.

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Chapter 4

Systematic reviews of cognition and stroke


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Chapter 4.1

Exploring strategies to operationalize cognitive reserve: A systematic review of reviews

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ABSTRACT

Background The cognitive reserve hypothesis suggests that across the lifespan higher education, regular participation in social or mentally stimulating activities and complexity of occupation increase an individual's resistance to dementia. However, there is currently no consensus regarding how to assess or measure cognitive reserve.

Method We performed a systematic review of reviews focused on the concept of cognitive reserve to examine key elements of the definition and highlight limitations. We searched Embase.com, MEDLINE (OvidSP), the Cochrane Library, Web of Science, Scopus, Google Scholar and PubMed.

Results Five systematic reviews were identified. These incorporated findings from cohort, cross-sectional and case-control studies and the outcomes examined included Alzheimer's disease, vascular dementia, non-specified dementia, all dementias and cognitive decline or cognitive impairment. Education, occupation and leisure or mentally stimulating activities were suggested to supply cognitive reserve and offer a protective effect against the risk of dementia. Premorbid IQ and socioeconomic status have not been investigated as thoroughly and showed inconsistent results. Two of the reviews showed that when combining different indicators in the analyses/definition, including education, occupation, mentally stimulating activities and premorbid IQ, cognitive reserve had a protective effect against cognitive decline. However, other indicators may also supply the reserve, including dietary habits and genetic indicators, but research is lacking with regards to creating a full cognitive reserve model.

Conclusions This review highlights the lack of consensus regarding a definition of cognitive reserve. Further research is required to clarify how the indicators already identified may provide cognitive reserve and offer a protective effect against dementia. Agreement on the indicators that constitute the cognitive reserve model is needed before testing possible interventions which may increase the reserve supply and improve cognition.

INTRODUCTION

The reserve hypothesis attempts to explain the neuropathological findings of advanced Alzheimer's disease pathology (e.g., amyloid-β plaques and neurofibrillary tangles) at autopsy which have been found in individuals who have remained cognitively healthy throughout their lives[1]. Further, individuals with equivalent levels of Alzheimer's disease pathology have shown differences in timing of dementia onset with some individuals having a shorter duration of the disease before death[1]. These findings suggest that some adults can endure the pathological changes related to Alzheimer's disease better than others, a concept known as cognitive resilience[2]. The explanation for the inconsistency between pathological damage and clinical expression of disease in different individuals is currently unclear, but the reserve hypothesis aims to fill this gap. Indeed, much research has focused on the various indicators which may contribute towards supplying the reserve[3]. Previous reviews have discussed the reserve hypothesis, however, there is a need for a review of these in order to pull together the concepts identified in the different reviews with the aim of arriving at consensus on how best to define reserve.

The reserve hypothesis can be broadly split into two concepts, brain and cognitive reserve. Cognitive reserve is an active model, which suggests that individuals differ in how the individual manages brain damage using protective mechanisms associated with cognitive abilities built up over the life course, and also via actively producing complex responsive processes to compensate for the damage caused by disease processes[4]. This has two components, neural reserve (the capabilities of the brain prior to damage) and neural compensation (using areas of the brain which were not previously used to compensate for damage)[5]. In the active model of cognitive reserve, certain experiences throughout the life course such as higher education, engagement in leisure activities and occupational attainments do not only increase cognitive ability, but also supply the cognitive reserve which may reduce the risk of dementia in old age[3]. Alongside the concept of cognitive reserve is the concept of brain reserve[6]. In contrast to cognitive reserve, brain reserve refers to a passive model of reserve, whereby a larger brain size or a larger number of neurons form the substrate[7-9]. Brain reserve is deemed passive as it implies that individuals only differ in the volume of their brain matter. It does not consider that individuals have differing cognitive abilities, which may lead to different outcomes from Alzheimer's disease pathology and other brain damage[7]. The concepts are not mutually exclusive. For example, a higher cognitive reserve could potentially lead to a protective mechanism, which results in less neuronal loss, and they may both contribute to the overall supply to reserve and assist the brain in minimizing the effects of brain pathology. However, whether the indicators of cognitive and brain reserve interplay remains unclear and has led to difficulty in differentiating between the two concepts[2].

Educational attainment, amongst other factors, has been proposed to contribute to cognitive reserve and has been a focus of much of the work regarding cognitive reserve in human studies. Several studies in older adults have shown slower rates of cognitive decline and a decreased risk of dementia amongst those with higher levels of education[7,10].

Various other indicators have also been linked to higher cognitive ability, such as occupational attainment, leisure activities and social engagement. However, currently there is no standard definition for cognitive reserve and, therefore, the indicators thought to contribute to cognitive reserve, and their weighted contribution to cognitive reserve, have not been adequately defined. This creates difficulty in comparing different studies of cognitive reserve and leads to heterogeneity across study designs. Additionally, there are issues with timing, as the cognitive reserve may incorporate numerous indicators across the life course from early life indicators, such as indicators relating to levels of education, to later life indicators, such as indicators relating to occupation. Further, there are indicators which continue throughout life, such as social engagement, which also have various proxy measures. Studies of cognitive reserve also tend to be limited to the data collected. For instance, degree of literacy has been suggested to be a better measure of cognitive reserve than number of years of education, yet few studies have collected this indicator and, therefore, years of education is more often used[9].

Although there has been considerable research into which factors contribute to reserve, the validity of the reserve hypothesis as an independent construct separate from other cognitive concepts has been questioned and is still under debate[5]. A study which evaluated the construct validity of cognitive reserve concluded that the concept had strong convergent validity and moderate discriminant validity, and could be appropriate to refer to cognitive reserve as a unique cognitive concept, but this may be limited to executive processing[5]. However, to further research the validity of the concept of reserve, identifying which factors contribute to the definition of reserve is essential.

The aim of this review is to pull together the existing literature regarding cognitive reserve and brain reserve and ascertain if there are any common elements, which may be key contributing indicators. Further, this work could contribute to future models of cognitive reserve, which could help to determine the indicators that may be the most important for defining cognitive reserve.

METHODS

Search strategy

We performed a systematic literature search in electronic databases. The online search strategies were designed and executed by the second author (AS) and an experienced

medical librarian (WMB). The search results were not restricted to articles using a phrase related to cognitive or brain reserve, but searched broader for prevention of cognitive impairment by cognitive training. A combination of controlled search terms (MeSH terms in MEDLINE and emtree terms in Embase) and words in title or abstract was used to search Embase.com, MEDLINE (OvidSP), the Cochrane library, Web of Science, Scopus, Google Scholar, and PubMed for recent articles added by the publisher. The search was last executed on November 10, 2014. Potentially eligible studies included English language systematic review articles on the concept of cognitive and brain reserve and how it has been mapped in population studies.

Scope of the Review

Cognitive reserve is defined as: "Innate intelligence or aspects of life experience like educational or occupational attainments that may supply reserve, in the form of a set of skills or repertoires that allows some people to cope with progressing Alzheimer's disease pathology better than others"[3]. Overlapping this concept is the concept of brain reserve, which adds indicators such as brain size and synaptic count, which may supply the overall reserve and account for some differences between individual outcomes to brain damage. As the aim was to gain a more comprehensive overview of the indicators of cognitive reserve, any systematic review articles which focused on the concept of cognitive reserve or brain reserve were eligible for inclusion.

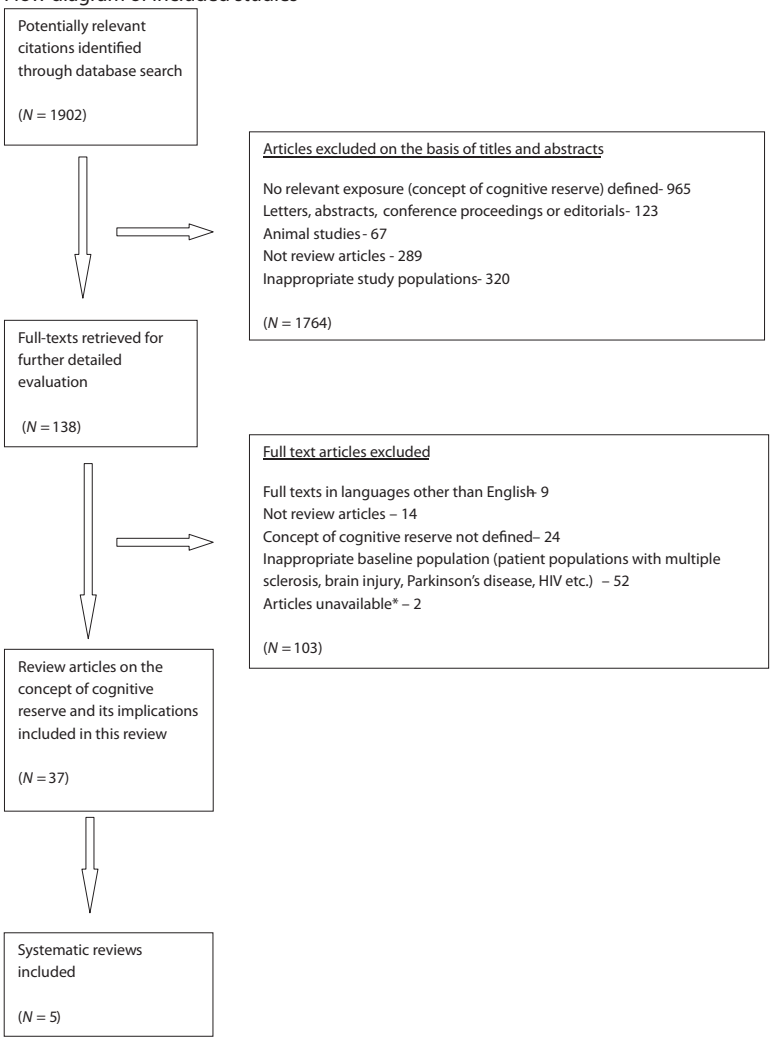
Article Selection

Two reviewers (AS and SLH) independently screened the titles and abstracts of the initially identified studies for their potential eligibility. Full-text versions of the identified studies were then screened using the selection criteria for final inclusion. An overview of the selection of studies and full eligibility criteria is presented in Figure 1. We also cross-referenced the selected studies to identify additional relevant studies.

Data collection and analysis

Using a pre-defined data collection form the following information was extracted from each paper: the number of studies reviewed, search date, sample size, age range of the included participants in the review articles, follow-up time, indicator of cognitive reserve identified such as education, occupation, lifestyle, mental activities, premorbid IQ etc., differentiation of the indicators of brain reserve (where differentiated), summary measures if meta-analyses were performed, limitations, suggestions for future research and the implications/conclusions of the concept of cognitive reserve in each review article. Summary statistics (if reported in a meta-analysis) were compared across studies.

Figure 1. Flow diagram of included studies



RESULTS

Number of studies

Searches of the different online databases stated previously resulted, after removing duplicate entries, in 1902 potentially relevant citations. After independent reviewing of titles and abstracts by two authors, 1764 articles were excluded, the main reasons for exclusion at this stage were no concept of cognitive reserve defined (N=965) and that they were not full reviews i.e. letters, abstracts, conference proceedings or editorials (N=123), further reasons for exclusion are shown in Figure 1. The full-text of 138 articles

were retrieved and further evaluated in more detail. In total, five articles were systematic reviews which were included in the results section of this review[10-14]. Furthermore, 32 papers were excluded as they were narrative reviews and were only to be used in the introduction and discussion. Other reasons for exclusion at the full-text review stage were that the studies were not review papers, the concept of cognitive reserve not defined; inappropriate baseline population (patient populations with multiple sclerosis, brain injury, Parkinson's disease, HIV etc) and articles being unavailable.

Characteristics and focus of included reviews

Table 1 shows the characteristics of the five systematic reviews included. Four of the five reviews also included a meta-analysis of results[10,12-14]. The number of articles included in the individual reviews ranged from 18[12] to 69[10]. Sample sizes of the original articles ranged from 28 to 23329[10]. The outcomes of the reviews were wide-ranging (e.g., Alzheimer's disease, vascular dementia, non-specified dementia, all dementias and cognitive decline or cognitive impairment). There was a mixture of cohort studies and cross-sectional studies based on the general population and case-control studies which has cases of with different types of dementia compared against healthy controls. Three of the systematic reviews included studies that were of a case-control, cohort or cross-sectional design[10,11,14], and two of the systematic reviews included only longitudinal cohort studies[12,13]. Follow-up years were not given in two reviews[10,14], and ranged in the other three reviews from 1[11] to 45 years[12]. The number of indicators of cognitive or brain reserve investigated varied from one indicator[10,14] to five indicators[11]; also, the indicators investigated varied, as did the definitions used for the indicators (e.g., mentally stimulating activities or complex leisure activities were similar, but had different categorization criteria). Further, the reviews differed as to whether they only analyzed cognitive reserve (N=3)[10,11,14] or used the term brain reserve (N=2)[12,13].

Quality of the systematic reviews

The quality of included studies was evaluated using the measurement tool to assess systematic reviews (AMSTAR) criteria[15]. Each review was individually assessed against the set criteria, which comprises of eleven questions focused on scientific quality of included studies assessed and if the methods used to combine findings of the study were appropriate. <5 points indicates low review quality, 5 to 8 points indicates moderate review quality and >8 points indicates high review quality. The majority of studies scored low on assessment of review quality (4 reviews scored 4 points on the AMSTAR checklist), and only one review met the AMSTAR criteria for moderate systematic review quality (one review scored 7 points)[10]. The main reasons for scoring points were having a comprehensive literature search and detailing characteristics of the studies provided. None of the studies gained points for detailing conflicts of interest, assessing and docu-

menting the scientific quality of included studies, providing a list of included and excluded studies or providing an 'a priori' design. Further, the meta-analyses results varied for what outcome was tested (Alzheimer's disease, vascular dementia, any dementia, non-Alzheimer's dementia, cognitive decline or cognitive impairment) and also the test statistic generated (odds ratios, risk ratios or φ).

Description by possible indicators of cognitive and brain reserve

Education

Education was the only reserve indicator investigated in all five of the systematic reviews, and was analyzed both in relation to cognitive and to brain reserve. The results of the meta-analyses found a lower level of education to be significantly associated with an increased risk of Alzheimer's disease, vascular dementia, any dementia or cognitive decline. Definitions for 'high' levels of education varied across studies, as did definitions for the reference category, i.e. some studies used low levels of education and some used all participants except those with high levels of education. Although degree of literacy is thought to be a better measure of educational attainment[9], this was scarcely used across included studies, with years of education being far more extensively studied. Yet, all studies concluded that the results of the review indicated that a low level of education was a risk factor for dementia, cognitive decline or cognitive impairment. Only one of the reviews systematically examined pathology and neuroimaging studies[10], and this was a review which only examined education as an indicator for cognitive reserve. This review reported that out of 14 imaging studies investigated, 10 studies reported an increased level of degradation and pathology amongst those with higher levels of education. This is in line with the idea that those with higher levels of education have the ability to cope with greater levels of brain pathology before experiencing symptoms of the disease.

Occupation

Occupational attainment was only investigated in two meta-analyses[12,13], and work complexity was investigated in one systematic review[11]. The results from the meta-analyses conflicted, as one meta-analysis found occupation to be significantly associated with an increased risk for incident dementia[13], whereas, the other did not find any association between occupation attainment and changes in cognition[12]. Variability between results may be due to the use of different categorizations to describe occupational attainment (e.g., low occupation was described as house duties, farmers, domestic, blue collar in one article[16] and unskilled, semi-skilled, housewife in others[17,18]). Further, the different outcomes assessed and different controls for confounding indicators (e.g., age, gender, education) may have led to the inconsistencies in results. Also, across all the reviews

which investigated occupation, different terms were used in the definition (e.g., work complexity or occupation).

Leisure activities

Leisure activities is a very broad phrase for what could include many different and diverse activities, which could be stimulating mentally, socially, physically or via a combination of these. Three review articles described leisure activities as a potential indicator of cognitive reserve[11-13]. One review concluded that leisure activities was a more robust measure of brain reserve compared to premorbid IQ, education and occupation, as increased involvement in leisure activities throughout life was significantly associated with a reduced risk of incident dementia in all of the studies investigated even after controlling for confounding factors (e.g., age, occupation, education)[13]. One review emphasized that previous studies which investigated leisure activities in relation to incident dementia have been heterogeneous with regard to both study design and types of activities studied, yet, despite this lack of comparability; the majority of studies reported an association between increased participation in leisure activities and a reduced risk of developing dementia[11]. All of these reviews highlighted that the leisure activities should be "mentally stimulating", and one review went further to conclude that activities which are more complex, such as those which combine physical, social and mentally stimulating aspects, appear to have the most benefit in the protection against dementia onset[11]. Many of the included studies within the reviews suggested that any sort of mentally stimulating activity would have to be partaken on a regular basis to have a positive effect, although they differed as how to define regular involvement (e.g., >3 times a month or >3 times a week).

Social networks

Two review articles included studies describing social engagement within the indicator of leisure or mental activities[12,13], however, one review defined "social networks" independently of leisure activities[11]. This review showed that much fewer studies have examined the relationship between a social network and risk of dementia (4 of the 51 included studies)[11]. Again, the term social network is quite broad and within this description are a number of different indicators (e.g., number of confidants, marital status, living alone or satisfactory relationship with children or friends). Yet, all of the included studies reported associations between a greater social network and a reduced risk of dementia.

Premorbid intelligence and socioeconomic status

Two other potential indicators of cognitive reserve (premorbid intelligence and socioeconomic status) were also discussed in two reviews[11,13], but the number of included studies for these indicators was more restricted (6 studies for socioeconomic status and

2 studies for premorbid IQ). One review article examining the concept of premorbid IQ (intelligence before dementia onset) only evaluated two studies, and found that higher premorbid intelligence was significantly associated with a reduced risk of dementia[13].

One review examined socioeconomic status and dementia incidence[11], but concluded that there was insufficient evidence of an association between socioeconomic status, at any stage of life, and risk of dementia. Although education is the most common measure for socioeconomic status, included studies encompassed many different definitions for socioeconomic status (e.g., father's occupation or a mixture of schooling, occupational status and income). Results from the studies were inconsistent, with some suggesting a positive association between higher socioeconomic status and reduced risk of dementia[19,20], whereas other studies suggested the association was confounded by education, further, conflicting results were also found amongst studies investigating early-life socioeconomic status and risk of dementia[21,22].

Overall effect of reserve indicators

Two of the included reviews examined the overall effect of brain reserve towards dementia incidence by combining the effects of multiple potential cognitive reserve indicators[12,13]. The first review looked at the effect of three reserve indicators (education, occupation and mental activities), and found that overall high cognitive reserve was associated with decreased longitudinal cognitive decline[12]. The authors stated that this effect size was large and significant (φ=1.70, $p<0.001$), and the included studies controlled for a number of confounding factors. The most common confounding factors adjusted for included age and gender; however, multiple other confounding factors, such as disease history and physical function, were also adjusted for. Yet, studies differed widely as to how many and which confounding factors were adjusted for, with some studies not reporting any adjustments. The second review combined education, occupation, premorbid IQ and mentally stimulating leisure activities and found that higher reserve was associated with a reduced risk of incident dementia (OR=0.54, 95% confidence interval: 0.49 to 0.59)[13].

DISCUSSION

Summary of findings

The most widely investigated potential indicators of the cognitive reserve hypothesis include education, occupation and leisure or mentally stimulating activities. Further, social engagement or social networks has been investigated both as their own indicator; separate from other mental activities, and in combination with other leisure activities. Reviews examining the cognitive reserve hypothesis have found that the majority of

current evidence indicates that higher educational attainment, occupation and regular participation in leisure and social activities are associated with a decreased risk of future dementia.

There appears to be less decisive evidence, as compared to education, that occupation is associated with reduced risk of dementia and, therefore, if this supplies cognitive reserve. Yet, occupation appears to overlap with some of the other indicators discussed in other reviews, such as extensive social networks or regular participation in intellectually stimulating activities.

Perhaps there is an inverse relationship between occupation and leisure time activity, such as those who are unemployed or retired may have more leisure time than those who are employed, which could affect results. Further, different leisure activities investigated seemed to be very diverse and included a wide-range of activities, such as participation in sport, daily gardening or travelling. Therefore, which leisure activities may have a greater positive effect on reducing the risk of dementia is not clear, and what specific aspects of these activities may be most beneficial (e.g., social, physical or mentally stimulating or a combination of these) also requires further clarification. A similar potential indicator of cognitive reserve was described as "regular participation in intellectually stimulating activities"[10]. Although, this review did not specifically use the term leisure activities there is obviously an overlap between any intellectually stimulating activities and leisure activities in terms of potential benefits to cognitive reserve. This demonstrates the issue of accurately defining indicators of cognitive reserve. Further indicators that have been investigated were premorbid intelligence and socioeconomic status, but few studies have been conducted for both of these indicators, and the findings for the relationship between socioeconomic status and risk of dementia have been inconsistent[11].

Studies that have combined multiple indicators of cognitive reserve have concluded that higher cognitive reserve is associated with a decreased risk of incident dementia, and this effect was large and significant[12,13]. Yet, it has been noted that it is difficult to distinguish if a higher reserve decreases the risk of dementia or only delays its onset[11]. This requires further investigation.

Limitations of reviews

32 narrative reviews examining the cognitive reserve hypothesis were identified in our initial search. These were excluded from this review. In contrast, fewer systematic reviews or meta-analyses evaluating indicators of the cognitive reserve hypothesis have been conducted. Systematic reviews are important as they ensure that the evidence contained within the review summarizes the current available literature and are representative of high-quality evidence. The indicators that were discussed in the reviews differed and there was also heterogeneity between included studies as to how they

categorized different reserve indicators (e.g., whether social and leisure activities were combined as one category).

Education, occupation and leisure activities have been more widely explored, and premorbid IQ and socioeconomic status were explored to a certain extent. However, the findings from neuroimaging studies were only systematically examined in one of the reviews, even though neuroimaging studies have provided important findings for the concept of cognitive reserve[23]. Indeed, neuroimaging studies have shown that for patients with Alzheimer's disease with similar levels of dementia severity, higher years of education, occupational attainment and increased leisure activities is associated with lower levels of cerebral blood flow, a marker for Alzheimer's pathology[2]. In addition, animal studies were excluded as the focus of this review was human studies. Animal studies have provided a great deal of information regarding cognitive reserve as such studies allow for greater control of the environmental factors which could influence cognitive reserve. Indeed, animal studies have shown that adult mice that have been exposed to an enriched environment have significantly more hippocampal neurons[24] and reduced amyloid-β levels[25]. A recent review summarizes the current evidence gained from animal studies regarding the potential mechanisms of cognitive reserve towards Alzheimer's disease and how it may affect neurogenesis, neuroplasticity and the locus coeruleus-noradrenergic system[26].

Other potential indicators that were identified within narrative reviews, that were not included in any of the systematic reviews, included: dietary habits[27-29], personality indicators[30,31], prenatal environment[32,33], childhood environment[32,34] and genetic indicators[33-35]. Also, when exploring a model for cognitive reserve it should be noted that studies have suggested that alcohol consumption, substance abuse, social isolation and medical conditions such as depression, anxiety, diabetes, hypertension and pulmonary disease may all lower cognitive reserve capacity[26,27,32,36]. The systematic reviews examining cognitive reserve or brain reserve found in this review did not include proxy measures for brain reserve capacity such as brain size, head circumference or neuronal count.

Article Quality

The systematic reviews investigated in this review were assessed using the AMSTAR tool and the quality of the majority of reviews was found to be low, with only one review meeting the criteria for moderate quality[10]. None of the reviews detailed the conflicts of interest of their included studies, assessed and documented the scientific quality of their included studies or provided a list of both their included and excluded studies. More high-quality systematic reviews examining the different indicators of cognitive reserve individually and as a combined effect would be beneficial.

Limitations of original studies

Included studies within the different reviews differed widely with regards to what criteria were used for the indicators (e.g., how to define high vs. low education), and also how the indicator was measured (e.g., degree of literacy vs. number of years of education). Further, there were differences as to what methods were used for analysis (e.g., cross-sectional or longitudinal design), and what confounding factors were controlled for (e.g., sex, age). Three of the five systematic reviews examined cross-sectional and longitudinal studies together, even though it is difficult to determine causal inference from cross-sectional studies. It was suggested in one review that the methods for analysis and the differences in the design of the studies (e.g., populations used) were a major source of heterogeneity[10]. As well as variations in the independent indicator, there were many different cognitive outcome measures, effect size estimates and follow-up ranges. Therefore, the heterogeneity of the studies made comparing them difficult.

CONCLUSIONS

Current evidence suggests that education, occupation and leisure activities, with a social and mentally stimulating aspect, supply the cognitive reserve and offer a protective effect against the risk of dementia. However, other indicators may also supply the reserve, but research is lacking with regards to creating a full cognitive reserve model and determining the overall effect this has towards cognitive function and dementia. Further research is also needed to clarify how the different indicators may supply the cognitive reserve and offer a protective effect against dementia, a delay the onset of dementia or influence the progression of dementia and cognitive symptoms. Determining the importance and relevance of cognitive reserve and the indicators which supply the reserve (e.g., modifiable indicators such as involvement with leisure activities) could lead to new intervention strategies to reduce the risk of dementia. Creating a set definition of the indicators of the cognitive reserve model, and further understanding which indicators are the most important for defining cognitive reserve, would be needed before testing possible interventions that may increase the reserve supply and hence the risk of dementia.

Table 1: Characteristics of the included systematic reviews

First author (year)	Search date	N reviewed	Sample size range	Age range	Follow-up range	Components of cognitive reserve and brain reserve (where differentiated) identified	Meta-analysis results	Conclusions and recommendations for future research where stated	Reported limitations	Quality
Caamaño-Isorna (2006)	1966 to October 2005	19 (13 cohort and 6 case-control)	Cohorts: 593 to 6827 and case controls: 144 to 4088	Not shown	Not shown	Education Brain reserve not differentiated	Outcome tested: Alzheimer's disease, non-Alzheimer's dementia and all dementias. Lowest education vs. highest education RR (95% CI), Ri, P value AD 1.80 (1.43, 2.27), 0.61, <0.0001 Non-AD 1.32 (0.92, 1.88), 0.22, 0.2816 All dementias 1.59 (1.26, 2.01), 0.88, <0.0001 Any education except highest vs. highest education RR (95% CI), Ri, P value AD 1.44 (1.24, 1.67), 0.47, 0.0730 Non-AD 1.23 (0.94, 1.61), 0.21, 0.3030 All dementias 1.33 (1.15, 1.54), 0.51, 0.0740	Results confirm that low education may be a risk factor for dementia, especially for Alzheimer's disease. Results are in accordance with the cognitive reserve hypothesis that postulates that some aspects of life experience may protect against the clinical manifestations of dementia.	Original studies: -measurement and categorization of the independent variable	4
Fratiglioni (2007)	1985 to December 2006	23 (12 cross-sectional or case-control and 11 are cohort studies)	Cohorts: 154 to 14165 and cross-sectional/case controls: 107 to 6434	Cohorts: 33+ to 75+ and cross-sectional or case-control 52+ to 75+	Cohort studies: 1 to 26 years	Education Socio-economic status Work complexity Social network Leisure activities (complex activities which include physical, social and mental seem to have the most benefit) Brain reserve not differentiated	No meta-analysis	Factors acting at different periods across the life course and have an intellectually stimulating nature may contribute in increasing the neural reserve.	Original studies: - No data to help decipher whether education, work complexity, and social network and leisure activities can decrease the lifetime risk of disease or merely postpone the onset of dementia. Review: -Difficult to estimate publication bias	4

Table 1: Characteristics of the included systematic reviews (continued)

First author (year)	Search date	N reviewed	Sample size range	Age range	Follow-up range	Components of cognitive reserve and brain reserve (where differentiated) identified	Meta-analysis results	Conclusions and recommendations for future research where stated	Reported limitations	Quality
Meng (2012)	January 1980 to June 2011	66 studies for qualitative synthesis and 69 studies for meta-analysis	134 to 20938 (quantitative studies) and 28 to 23329 (qualitative studies)	55+ to 85+ (qualitative studies)	Not shown	Cognitive reserve: Education and lower incidence of dementia (although higher education leads to a more rapid decline once dementia threshold reached)	Outcome tested: Alzheimer's disease, vascular dementia, and non-specified dementia. Low education vs. high education Prevalence studies OR (95% CI), P value Any dementia 2.61 (2.21, 3.07), 0.001 AD 2.62 (2.06, 3.33), 0.001 VaD 2.11 (1.40, 3.19), 0.001 Unspecified dementia 2.79 (2.13, 3.66), 0.001 Incidence studies OR (95% CI), P value Any dementia 1.88 (1.51, 2.34) 0.001 AD 1.82 (1.36, 2.44) 0.001 VaD 2.75 (2.20, 3.45), 0.001 Unspecified dementia 1.48 (1.17, 1.86), 0.001	Quantitative and qualitative findings consistent with the cognitive reserve hypothesis that early education and stimulation may be seen to effect brain structure. Further research is needed to elucidate the underlying biological mechanisms by which education protects against the onset of dementia and influences its course and outcome. Further research: -determine the underlying biological mechanisms by which education protects against the onset of dementia and influences its course and outcome	Original studies: -differences in the design of observational studies are a major source of heterogeneity Review: -language bias -heterogeneity	7

Table 1: Characteristics of the included systematic reviews (continued)

First author (year)	Search date	N reviewed	Sample size range	Age range	Follow-up range	Components of cognitive reserve and brain reserve (where differentiated) identified	Meta-analysis results	Conclusions and recommendations for future research where stated	Reported limitations	Quality
Valenzuela (2006)[1]	September 2004	22	314 to 3608	Mean 68 to 81.5 years	2 to 22 years	Only mentions brain reserve. Brain reserve: Education Occupation Premorbid IQ Mentally stimulating leisure activities Overall effect	Outcome tested: incident dementia. OR (95% CI), P value for incident dementia. All high compared to low. Education: 0.53 (0.45, 0.62), <0.0001 Occupation: 0.56 (0.49, 0.65), <0.0001 Premorbid IQ: 0.58 (0.44, 0.77) Mentally stimulating leisure activities: 0.50 (0.42, 0.61), <0.0001 Overall brain reserve: 0.54 (0.49, 0.59), <0.0001	Complex patterns of mental activity in early-, mid- and late-life stages are associated with significant reduction in dementia incidence. Future research: -testing interventions that increase behavioural brain reserve in randomized control trials. -further neurobiological understanding of the brain reserve effect in humans	Review: Covariate control was not possible in the meta-analysis	4
Valenzuela (2006)[2]	September 2004	18	132 to 14883	18+ to 75+	1 to 45 years	Only mentions brain reserve. Brain reserve: Education Occupation Mental activities Overall effect	Outcome tested: Cognitive change as defined by the source publication. φ (effect size corrected for sample size) Education: 2.63 p<0.0001 Occupation: Not significant Mental activities: 1.39 p=0.003 Overall brain reserve: 1.72 p<0.0001	Affirms that the link between behavioural brain reserve and incident dementia is most likely due to fundamentally different cognitive trajectories rather than confound factors.	Original studies: - many different neuropsychological outcome measures and effect-size estimates -wide range of follow-up ranges -ascertainment bias	4

Abbreviations: AD, Alzheimer's disease; VaD, Vascular dementia.

Quality score assessed using A measurement tool to assess systematic reviews (AMSTAR); <5 points low review quality and 5 to 8 points moderate review quality. [1]Brain reserve and dementia: a systematic review; [2] Brain reserve and cognitive decline: a non-parametric systematic review

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Chapter 4.2

A systematic evaluation of stroke surveillance studies in low- and middle-income countries

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ABSTRACT

Background Reliable quantification of the burden of stroke in low- and middle-income (LMI) countries is difficult as population-based surveillance reports are scarce and may vary considerably in methodology. We aimed to evaluate all available primary stroke surveillance studies by applying components of a benchmark protocol [World Health Organization (WHO) 'STEP-wise' approach to stroke surveillance] and quantify the 'reported' burden of stroke in LMI settings.

Methods Electronic databases Medline, EMBASE, Scopus and Web of Knowledge were searched for population-based surveillance studies. Studies conducted in the LMI countries that reported on incident stroke were included. Data was extracted from each study using a pre-structured format. Information on epidemiological measures including crude and age-adjusted incidence rates, person-years, admission rates, case fatality rates, death certification, autopsy rates, measures of disability and other study specific information, in line with WHO-STEPS-stroke protocol, were recorded. Age-adjusted incidence rate data of stroke were combined using random-effects meta-analyses.

Results We identified seven studies that reported on burden of stroke in nine LMI countries, including aggregate information from 1,711,372 participants collected over 5,240,923 person-years. The age-adjusted incidence rates across the LMI countries varied widely, with the burden of total first-ever strokes ranging from 41 to 909 events per 100,000 person-years.

Conclusions Systematic evaluation of all available primary surveillance studies, particularly in the context of WHO-STEPS guidelines indicate inadequate adherence to standardized surveillance methodology in the LMI countries. Incorporation of standardized approaches is essential to enhance generalizability and estimate stroke burden accurately in these resource-poor settings.

INTRODUCTION

Stroke remains a major cause of death and disability globally, claiming more lives each year than HIV-AIDS, tuberculosis and malaria put together[1–3] and accounting for 46.6 million Disability-adjusted life years (DALYs) worldwide.[4] In 2005, mortality due to stroke was 5·8 million and more than 85% of all stroke-related deaths were estimated to occur in low- and middle-income (LMI) countries.[5-8] Accurate estimation of stroke burden in these settings is difficult due to shortage of resources to establish sustainable, reliable and standardized surveillance systems essential to generate comparable data.

In this context, the World Health Organization (WHO) has devised a tool for stroke surveillance that comprises six modules arranged in three different 'STEPS' based on the initial presentation and outcome of a stroke patient.[9] As the hierarchy of the modules is climbed, the costs and complexity of data collection processes increases. In LMI countries, where resources are limited the importance of seeking an optimum balance between feasibility and practicality is considered the mainstay of this system to gather stroke epidemiology data.[9] Interpretation of available reports in LMI regions is also complicated as data collection methods vary widely across studies. Systematic assessment of these studies, particularly in the context of the WHO surveillance guidelines, could potentially help (1) collect more reliable stroke estimates in LMI countries, (2) allow international comparisons across populations, and (3) shape public health interventions and policies aimed to improve the prevention and management of stroke.

Therefore, the purpose of this review is to: (1) systematically evaluate published studies on stroke surveillance in LMI countries in order to compare the strategies adopted by these studies with that of the stroke surveillance protocol proposed by WHO, and (2) quantify the burden of first-ever stroke in LMI countries reported in available studies from these settings.

METHODS

This review was conducted using a predefined protocol and in accordance to the PRISMA guidelines.

Search strategy

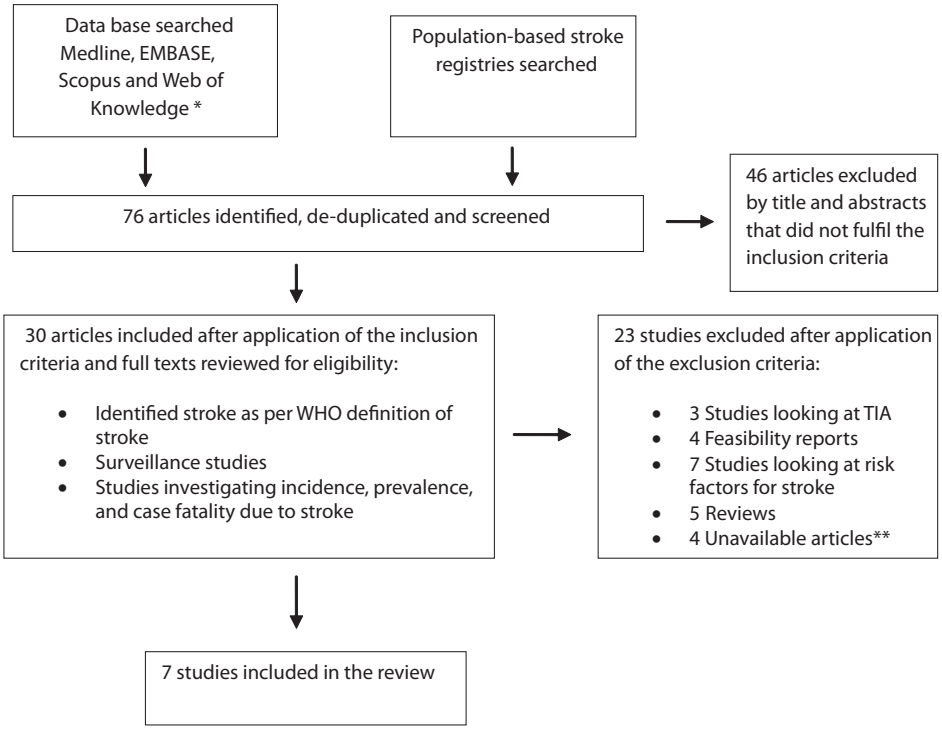
We conducted a systematic literature search to corroborate surveillance data and to identify candidate studies on stroke surveillance in low- and middle-income countries. A combination of controlled vocabulary and keywords were used in the search strategy in electronic databases: Medline, EMBASE, Scopus and Web of Knowledge and no restrictions were placed by date of publication. The search strategy was restricted

to English Language. Stroke surveillance registries were also searched for local data. The computer-based searches combined search terms related to the surveillance (e.g. "population surveillance", "surveillance", "sentinel surveillance", "survey"), outcome (e.g. "stroke", "cerebrovascular disease") and LMI countries (eg, "low-and middle-income countries" and "names of all LMI countries individually (Appendix)").

Study selection

Identification of LMI countries was based on World Bank groups by income per capita. Income categories used were for 2006-2010 according to Gross National Income (GNI) per capita.[10] Studies were included that identified stroke as per WHO definition: a clinical syndrome characterized as "rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin".[9] Population-based surveillance studies recording incidence and case-fatality were included in this review. Selection of studies and full eligibility criteria are presented in Figure 1. We cross-referenced all papers selected to identify further relevant studies.

Figure 1. Flow-diagram of literature search



* See Appendix

** Studies were not available in the archives and no contact with the authors was established to retrieve full texts.

Data extraction

We extracted data from each study using a pre-structured format. Specific epidemiological measures including crude incidence rates, person-years, age adjusted incidence rates, hospital admission rates, case fatality rates, death certification, autopsy rates and measures of disability were recorded. Incidence rates were age-standardized per 100000 individuals by use of direct method using the WHO World Standard population[11]. Usage rates of MRI or CT scan in stroke diagnosis, determination of stroke subtypes and whether it was a first-ever or recurrent event were also extracted. We also recorded and compared the dates of publication, year in which surveillance was conducted, location, study duration, specific aims and the strengths and limitations of each study.

Application and comparison of STEPS Stroke surveillance criteria to each study

The WHO STEPS - stroke surveillance protocol[9] employs three steps for stroke surveillance as described below:

STEP 1. Events in the hospital: Gives frequency of hospital admissions due to stroke. It consists of three modules that obtain core data. Module 1 includes basic history of the patient and records whether it's a first time or a recurrent stroke. Module 2 measures patient's functional level and in-hospital medical treatment. Module 3 consists of utilization of imaging facilities to classify the type of stroke.
STEP 2. Fatal events in the community: Enables calculation of mortality rates. This records the stroke events that result in fatality but do not reach the health facility. Information about these events is gathered from death certificates or verbal autopsies (Module 4). In countries where death certification is either not used routinely or uncertain in validity, verbal autopsies are used to ascertain the cause of death. Module 5 is the data collection from death records undergoing medical autopsies.
STEP 3. Non-fatal events in the community: Provides incidence rates and case-fatality. It represents the highest level of complexity in data collection. This involves medically recognizing all the stroke patients that are cared for entirely within the community and are not managed in the hospital at any time during or after the onset of stroke (Module 6).

Depending on the availability of resources, layers of information can be built upon the basic framework of these three steps adding detailed surveillance information in the form of modules.

The surveillance strategy and characteristics of each study were scrutinized and further divided to fit the guidelines proposed in the form of these three fundamental STEPS and various sub-modules of the WHO STEPS – stroke surveillance protocol. Even if the study was not based on the STEPS-protocol, we used it as a template by which the methodology of each study was compared (Supplementary table 1 and table 1).

Table 1: Incidence rates and case fatality (%) of population-based studies.

Country	Year	Study duration (years)	Person years	Age range	Total number incident strokes	Crude incidence of total strokes/ 100,000 (95% CI)	Age-adjusted incidence of total first ever strokes/ 100,000 (95% CI)	Hospital admission proportion	1 month case fatality proportion	CT/MRI Autopsy proportion	Timing of CT/MRI after stroke	Data of stroke types by age and sex
Nigeria	1971-1974	3	2409300	All	300	15 (13-17)	41 (36-45)	89%	NR	NR	NR	Total strokes
Mongolia	1971-1974	3	783900	All	653	50 (46-54)	78 (71-84)	51%	NR	NR	NR	Total strokes
India (Rohtak)	1971-1974	1	124700	All	82	27 (21-33)	48 (38-59)	55%	NR	NR	NR	Total strokes
Sri Lanka	1971-1974	1	562400	All	163	24 (20-27)	41 (35-47)	98%	NR	NR	NR	Total strokes
Ukraine	2000	1	125482	All	352	281 (248-313)	238 (213-263)	66%	23%	41%	NR	Total strokes
Georgia	2003	2·5	140926	All	233	165 (144-186)	103 (72-133)	66%	35%	66.7%	NR	Total, IS, PICH,SAH
Brazil	2004	1	75053	All	81	108 (84-131)	130 (125-220)	100%	19%	100%	2 days	Total, IS, PICH
India (Mumbai)	2006	2	313722	25->94	456	145 (132-159)	151 (137-165)	67%	30%	89%	NR	Total strokes
Bulgaria	2002	1	56447	45-84	157	622 (560-690)	RM- 909 (712-1105) UM-597 (482-712) RF -667 (515-818) UF-322 (248-395)	46%	35%	36%	1 week	Total strokes
Tanzania	2003-2006	3	648993	All	636	Urban-107·9 (88·1-129·8) Rural-94·5 (76-115)	Urban-315·9 (281·6-352·3) Rural-108·6 (89·0-130·9)	32%	NR	79%	15 days	Total strokes

IS Ischaemic Stroke, PICH Posterior Intracerebral Hemorrhagic Stroke, SAH Subarachnoid Hemorrhage, NR Not Reported

Statistical methods

Summary incidence rates (and corresponding 95% confidence intervals) were calculated by pooling the study-specific estimates using a random-effects model that included between-study heterogeneity (parallel analyses used fixed-effect models). Heterogeneity of findings across studies was primarily assessed by standard χ^2 tests and the I^2 statistic.[12]. Additionally, further detailed assessments of heterogeneity were carried out by comparing results from studies sub-grouped according to different study characteristics using meta-regression technique, and by performing sensitivity analyses to evaluate the influences of selected study or characteristics on the overall results. All statistical tests were two-sided and used a significance level of p <0·05. All analyses were performed using Stata release 11 (StataCorp, College Station, Texas).

RESULTS

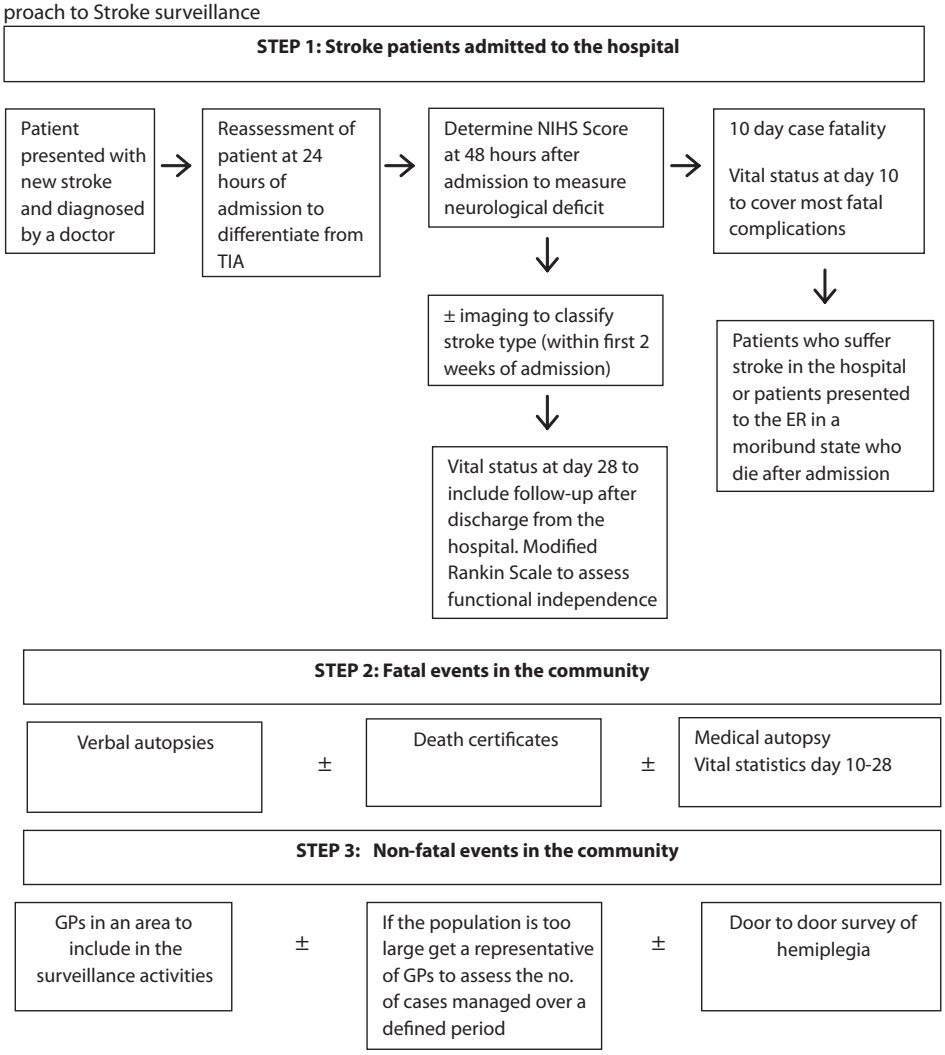
Study selection and characteristics

The initial search yielded 76 articles, the titles and abstracts of which were reviewed, out of which 30 fit the inclusion criteria and were subjected to further review. After application of inclusion and exclusion criteria to the full texts of these articles, we included a total of seven articles in this review (Figure 1). Their summary characteristics are provided in Supplementary table 1 and Table 1. According to the WHO regions, within the LMI countries, surveillance of stroke has been undertaken (and published) in Sri Lanka and India (South Asia);[13 14] Ukraine, Georgia, and Bulgaria (Europe);[15 16 18] Brazil (South America);[17] Mongolia (Western Pacific);[13] Nigeria (West Africa),[13] and Tanzania (East Africa).[19] Majority of the included studies were conducted primarily in urban populations with only two studies (Powles et al.[18] (Bulgaria) and Walker et al.[19] (Tanzania)) that included both urban and rural participants. Most studies restricted their study population to 45-85 years age group in which most strokes typically occur (population at risk).

Comparison with WHO STEPS surveillance protocol

We applied the three-step WHO STEPwise approach (Figure 2) to optimize stroke surveillance for LMI countries to individual studies (Supplementary table 1 and Table 1). All studies covered at least one part of the STEPS approach, but beyond that adherence to the STEPS protocol was patchy. Dalal et al.[14] based in urban India was the only study that incorporated the STEPS-stroke exclusively as its operational protocol. All studies established surveillance sites at local hospitals. Although patient core data, functional assessment and medical treatment were recorded comprehensively, the majority of these studies were generally limited by resources or availability of neuro-imaging to adequately classify and validate stroke subtypes. CT scanning rates varied substantially in

Figure 2. Descriptive protocol of an ideal stroke surveillance strategy adapted from WHO Stepwise approach to Stroke surveillance

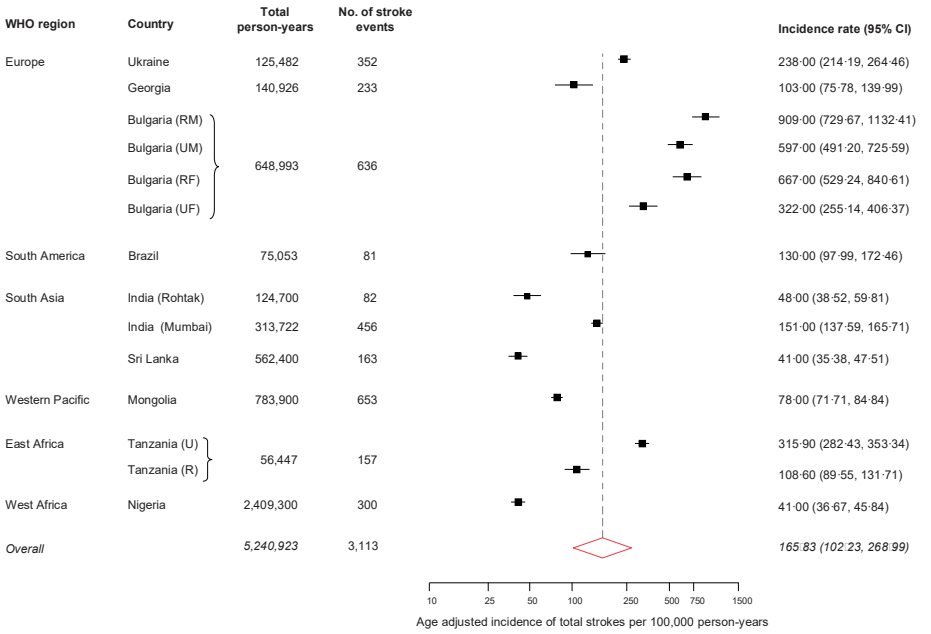


these studies (ranging from 0 to 100%). Minelli et al.[17] was the only study that performed CT scan on all of its recruited participants. By contrast, Aho et al. state that they did not have any access to CT scan facilities to enroll imaging-confirmed strokes at any of the participating centers due to unavailability (Supplementary table 1 and Figure 4).[13]

All studies recorded one-month case fatality data and three[13 17 19] followed up patients for six-month case fatality. While disability assessments were not adequately determined in the majority of studies, Minelli et al.[17] and Dalal et al.[14] were notable exceptions; they used Barthel scale for disease prognosis and Modified Rankin Scale (MRS) at day 28 to predict long-term disability respectively. (Death certification and autopsy

records were screened efficiently for mortality data in all studies. WHO-validated verbal autopsy method to ascertain fatal outcomes in LMI settings were employed by two studies.[16 19] The most complex level of data collection for non-fatal events in the community remained a challenge in all included studies, although efforts were taken to contact local general practitioners and community health workers in some studies. The door-to door survey method, typically aiming to provide prevalence data of stroke, was used by Tsiskaridze et al.[16] Walker et al.[19] aimed to raise awareness of stroke, and potential preventative and treatment measures in the general population in order to enhance overall stroke recognition.

Figure 3. Age adjusted incidence rates (95% CI) for incident stroke in low- and middle-income countries, based on available studies



RM,rural male; RF,rural female; UM,urban male; UF,urban female; U, urban; R, rural

Random-effects meta-analyses of age-adjusted incidence rates in low-and middle-income countries included in this review. Studies included in this meta-analysis collected stroke incidence data on all ages in their respective populations except for Bulgaria and Mumbai which were based on restricted age-bands. Age bands for each study: Ukraine: 0-45, 45-54, 55-64, 65-74, 75-84, >85; Georgia: 0-44, 45-54, 55-64, 65-74, 75-84, ≥ 85; Bulgaria: 45-54, 55-64, 65-74, 75-84; Brazil: <45, 45-54, 55-64, 65-74, ≥75; India (Rohtak): ≤ 44, 45-54, 55-64, 65-74,≥75; India (Mumbai) 25-34, 35-44, 45-54, 55-64, 65-74, 75-84, 85->94; SriLanka: ≤ 44, 45-54, 55-64, 65-74,≥75; Mongolia: ≤ 44, 45-54, 55-64, 65-74,≥75; Tanzania:0-44, 45-54, 55-64, 65-74, 75-84, ≥85; Nigeria: ≤ 44, 45-54, 55-64, 65-74,≥75.

Burden of stroke in LMI countries

In aggregate, included studies in this review involved 3,113 incident stroke events collected over 5,240,923 person-years from 1,711,372 participants. While all seven studies investigated the incidence of first-ever stroke (Figure 3 and Supplementary figure 1) only two[13 19] provided additional data on recurrent stroke events. Based on WHO global regions, age-adjusted stroke incidence rates are presented in figure 3 and table 1. Only two studies gathered surveillance data differentiating between urban and rural settings.[18,19] The age adjusted incidence rates were approximately three times higher in the urban population of Tanzania (315·9 per 100,000 person-years) than the rural population (108·6 per 100,000 person-years). By contrast, in Bulgaria, stroke incidence estimate in the urban residents was almost half (919 per 100,000 person-years) compared to their rural counterpart (1576 per 100,000 person-years). Combined age-adjusted incidence rate, based on all nine LMI countries, was 165·8 per 100,000 person-years (Figure 3). Upon removal of the estimates from Bulgaria (ie, the only study based on restricted age bands) in the sensitivity analyses, the age-adjusted incidence rate was reduced to 100.45 per 100,000 person-years (Supplementary figure 1). There was evidence of substantial heterogeneity across studies in both main and sensitivity analyses (I- squared estimates >50% and P <0.001). In the subsequent subgroup analyses, conducted to explain the observed overall heterogeneity, there were important differences in the estimates across several study level characteristics. For instance, studies published after 2005 (i.e. year when WHO-STEPS was formally operationalized), studies that had lower hospital admissions, those that had lower case fatality and higher CT/MRI autopsy proportions tended to have significantly higher stroke incidence rates (metaregression P<0·05 for all; Supplementary figure 2).

Figure 4. Features of studies included in the review

Study	LMI country	Events in hospital							Fatal events in the Community			Non-Fatal events in the community		
		First time stroke	Imaging to confirm stroke	Type of stroke	Recurrent stroke	10 day vital status	1 month case fatality	Death certificates	Verbal autopsy	Medical autopsy	6 month mortality	Disability assess-ment	Barthel Index	MRS
Aho et al	Nigeria	●	○	●	●	●	●	●	○	●	●	●	○	○
	Mongolia	●	○	●	●	●	●	●	○	●	●	●	○	○
	India	●	○	●	●	●	●	●	○	●	●	●	○	○
	Sri Lanka	●	○	●	●	●	●	●	○	●	●	●	○	○
Mihalka et al	Ukraine	●	●	●	○	●	●	●	○	●	○	○	○	○
Tsiskaridze et al	Georgia	●	●	●	○	●	●	●	●	○	○	○	○	○
Minelli et al	Brazil	●	●	●	○	●	●	●	○	○	●	○	●	○
Powles et al	Bulgaria	●	●	●	○	●	●	●	○	●	○	○	○	○
Dalal et al	India	●	●	●	○	○	●	●	○	○	○	●	○	●
Walker et al	Tanzania	●	●	●	●	●	●	●	●	●	●	○	○	○

● Relevant data collected in the study

○ Relevant data not collected in the study

DISCUSSION

This comparative review is the first attempt to systematically compare the methodology used across published primary reports of stroke surveillance studies. In context of the WHO STEPS stroke surveillance protocol as a benchmark approach especially in the low- and middle-income countries revealed a lack of standardized approach towards stroke surveillance. Additionally, evidence from these population-based surveillance studies showed that the apparent discordance in the burden of incident stroke events across the LMI countries can be due to inherent differences in the population groups involved and potentially to the large variations in the underlying methods used. For instance, the age-adjusted rate of new stroke events in Bulgaria was much higher than that of other countries. It is possible that this resulted from an apparent "hot pursuit" nature of this study that adopted a more vigorous approach and restricted age bands.

Furthermore, our subgroup analyses provide further support towards the potential role of heterogeneity between the studies that might affect the true disease burden estimates of stroke in the LMI countries. It is likely that the burden of stroke is under-estimated in the LMI countries, as there have been few large-scale systematic efforts to monitor the actual occurrence of cerebrovascular diseases in these settings. Limited numbers of nationally-representative surveys, lack of adherence to standardized optimum protocols, and limited disability information in the LMI countries have meant that conventional disease burden estimates have had to be derived from statistical modeling studies.[20 21] Ability to accurately quantify the disease and causative risk factors for stroke will make the development of locally appropriate prevention and treatment strategies easier to shape and the data collected more reliable.

In our review, most primary LMI countries studies only recorded hospital morbidity and mortality of acute stroke, which makes accurate estimation of the burden of disease at a population level difficult. While hospital based data collection has shown to be feasible and easy to accrue in the LMI nations[7], their interpretation can only be used to characterize patients who were admitted to a specific health facility. Hospitalization rates in LMI countries differ due to access barriers and hospital patient samples may not be representative of the population. These rates vary among urban and rural populations, and between countries - making meaningful comparisons difficult to achieve. Hospital-based studies are also more likely to under-diagnose minor strokes. Linking up hospital events to death certificates in order to record cause-specific mortality is another challenge in LMI countries, as the majority of these countries do not have comprehensive certification of death. The data that are available are difficult to use for cross-national comparison since they often use different methods, varying criteria, and time periods.

Disability remains an important attribute resulting from cerebrovascular disease and has enormous economic implications.[21] DALYs provide a useful indication of economic

and social burden of the disease as this records both morbidity and mortality.[23-25]. Several measures of disability assessment such as in-hospital disability scales (NIH Stroke Scale, and Glasgow coma scale) and post-acute event disability scales (Barthel index and MRS) have been formulated which provide disability scales encompassing the time of stroke onset and admission to the health care facility till the time of discharge and long term disability ascertainment. In future studies attention should be paid to inclusion of DALYs along with mortality and case fatality estimates.

It is well established that the rising burden of stroke in the LMI countries poses a high economic burden on the society as the event is typically followed by an array of expensive diagnostic measures, long hospital stays and often requires long-term rehabilitation.[26] One method to estimate this associated financial burden is through Cost of Illness studies (COIs).[27] COIs use prevalence and incidence data from surveillance studies to compose models in estimating costs. An earlier review estimated economic losses in 23 LMI countries to be approximately $84 billion in economic output in 2006-2015 from coronary disease, stroke and diabetes combined.[28] However, no such study is currently available to quantify economic losses attributed to stroke alone. Therefore, accurate cerebrovascular disease burden estimation (a prerequisite for reliable economic assessment) would help inform health policy in the LMI countries.

Strengths of our study include its systematic approach. Additionally, we relied exclusively on "nationally conducted" population-based surveillance studies that use "reported" (rather than "estimated" national data from the global burden of disease study) data that gives a more indubitable reflection of the actual burden of stroke in LMI countries. We have included studies that conducted national surveillances of stroke in their representative population subsets that varied considerably in methodology, outcomes ascertained and population settings. Some limitations merit careful consideration: As countries have gone through economic metamorphoses in the past decades, e.g. Russia, there may be some discrepancies between their current economic status and their status at the time the study was conducted. In order to minimize the disconformities we adhered to the categorization of the economic status of countries according to the 2006-2010 World Bank classification[10]. In our study, comparison has been made with the WHO STEPS stroke surveillance protocol as an operational guideline to collect stroke surveillance data in the LMI settings; however, there are other processes such as census data, from which burden of stroke can also be ascertained that could have not been sufficiently covered by the search strategy implemented in our review and limited the scope of our findings.

CONCLUSION

This study systematically evaluates the methodology protocols adopted for stroke surveillances and compared the available surveillance studies to generate an overall comparative scenario. Available literature generally indicates lack of standardized surveillance systems in low-and middle-income countries. Although, primary surveillance studies have been conducted in most global regions, they remain difficult to be compared across nations or regions due to heterogeneity in underlying methodologies. If a more standardized benchmark approach for stroke surveillance was adopted, better estimates of the current and future burden of stroke would become available, which could inform future health policy. As resources are limited in LMI countries it might be considered to focus on establishing sustainable systems to gather reliable and accurate data on stroke mortality and risk factors shared by other non-communicable diseases. Wider application of comprehensive and practical, yet feasible surveillance protocols such as WHO-STEPS-stroke could potentially maximize available local resources and produce comparable data to help shape effective stroke reduction programs and policies in resource-poor settings.

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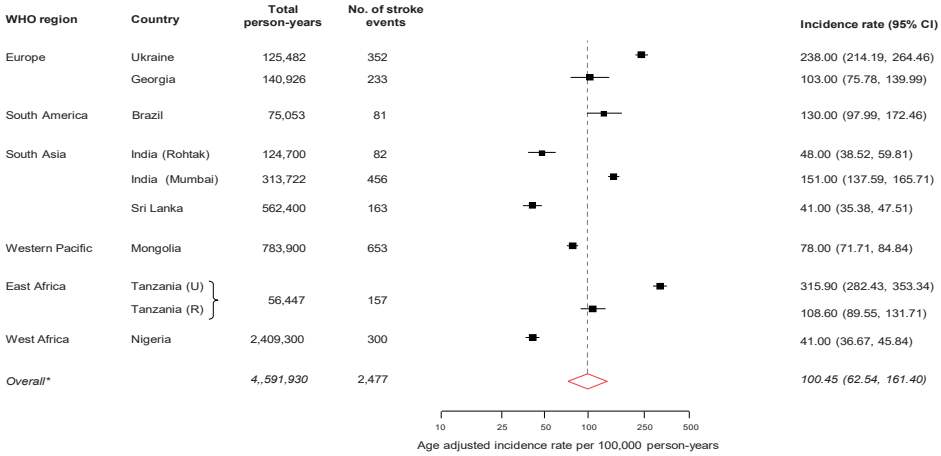
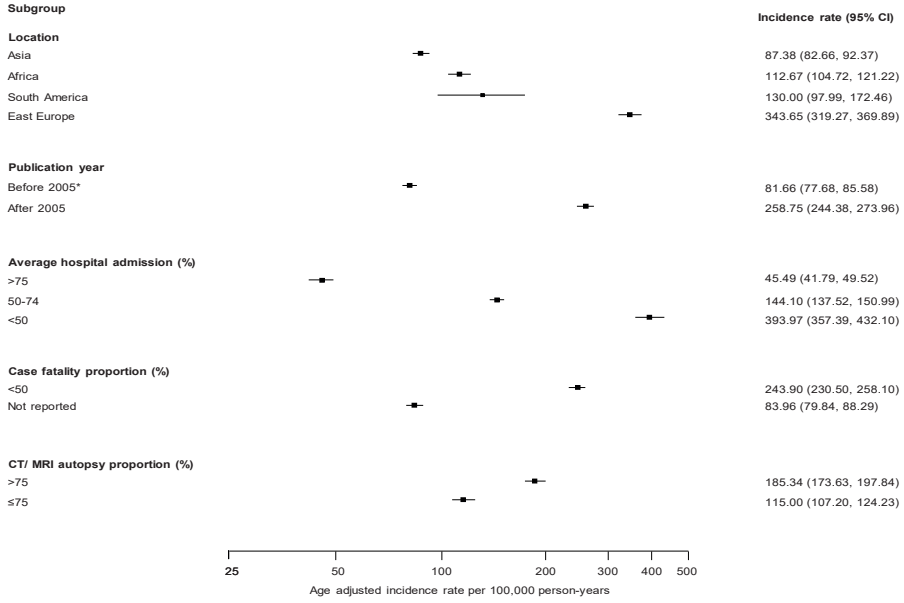
SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Age adjusted incidence rates (95% CI) for incident strokes in low- and middle-income countries, based on available studies



From random-effects meta-analyses; * Based on 6 studies and excluding Bulgaria; U, urban; R, rural; P for I- squared estimate <0.001

Supplementary Figure 2. Age-adjusted incidence rates (95% CI) for incident stroke in low- and middle-income countries, by different subgroups



Meta-regression P <0.05 for all subgroups; * year when WHO-Steps was formally introduced

Supplementary Table 1. Summary of studies included in this review

Study (Date of publication)	Country	Study Population	Study Aims	Adherence to WHO STEPS stroke criteria			Strengths (a) and/ or Limitations (b)
				STEP-1	STEP-2	Step-3	
Aho et al (1980) [13]	Nigeria, Mongolia, India, Sri Lanka	Urban population aged ≤ 44-≥75 Total 1198 in all areas WHO collaboration of implementing stroke registers in local hospitals and health centers in several populations.	To collect accurate data on: • the magnitude of stroke in each community • social and clinical profile of stroke patients • preventive measures, diagnostic procedures and rehabilitation applied to the patient • natural history of stroke	Module 1 Incident cases in the hospitals of first-ever and recurrent stroke Module 2 Functional level assessment and type of management	Module 4 Death certificates Vital status at 3 weeks. Module 5 Autopsy findings	Module 6 Survey of general practitioners and nursing institutions. Rehabilitation, disability grade, functional progress and return to work at 1 year	**(a)**Incidence of stroke was measured in different populations on a uniform scale to provide comparable data. This data provides an estimate for resources required for stroke treatment and rehabilitation. The study provides case fatality rates and risk factor profiles in individual populations **(b)** No data on stroke prevalence was provided. No CT scan was provided due to unavailability at any of the participating centers.
Dalal et al (2008) [14]	India	156861 inhabitants of H-district of Urban Mumbai aged between 25 – over 94 years.	This study aimed to establish prospective community-based stroke registry of patients presenting with first ever stroke and to collect standardized data on annual incidence, stroke subtypes, and 28-day case fatality rate during 1-year period.	Module 1 Data collected from medical practitioners, major hospitals, nursing homes, CT diagnostic centers and municipal health authorities Module 2 NIHSS scale for neurological deficit on admission and follow up and MRS evaluation for performance of specific tasks at day 28 was recorded Module 3 Stroke sub typing by CT scan	Module 4 Death certificates were scrutinized from the death record office and vital status at day 28 was recorded. "Verbal autopsies" were a vital component in the research protocol Module 5 Medical autopsy records not included	Module 6 Surveillance by general practitioners in the area	**(a)**Data collection by "hot pursuit" (prospective case registration) and "cold pursuit" (retrospective case registration) methods **(b)** No data on recurrent stroke No surveys to determine non-fatal events in the community e.g. prevalence of hemiplegia in the community.

Supplementary Table 1. Summary of studies included in this review (continued)

Study (Date of publication)	Country	Study Population	Study Aims	Adherence to WHO STEPS stroke criteria			Strengths (a) and/ or Limitations (b)
				STEP-1	STEP-2	Step-3	
Mihalka et al (2001) [15]	Ukraine	125482 inhabitants of Urban Uzhgorod in west Ukraine. Age range <45-≥85	A prospective population-based study to evaluate stroke incidence and 30 day case fatality. The study results conflicted with the statistics estimated by the WHO. The study concluded low incidence than that expected from WHO statistics.	Module 1 Core data on patient demographics. Cases identified from hospital records, registry of calls of the emergency neurology service and registries of the city's neurology service and home calls Vital status at 1 week. Module 2 Therapeutic interventions Module 3 Stroke subtypes by CT	Module 4 Death certificates Vital status at day 30 Module 5 Autopsy records of the hospitals and the county forensic department	Module 6 District registries of GP zones. Follow up by regular personal contact with all GPs and their nurses for 12 month period	**(a)** Risk factor assessment carried out by enrolling neurologist **(b)** No record of first time or recurrent stroke No disability grade and rehabilitation No estimate of burden of stroke in the community by survey of hemiplegia in the community
Tsiskaridze et al (2004) [16]	Georgia	1080000 people living in the suburban town of Tbilisi. Age range: <45-≥85	To establish a population-based registry to determine the incidence and case-fatality rates of first-ever and recurrent stroke in a defined urban population.	Module 1 Daily checking of hospital registrations and refusals Daily checking of emergency medical service calls Daily checking of outpatient data in polyclinics serving the districts Module 2 In hospital treatment protocols Module 3 CT/MRI performed within 30 days of stroke onset.	Module 4 Weekly checking of death certificates, verbal autopsies: contact with volunteers. 30 day case fatality Module 5 Weekly checking of autopsy protocols	Module 6 Daily rounds in the study region (field work), including door to door survey	**(a)**Door to door surveillance to assess non-fatal stroke events in the community. "Hot pursuit" study to find out the majority of stroke cases not hospitalized Diagnostic techniques were utilized to determine the cause of stroke and associated risk factors **(b)** No data was gathered for disability grades and need for rehabilitation

Supplementary Table 1. Summary of studies included in this review (continued)

Study (Date of publication)	Country	Study Population	Study Aims	Adherence to WHO STEPS stroke criteria			Strengths (a) and/ or Limitations (b)
				STEP-1	STEP-2	Step-3	
Powles et al (2002) [17]	Bulgaria	Inhabitants of Varna city aged 45-84 37791 – Urban population 18656 – Rural Population	A "hot pursuit" prospective study to measure stroke incidence and case fatality rates in urban and rural populations, as the large number of stroke cases did not receive specialist attention in this region. To confirm the hypothesis that stroke incidence was high in this region and was higher in rural than urban population	Module 1 Data collected from emergency centers, hospital emergency room, duty doctors and nurses in neurological units in the area. Vital status at 1 week was recorded Module 2 Glasgow coma score was assessed Discharge notes, clinical records and records of disability within the hospital were monthly checked Module 3 Stroke distinguished from TIA Classification by subtypes was made for minority of cases who received ancillary investigations	Module 4 Death registrations were scanned weekly Vital status at day 28 was recorded Module 5 Autopsy protocols	Module 6 Monthly ambulance records were screened for missed cases Residential homes for elderly	**(a)** This "hot pursuit" study maximized case ascertainment of stroke events and determined first-ever stroke accurately. **(b)** No data was gathered for survey of prevalence of hemiplegia/ hemiparesis in the community as a determinant of stroke events in the community
Minelli et al (2007) [18]	Brazil	75053 inhabitants of the city of Matao aged <45 - >75	To determine the incidence of stroke, stroke subtypes, case fatality and prognosis after 1 year of follow-up	Module 1 All hospital admissions and discharge lists were checked weekly Module 2 Barthel scale was assessed for prognosis Module 3 30 day Case fatality was assessed and followed up for 1 year Stroke subtypes were determined	Module 4 Death certificates from the study period were checked monthly to search for patients who died at home and had not been referred to the hospital Module 5 Autopsy findings were recorded where applicable	No data recorded	**(a)** A research team comprising of neurologists and nurses was set up. All patients were assessed by a member of the research team Radiology records were checked for suspected stroke cases CT was done on every suspected case of stroke **(b)** Only data on first ever stroke was used in the analysis. No data on recurrent strokes. The patients with clinical evidence of a previous stroke were excluded from the study

Supplementary Table 1. Summary of studies included in this review (continued)

Study (Date of publication)	Country	Study Population	Study Aims	Adherence to WHO STEPS stroke criteria			Strengths (a) and/ or Limitations (b)
				STEP-1	STEP-2	Step-3	
Walker et al [19]	Tanzania	159814-Rural population of Hai and 56517 Urban population of Dar es-Salaam in all age ranges	A methodological study aimed to provide reliable data on the incidence of stroke in urban and rural Tanzania	Module 1 Medical ward admission books and discharge lists were examined every 2 weeks Module 2 Not conducted Module 3 CT scan was done within 15 days of stroke onset to distinguish between haemorrhagic and ischemic stroke	Module 4 As death certification is incomplete in sub-Saharan African regions, verbal autopsies were conducted within 1 month of death with the care givers or relatives of the deceased. Module 5 Not conducted	Module 6 Awareness about stroke was raised in the general population and within community structures at the time of censuses. Patients identified by enumerators passed information to clinical officers so that patients can be assessed at home.	**a)** Measured first-ever stroke and recurrent stroke. Compared urban/rural incidence rates. Verbal autopsies were used to identify events in the community Risk factor assessment was undertaken. 'Enumerators'- nurses, teachers, and community development workers were trained to identify people with stroke. **b)** No treatment and discharge protocols were recorded. No measure of disability was assessed for the patients. Stroke subtypes were not recorded

Appendix. Search terms and names of low income and lower-middle income countries included as headings in the literature search in accordance with World Bank's criterion for classifying economies as per gross national income (GNI) per capita.[1]

Published studies were identified through electronic searches limited to the English language using MEDLINE, EMBASE, Scopus and Web of Knowledge databases. Electronic searches were supplemented by scanning reference lists of articles identified for all relevant studies (including review articles), by hand searching of relevant journals and by correspondence with study investigators. Published reports of local stroke registries were also searched for.

(i) MEDLINE strategy to identify relevant exposures and outcomes:

("stroke"[Mesh] OR "cerebrovascular disorders"[Mesh] OR ("population surveillance"[Mesh] OR "surveillance"[All Fields] OR "sentinel surveillance[All fields]" OR survey"[All Fields]) AND ("countries names"* OR "Low and Middle Income countries")

Each term was specifically translated for searching alternative databases.

Below is the list of all the countries' names that were used in the search strategy with "OR" in between in each country name.

LOW-INCOME COUNTRIES
Afghanistan, Bangladesh, Benin, Bhutan, Burkina Faso, Burundi, Cambodia, Cameroon, Central African Republic, Chad, Comoros, Congo, Côte d'Ivoire, Democratic People's Republic of Korea, Democratic Republic of the Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Guinea-Bissau, Haiti, India, Kenya, Kyrgyzstan, Lao People's Democratic Republic, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Moldova, Mongolia, Mozambique, Myanmar, Nepal, Nicaragua, Niger, Nigeria, Pakistan, Papua New Guinea, Rwanda, Sao Tome and Principe, Senegal, Sierra Leone, Solomon Islands, Somalia, Sudan, Tajikistan, Timor-Leste, Togo, Uganda, United Republic of Tanzania, Uzbekistan, Viet Nam, Yemen, Zambia, Zimbabwe
LOWER-MIDDLE INCOME COUNTRIES
Albania, Algeria, Angola, Armenia, Azerbaijan, Belarus, Bolivia, Bosnia and Herzegovina, Brazil, Bulgaria, Cape Verde, China, Colombia, Cuba, Djibouti, Dominican Republic, Ecuador, Egypt, El Salvador, Fiji, Georgia, Guatemala, Guyana, Honduras, Indonesia, Iran (Islamic Republic of), Iraq, Jamaica, Jordan, Kazakhstan, Kiribati, Maldives, Marshall Islands, Micronesia, Morocco, Namibia, Paraguay, Peru, Philippines, Romania, Samoa, Serbia and Montenegro, Sri Lanka, Suriname, Swaziland, Syrian Arab Republic, Thailand, The former Yugoslav Republic of Macedonia, Tonga, Tunisia, Turkmenistan, Ukraine, Vanuatu, West Bank and Gaza Strip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Chapter 5

Discussion

In this chapter, I discuss the main findings of our research on cognitive outcomes in different populations along with methodological issues. Furthermore, I present the clinical implications of findings and considerations for future research.

MAIN FINDINGS

Breastfeeding

The World Health Organization (WHO) recommends six months of exclusive breastfeeding as a global public health goal for infant's overall health and wellbeing.[1,2] Benefits of breastfeeding vs. formula feeding have been shown for infant diarrhea, otitis media, respiratory diseases and insulin dependent diabetes mellitus.[3,4] However, results of studies examining the association of breastfeeding duration with cognitive development are still strikingly inconsistent. Anderson et al. concluded in a meta-analysis that reported IQ differences favor breastfeeding over formula feeding.[5] On the contrary, in a recent review, Kramer et al. concluded that there were no advantages in child cognition in children exclusively breastfed for 6 months; neither compared to children breastfed partially for 6 months nor compared to children with shorter than 6 months of breastfeeding duration. Results were similar for developed and developing countries.[1,6] One of the explanations of this controversy has been confounding of the association between breastfeeding and cognitive outcomes by factors such as maternal intelligence and socioeconomic status. These factors not only affect cognitive performance of the child but also the mother's decision whether or not to breastfeed. Additionally, some studies did not address questions of duration of breastfeeding, making the dose-response relation difficult to assess.[7] Finally, formula milk has been fortified with long chain polyunsaturated fatty acids in the last ten years, the composition of which mimics breast milk very closely. Studies comparing fortified formula milk to breast milk are, however, scarce. In Chapter 2.1, we describe the associations between breastfeeding duration and child non-verbal IQ attenuate after adjusting for various confounders. Our study is one of a few that prospectively assesses breastfeeding throughout the first year of life, thereby eliminating potential recall bias. Furthermore, we were able to adjust our analysis for maternal diet. It has been argued that LCPUFA content of maternal milk is dependent on maternal diet;[8] with higher quantities reported in mothers who adhere to "healthy diet". Previously, many studies have chosen fish intake and maternal folate levels as markers for maternal health reporting beneficial effects on the associations between breastfeeding and child cognitive development.[9,10] Our adjustment for maternal dietary patterns did not affect the results. However, since the availability of maternal dietary patterns was only limited to mothers of Dutch origin, these results cannot be generalized to other ethnicities and thus should be interpreted with caution. In light of these findings,

we conclude that the association between longer breastfeeding duration (regardless of exclusivity) and child IQ is confounded by maternal intelligence and sociodemographic factors such as maternal age, maternal BMI, parental education, and family functioning.

Arterial stiffness and blood pressure

It is well established in previous lines of research that cardiovascular markers are primary modifiable risk factors that affect cognitive outcomes in the elderly with particular emphasis on cognitive decline.[11] Arterial stiffness is of particular interest because it is an easy measure of structural and functional changes in the vessel wall.[12] Arterial stiffness is also related to hypertension and both are risk factors for cognitive decline and dementia.[13,14] These cardiovascular risk factors may have their correlates of subtle vascular damage very early in life which may affect late-life health, in our case late-life cognition. In Chapter 2.2, we tested whether arterial stiffness, measured by carotid-femoral pulse-wave velocity and blood pressure affect cognition in early life. We found no evidence for an association between arterial stiffness and child IQ at age 6 years. A negative association between diastolic blood pressure and child non-verbal IQ was observed that attenuated after adjustment for maternal factors. We concluded that the association of cardiovascular risk factors with child IQ is not evident in early age.

In the elderly, we simultaneously investigated the association between arterial stiffness, blood pressure and general cognitive factor (g-factor)[15]. Next to higher age being known as the strongest risk factors for cognitive impairment, arterial stiffness was also suggested to be an early marker of subclinical vascular damage with high predictive accuracy.[16] Previous literature highlights that the association between vascular risk factors, particularly blood pressure and cognitive impairment may differ for the very old (>75 years) and the young-old (<75 years).[17,18] It is therefore important to distinguish between the young-old and the old-old, given differences in prevalence and risk of disease comorbidity, frailty, sensory and motor disabilities, cognitive impairment and dementia between the two groups.

In our study, we found that each unit increase in arterial stiffness as measured by carotid-femoral pulse-wave velocity is associated with a 0.40 decline in g-factor in the fully-adjusted model. This association observed is primarily driven by elderly <75 years old. For persons >75 years we found no association between arterial stiffness and g-factor. We observed similar associations between systolic blood pressure and g-factor. The associations between diastolic blood pressure and g-factor in persons <75 years old attenuated after adjusting for education, BMI, smoking, diabetes and blood pressure lowering medication.

These findings highlight that accumulation of subclinical vascular risk factors throughout the ageing process are responsible for cognitive changes in late life. We were not able to track their basis into early childhood.

Subjective well-being and mortality

The vast majority of literature related to clinical research is based upon objective measures to assess health and well-being. However, recent evidence has pinpointed that subjective indicators of well-being are of equivalent importance as objective measures in the assessment of health outcomes.[19] We conceptualised subjective well-being as a continuum consisting of parameters of physical functioning at one end and mental well-being at the other. We included basic activities of daily living, which is a subjective indicator of locomotive health, and instrumental activities of daily living that takes into account the cognitive attributes of performing daily tasks. Together, they cover a large aspect of overall physical and cognitive functioning. Mental health was conceptualized as a combination of somatic symptoms (i.e. physical manifestations of depression), negative affect and positive affect. Finally, quality of life is studied as a measure of subjective well-being that aims to bridge the physical and mental attributes of overall well-being. In chapter 3.1 we aimed to determine whether various measures of subjective well-being are associated with all-cause mortality.

We found that all measures of subjective well-being were related to mortality after adjustment for age, gender, education, cognition, prevalent chronic diseases (i.e. stroke, myocardial infarction, heart failure, diabetes, COPD, and joint problems), cardiovascular risk factors and lifestyle factors such as BMI, smoking and drinking. However, once mutual adjustment was carried out to determine the independent association of each measure of subjective well-being with mortality, we found that only impairment in self-reported BADL and IADL were independently related to all-cause mortality; QoL, somatic symptoms, negative affect and positive affect were not independently related to mortality after mutual adjustments.

Subjective memory and stroke

Cognitive impairment and dementia are well known to occur as a long-term consequence of damage to the brain parenchyma after a stroke event.[20] Stroke, cognitive impairment, and dementia share common vascular risk factors that influence each other in their pathogenesis.[21] In light of this evidence, we aimed to determine the association between early cognitive impairment, which can present as subjective lapses in memory, and future risk of stroke in older persons.

In chapter 3.2 we found a higher risk of total stroke in persons who answered "yes" on the subjective memory complaints question than in persons who did not complain about their memory. We simultaneously investigated the association of an objective test of memory, the MMSE with the risk of stroke and found no significant associations. We scrutinized our data further by censoring participants in whom dementia developed during follow-up and excluded those who had an MMSE score lower than 26 at baseline to preclude that subclinical dementia may affect the association between subjective

memory complaints and stroke. We found similar results in these subgroups. In addition, we found significant interaction with education. Only in persons with a high vocational or university level of education, those who complained about their memory were at increased risk for stroke compared with persons who had no memory complaints.

These results highlight the significance of subjective memory complaints, especially in highly educated persons who may be protected by their cognitive reserve.[22] Higher cognitive reserve may mask early cognitive changes that may have resulted from subclinical vascular insults to the brain. Despite complaining about their memory, these persons may still perform well on objective cognitive tests. Therefore, complaints about memory in highly educated persons may be predictive of an increased risk of stroke.

Cognitive reserve

In clinical practice, the cognitive functional impairment in patients after enduring a stroke of a given magnitude can present in different ways; in some patients having profound impairment while in others producing minimal effects. This clinical phenomenon highlights that certain individuals endure neurodegenerative disorders that affect cognition such as stroke or dementia better than others. These differences in cognitive outcomes associated with similar brain pathology is hypothesized to be driven by the concept of cognitive reserve.[23] Notwithstanding several attempts to produce a coherent account of the concept of cognitive reserve, there is still lack of consensus in its definition. Previous lines of literature have proposed different ways to assess or measure cognitive reserve, but to date none has come to a conclusive recommendation.

In chapter 4.1 we attempted to pull together and review all available systematic review literature on the concept of cognitive reserve through systematic literature search in seven databases. We identified five systematic reviews that incorporated findings from cohort, cross-sectional and case-control studies. Examined outcomes included Alzheimer's disease, vascular dementia, non-specified dementia, all dementias and cognitive decline or cognitive impairment. Education, occupation and leisure or mentally stimulating activities were suggested to supply cognitive reserve and offer a protective effect against the risk of dementia. Premorbid IQ and socioeconomic status have not been investigated as thoroughly and showed inconsistent results. Other indicators such as dietary habits and genetic indicators are understudied. Although it is shown that cognitive reserve has a protective effect against cognitive decline and dementia, the available research to date is insufficient to create a full cognitive reserve model.

Extrapolating cognitive outcomes to populations in low- and middle-income countries

Stroke is one of the most debilitating neurological diseases accounting for approximately 5.5 million deaths per year globally with a projected 61 million disability-adjusted

life years lost by 2020.[24,25] 85% of all stroke mortality is estimated to occur in low- and middle-income countries. Furthermore, the incidence rates of stroke in low- to middle-income countries are estimated to exceed that in high-income countries.[26] It is crucial to accurately estimate stroke burden in these low- and middle-income countries, both for risk factor identification, and to establish prevention strategies and health policies to reduce this burden. There is an urgent need for accurate and reliable stroke surveillance systems to generate comparable data. Also, existing population-surveillance systems of stroke in these low- and middle-income countries are scarce and vary in methodology. Therefore, in chapter 4.2, we conducted a systematic literature search and identified seven studies from nine low- and middle-income countries that reported the burden of stroke. We meta-analysed the data to report the age-adjusted incidence rates of stroke in order to determine the burden of first-ever stroke in these countries. We report 41 - 909 first-ever stroke events per 100,000 person-years. Due to limited numbers of nationally representative surveys, lack of adherence to standardized optimum protocols, and limited disability information in low- and middle-income countries, conventional disease burden estimates had to be derived from statistical modelling. We conclude that burden of stroke is underestimated in these countries, as there have been few large-scale systematic efforts to monitor the actual occurrence of cerebrovascular diseases in these resource-poor settings.

METHODOLOGICAL CONSIDERATIONS

Cognitive tests in different populations

With the rise in patients with dementia due to changes in the global age demographics, there is a clear need for valid and reliable cognitive tests that can be applied in population studies on a large-scale. The large domain of cognitive function has previously been categorised into 1) crystallised abilities such as reading, general knowledge, and language abilities[27], all of which are less likely to change with ageing and cognitive deterioration, and 2) fluid abilities such as memory, attention, and general cognitive speed that have a tendency to change with the ageing process or a disease state with clinical or sub-clinical cognitive changes.

The most commonly used objective measurement of cognitive function is the Mini mental state examination (MMSE).[28] However, Houx et al.[29] pointed that the MMSE includes many crystalline functions, which do not change much over time. Other drawbacks are the fact that subtle improvements or deterioration over time are not captured in the over-all score. Moreover, subjects score higher on subsequently repeated tests, which hinders the reliability. We used the MMSE as well as a simple question on subjective memory, i.e. "do you have memory complaints?" (yes/no), and associated these

measures with stroke. We found that MMSE was not associated with the risk of stroke while the subjective memory complaints were significantly associated. The importance of these results is two-fold: a) subjective health and health perception may be as important as objectively measured health, and b) as discussed above, the use of MMSE may be disadvantageous in large-scale population studies. Other tests that are more sensitive to detect small changes in cognitive function in various domains should be employed. General cognitive factor (g-factor) is an example of such a test. In chapter 2.2 g-factor was utilised as a cognitive testing battery which constituted a colour-word interference subtask of the Stroop test, LDST, verbal fluency test, delayed recall score of the 15-WLT, and Purdue pegboard test. In its construct, g-factor utilizes the shared variance between individual cognitive tests, and can be interpreted as a common underlying factor to a variety of cognitive domains that is independent of each domain separately and is linked to general cognitive function or general intelligence.

In children, choosing tests to determine cognitive function is even more challenging. In early childhood, brain maturation and behaviour skills are developing simultaneously and synergistically. These processes are interactive and intertwined with each other and external environmental factors.[30] Furthermore, infant and child cognition changes dramatically with age requiring several measurement tools of cognitive function across different age groups. For instance, several intelligence tests incorporate tests that require verbal output; whereas language development is largely different in a 2-year-old, when small sentence formation is the peak performance, or a 4-year-old, where vocabulary enhancement is the most crucial linguistic developmental milestone. Furthermore, in population studies with different ethnic groups who speak different languages, early childhood verbal tests cannot be implied without the risk of misclassification. On this basis, we chose to measure child IQ in chapter 2.1 using two subtests of a Dutch nonverbal intelligence test: the Snijders-Oomen Niet-verbale intelligentie Test–Revisie (SON-R 2½ - 7)[31] in children aged 6 years. This was designed to minimize the reliance on acquired knowledge and verbal ability. The test allows a scaled total score to be calculated for any combination of subtests with the same distribution characteristics as the IQ score. Two subsets were chosen for Generation R: Mosaics, which assesses spatial visualisation abilities, and Categories, which assesses abstract reasoning abilities. In a different sample of 626 children, aged 4.5–7.5 years, the correlation between the total scores derived from the Mosaics and Categories subsets, and the IQ scores derived from the complete test, was $r = 0.86$.[32] For our study, raw test scores were converted into nonverbal intelligence scores using norms tailored to the child's exact age.[33] Using nonverbal IQ as our main outcome increases generalizability of our study results to different populations.

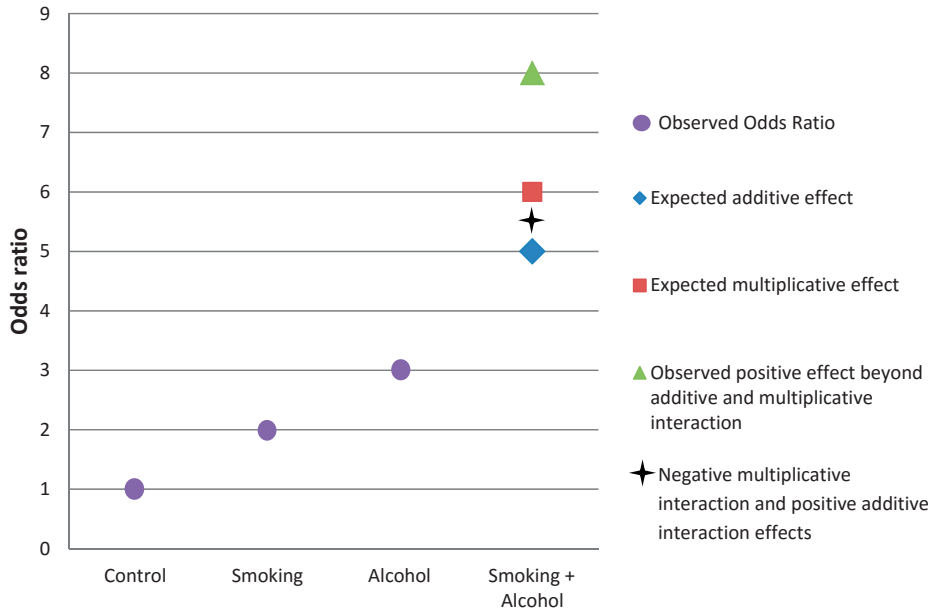
Interaction

Interaction occurs when the effect of a determinant on the outcome is different across strata or the continuum of another variable, which can be both a background or risk factor. According to Rothman, there are two types of interaction: statistical and biological.[34] Statistical interaction can be assessed by adding a product term to the models used in the analysis. The interpretation of regression coefficients of this product term depends on the type of statistical model used. In linear regression, the regression coefficient of this product term is interpreted as departure from additivity, while in logistic regression and Cox regression the regression coefficient of the product term indicates the degree of departure from multiplicativity.

Biologic interaction is typically defined as the interdependent operation of two or more causes in the same causal model.[35] Consider a simple hypothetical example – the causal model for the incidence of oesophageal cancer: smoking doubles the risk of oesophageal cancer while drinking alcohol triples the risk of oesophageal cancer (figure 1). Smoking and alcohol are both causes. They may act independently or may have a synergistic effect on the development of oesophageal cancer. The synergistic interdependence is subject to evaluation by assessment of interaction. In a multiplicative model, interaction is evaluated as deviation from multiplicativity (deviation from the expected odds ratio (OR): 2 x 3 = 6); In an additive model, interaction is evaluated as deviation from additivity (deviation from the expected OR: 2 + 3 = 5). If the observed OR were 8, then the interaction is so strong and positive that it does not matter on which scale (multiplicative or additive) interaction is evaluated. If the observed OR were 5.5, this would suggest negative interaction on a multiplicative scale, i.e., smoking and drinking together would have less than the expected effect predicted for the incidence of oesophageal cancer on the basis of separate analyses. However, on an additive scale, this would suggest a slight positive interaction as the separate OR add up to OR = 5 only. In etiologic epidemiology, biologic interaction should be considered by assessing interaction on an additive scale.[34]

In chapter 3.2, I tested interaction on a multiplicative scale to investigate whether education level affected the association between subjective memory complaints with the risk of stroke. I stratified the analysis on low, intermediate and high level of education. This did not reflect an etiological concept but was "statistical default practice". Alternatively, in the Cox proportional hazards model that I used in the analysis, interaction on an additive scale could also have been considered. The interaction on the additive scale can be tested by either relative excess risk for interaction (RERI), attributable proportion due to additive interaction (AP) and synergy index (SI) with no additional assumption beyond those required for the Cox proportional hazard.[36]

Figure 1: Example showing the causal relationship of smoking and alcohol on the incidence of oesophageal cancer.



For assessment of interaction on an additive scale, the simplest way is to calculate the Synergy Index (SI). SI is calculated as following:

SI = Observed hazard ratio/Expected hazard ratio.

where observed is the HR for the joint effect of A and B (HR(AB)) and expected is obtained from the main effects of A and B as follows: [HR(A) + HR(B) – 1] under the assumption of additivity recommended in the STROBE statement.[37] It is also suggested that reporting of *P* value of included product term for testing interaction is insufficient to evaluate departure from additivity when considering causal interaction effect.[38]

Based on the joint-effect results from the Cox model, I calculated the expected hazard ratios under the assumption of additivity using the original data used in chapter 3.2. We found that the association between subjective memory complaints and stroke was modified by the effect of educational level in the fully adjusted model. The strongest association between subjective memory complaints and the risk of stroke were observed in the highly educated group. For ease of interpretability, I eliminated the intermediate education group from the education variable and will define the education variable dichotomously : "low education " and "high education". The hazard ratios for the association with the risk of stroke which are as follows:

Education (high)/no subjective memory complaints (no) : HR= 1.05
Education (low)/Subjective memory complaints (yes): HR= 1.07
Expected HR under the assumption of additivity: 1.05 + 1.07 – 1 = 1.12
Observed HR: 1.48
Synergy Index (SI) = 1.48/1.12
= 1.32

If the Synergy Index is different from 1, there is additive interaction. Therefore, in our study, we also observe additive interaction. This is analogous to the OR = 8 described in the example above. Thus, in our study, it does not matter on which scale interaction is tested; whether multiplicative or additive. The observed interaction is strong and positive beyond additive and multiplicative interaction.

Heterogeneity in meta-analysis

Meta-analysis is the statistical synthesis of results from individual studies aimed to integrate findings. A systematic review may be accompanied by a meta-analysis. In this thesis, we used systematic reviews to identify gaps in literature. In chapter 4.1 and 4.2 we conducted two systematic reviews in an attempt to identify all relevant studies fitting our predefined criteria; one of which also included a meta-analysis.

Meta-analysis provides a powerful tool to understand differences and similarities of results across multiple studies. Performing a meta-analysis involves: 1) calculation of a summary statistic for each included study and 2) combination of these summary statistics into a weighted average.[39] The aim was to summarize the validity and findings of the included studies which offers a more generalizable result than results of single studies.[40] However, the conclusions are less clear when the effects across the included studies are inconsistent. Consistency of effects across studies in a meta-analysis can be assessed by a statistical test of heterogeneity.[41]

Heterogeneity is encountered when summary statistics from different studies are pooled. Heterogeneity can be due to variations in study sample, outcomes, locations or differences in study design and measurements or a combination of any of the above.[42] There are various ways to deal with heterogeneity by statistical tests. We used I^2 statistic that measures the proportion of the total statistical heterogeneity due to variation between studies rather than chance.[43] It is also viewed as a measure of inconsistency across studies. The estimation of heterogeneity is useful because it is calculated as a percentage enabling comparison across meta-analyses. However, there is no fixed categorisation of heterogeneity; roughly 25%, 50% and 75% are defined as “low”, “moderate” and “high” heterogeneity. These categorisations are tentative and quantification and interpretation of variability across studies also depends upon clinical and methodological diversity in the included studies.[41]

In chapter 4.2 we found substantial heterogeneity (>50%) across studies included in the meta-analysis. This is because all studies were conducted in different populations and differences were present in data collection and case identification of stroke across the included studies. We conducted a subgroup meta-analysis in order to stratify the studies and to explain the observed heterogeneity. We were able to deduce differences in the meta-analysed incidence rates of strokes on the basis of location, publication year, average hospital admission, case fatality proportion and CT/MRI autopsy proportion. These findings point to gaps in stroke surveillance methodologies adopted by individual countries. Optimisation of stroke surveillance techniques may generate more comparable findings across different populations.

CLINICAL IMPLICATIONS

Results from population-based observational studies like those presented in this thesis can provide insights upon shared risk factors for pathologies of cognitive function in different age groups. This may provide the window of opportunity to target specific groups in the general population for risk factor screening and further focusing primarily on risk factor reduction among the high-risk groups. Some conclusions drawn from the research of this manuscript have important clinical implications and are presented here:

The associations between risk factors for cognitive decline and dementia in the very-old (>75 years) are not always consistent with those observed in the young-old (<75 years). Pharmacological and non-pharmacological interventions to lower blood pressure and/or to reduce arterial stiffness should be administered with caution in persons aged more than 75 years, as lower blood pressure and lower arterial stiffness do not necessarily translate to better cognitive function in very old persons. Current recommendations to treat hypertension or maintain blood pressure at optimum levels are challenged by our findings and may be modified in order to target reduction in the risk of a cardiovascular event specifically in persons >75 years.

Early identification and modification of cardiovascular risk factors in persons who complain about their memory may help prevent future stroke, particularly in persons with a high level of education. In practice, simple screening by inquiring about subjective complaints about memory in older individuals during routine clinical examination may identify persons who are at higher risk for stroke. Once identified, these individuals may benefit from targeted interventions to reduce risk factors that are associated with stroke. Also it is important that caregivers should vigilantly monitor older persons, especially those who have attained a high level of education, about their memory status. If complaints about memory become evident, these persons should promptly be directed

to a general practitioner in order to assess their risk factor profile that may place them at a higher risk of stroke.

The degree of impairment in basic and instrumental activities of daily living may be used as screening tools to identify persons who are at a higher risk of death. Therefore, clinical and rehabilitative interventions aiming at improving survival could focus on basic and instrumental activities of daily living. Quality of life and symptoms of depression may be less optimal screening tools.

RECOMMENDATIONS FOR FUTURE DIRECTIONS OF RESEARCH

Recommendations for cessation of research activity on the association between breastfeeding and IQ in children

Given the many studies on the association of breastfeeding with child IQ to date and the demonstrated huge confounding by maternal IQ, I recommend cessation of smaller studies within this field which do not take into account the important parental sociodemographic factors.

Recommendations for optimizing measurement of cognitive reserve

Longitudinal studies with large sample sizes will be important to further define cognitive reserve and its indicators. For comparison, studies should incorporate similar outcome measure and similar categorization and measurement of independent indicators. It is also important to explore optimization of measurement of cognitive reserve. Systematic reviews and meta-analyses may be helpful to thoroughly explore the different indicators of cognitive reserve that have been identified in single studies to date.

Recommendations for standardization of stroke surveillance protocols in low- and middle-income countries

Once a more standardized benchmark approach for stroke surveillance is adopted, better estimates of the current and future burden of stroke would become available, which could inform future health policies. As resources are limited in low- and middle-income countries, I recommend to focus on establishing sustainable systems to gather reliable and accurate data on stroke mortality and risk factors shared by other non-communicable diseases. Moreover, I recommend applicability and adherence to WHO STEPS guidelines for stroke surveillance in these countries to generate comparable data. This helps not only to devise stroke reduction programs and policies but also to implement risk factor identification and reduction strategies. Clearly, the risk factor profile is different in populations residing in these resource-poor settings than in the western world.

CONCLUSIVE REMARKS

The conclusions of this thesis offer a contribution to understanding developmental and degenerative aspects of cognitive outcomes in children and elderly respectively. Some associations observed have conveyed a profound public health message especially on the importance of subjective health. Hopefully, this thesis lays the foundation for future researchers to decipher epidemiological aspects of cognition across the life-course on a gradual scale. This could be made possible with the ongoing inclusion of participants at younger ages in the Rotterdam Study and the follow-up of Generation R children into adolescence.

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Chapter 6

Summary/Samenvatting

SUMMARY

When people age, accumulating diseases and biological changes may result in cognitive deterioration. The degree of cognitive decline partly depends on early cognitive development. Several environmental and pathophysiological factors are assumed to be related to cognitive development in early life as well as to cognitive decline and survival in late life. The main aims of this thesis were 1) to extend existing knowledge on risk and protective factors of cognitive development in early childhood, and 2) to determine risk factors pertaining to subjective health and subclinical vascular disease that may be associated with cognitive outcomes and survival in late-life.

The studies in this thesis were conducted within two population-based studies in Rotterdam, the Netherlands; namely the Generation R Study,[14] a children's cohort from fetal life onwards and the Rotterdam Study,[15] a prospective study among people of 45 years and older.

In **chapter 2.1**, we studied the effect of longer duration of any or exclusive breastfeeding in infancy on non-verbal IQ in children. We took several maternal and paternal life-style factors, socio-demographic factors, child factors and maternal IQ into account. Maternal IQ primarily accounted for the association between breastfeeding duration and child non-verbal IQ. Although breastfeeding may be beneficial for many health outcomes in children, we found no evidence for a direct effect of breastfeeding on non-verbal IQ.

In **chapter 2.2,** we explored whether arterial stiffness and blood pressure affect child IQ or general cognition in an elderly population. Arterial stiffness and blood pressure were not associated to child IQ at age 6 years. However, arterial stiffness and systolic blood pressure were strongly associated with general cognition in the elderly up till age 75 years. These results suggest that subclinical vascular risk factors accumulate with increasing age which may affect cognition in late-life.

In **chapter 3.1,** we studied various measures of subjective well-being as predictors of all-cause mortality among the elderly. We found that subjective measures of physical and mental health strongly predicted mortality. Subjective measures of physical functioning were independently related to mortality once we accounted for each measure of well-being in a mutually adjusted model.

In **chapter 3.2,** we investigated the association between subjective memory complaints and the risk of stroke. Our results showed that subjective memory complaints were associated with a higher risk of stroke especially in persons with a higher level of education. Objective measures of cognition, such as the MMSE, was not related to the risk of stroke. These results suggest that subjective reports of health and well-being may be strongly associated with health outcomes.

In **chapter 4.1,** we systematically reviewed literature on the concept of cognitive reserve and its operationalisation in the general population. From the existing body of literature on cognitive reserve, we conclude that education, occupation and leisure activities, with a social and mentally stimulating aspect, supply the cognitive reserve and offer a protective effect against the risk of dementia. Other indicators may also supply the reserve, but research is lacking with regards to creating a full cognitive reserve model and determining the overall effect on cognitive function and dementia.

In **chapter 4.2,** we systematically reviewed literature on methodologies adopted for stroke surveillance and meta-analyses incidence rates of stroke reported in previous studies conducted in low-and middle-income countries. Available literature suggests lack of standardized stroke surveillance systems in low and middle income countries. Against this background and a highly variable burden of stroke reported in previous studies, we propose adherence to the World Health Organization stepwise stroke surveillance system for accurate and reliable collection of stroke surveillance data in low and middle income countries.

In **chapter 5**, the main findings of the studies in this thesis are summarized, together with their methodological considerations. This chapter is concluded with some clinical implications and suggestions for future directions for research.

SAMENVATTING

Met het ouder worden kan de optelsom van ziektes en biologische veranderingen leiden tot verslechtering van het cognitief functioneren. De mate en snelheid van cognitieve achteruitgang hangt mogelijk deels af van de vroege cognitieve ontwikkeling in de kindertijd. Diverse omgevingsfactoren en pathofysiologische factoren lijken zowel gerelateerd aan cognitieve ontwikkeling in het begin van het leven als aan cognitieve achteruitgang en overleving in het latere leven. Dit proefschrift had tot doel om 1) bestaande kennis van risico- en beschermende factoren van cognitieve ontwikkeling in de vroege kindertijd uit te breiden, en 2) om risicofactoren als subjectieve gezondheid en subklinische vasculaire ziekte te relateren aan cognitieve uitkomsten en overleving in het latere leven.

De studies in dit proefschrift werden uitgevoerd in twee populatiecohorten in Rotterdam, namelijk in de Generation R studie,[14] een kindercohort gevolgd vanaf de intrauteriene fase, en in de Rotterdam Study,[15] een cohort studie bij mensen van 45 jaar en ouder.

In **hoofdstuk 2.1** bestudeerden we het effect van borstvoeding in het eerste levensjaar op non-verbaal IQ op de leeftijd van 6 jaar. Bij het onderzoeken van deze relatie hielden we rekening met verschillende leefstijlvariabelen gemeten bij vader en moeder, sociaaldemografische factoren, kind factoren en IQ van moeder. We vonden dat IQ van moeder de belangrijkste factor was die de associatie tussen de duur van borstvoeding en non-verbaal IQ bij kinderen kon verklaren. Hoewel borstvoeding waarschijnlijk vele gunstige gezondheidseffecten heeft, vonden wij dus geen bewijs voor een direct effect van borstvoeding op non-verbaal IQ.

In **hoofdstuk 2.2** onderzochten we of vaatwandstijfheid en bloeddruk van invloed waren op IQ op de kinderleeftijd en/of algemene cognitie in een oudere populatie. We vonden geen bewijs voor een associatie tussen vaatwandstijfheid en bloeddruk enerzijds en IQ op de leeftijd van 6 jaar anderzijds. Wel vonden we sterke verbanden van vaatwandstijfheid en systolische bloeddruk met algemene cognitie bij ouderen tot de leeftijd van 75 jaar. Deze resultaten suggereren dat subklinische vasculaire risicofactoren met oplopende leeftijd in toenemende mate de cognitie kunnen beïnvloeden.

In **hoofdstuk 3.**1 bestudeerden we diverse subjectieve maten en constructen van welzijn als voorspellers van mortaliteit bij ouderen. We vonden dat mortaliteit voorspeld kan worden door subjectieve rapportages van zowel lichamelijke als geestelijke gezondheid. Alleen subjectieve rapportages van fysiek functioneren werden onafhankelijk in verband gebracht met mortaliteit.

In **hoofdstuk 3.2** onderzochten we de relatie tussen subjectieve geheugenklachten en het risico op beroerte. Onze resultaten laten zien dat subjectieve geheugenklachten worden geassocieerd met een hoger risico op beroerte, vooral bij personen met een

hoger opleidingsniveau. Objectieve metingen van cognitie, zoals de MMSE, konden daarentegen niet gerelateerd worden aan het risico op een beroerte. Deze resultaten suggereren dat persoonlijke rapportages over gezondheid en welzijn mogelijk sterk geassocieerd zijn met gezondheidsuitkomsten.

In **hoofdstuk 4.1** hebben we in een systematische review de literatuur over het concept 'cognitieve reserve' en de operationalisering in de algemene bevolking beschreven. We concludeerden dat opleiding, beroep en vrijetijdsactiviteiten, via een sociaal en mentaal stimulerende werking, de cognitieve reserve bepalen en een beschermend effect tegen het risico van dementie bieden. Ook andere indicatoren dragen bij aan de cognitieve reserve, maar het onderzoek is nog onvoldoende om te komen tot een volledig 'cognitieve reserve'-model en het bepalen van het totale effect daarvan op cognitieve functies en dementie.

In **hoofdstuk 4.2** beschreven we systematisch de literatuur over methodes voor het herkennen en signaleren van beroerte en de meta-analyseerden wij de incidentie van beroerte zoals gerapporteerd in eerdere studies uitgevoerd in lage en midden inkomens landen. De beschikbare literatuur suggereert een gebrek aan gestandaardiseerde beroerte-surveillancesystemen in lage en midden-inkomenslanden. Tegen deze achtergrond en de hoge mate van variabiliteit in beroertelast, stellen wij voor om het stapsgewijze beroerte surveillance systeem van de World Health Organization (WHO) na te leven.

In **hoofdstuk 5** worden de belangrijkste bevindingen van de studies in dit proefschrift samengevat, samen met hun methodologische overwegingen. Dit hoofdstuk wordt afgesloten met een aantal klinische implicaties en suggesties voor toekomstige richtingen van onderzoek.


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Chapter 7

Addendum

PUBLICATIONS AND MANUSCRIPTS

Sajjad A, Freak-Poli RL, Hofman A, Roza SJ, Ikram MA, Tiemeier H. Subjective well-being and all-cause mortality. *The Rotterdam Study. Submitted.*

Sajjad A, Leening MJG, Gaillard R, Hofman A, Franco OH, Mattace-Raso FU, Jaddoe VW, Roza SJ, Tiemeier H, Ikram M.A. The association of arterial Stiffness with cognition in the young and old. Results from the Generation R Study and the Rotterdam Study. *To be submitted.*

Harrison SL, **Sajjad A**, Bramer WM, Ikram MA, Tiemeier H, Stephan BC. Exploring strategies to operationalize cognitive reserve: A systematic review of reviews. *Journal of Clinical and Experimental Neuropsychology. 2015;37(3):253-64.*

Sajjad A, Tharner A, Kiefte-de Jong JC, Jaddoe VW, Hofman A, Verhulst FC, Franco OH, Tiemeier H, Roza SJ. Breastfeeding duration and non-verbal IQ in children. *Journal of Epidemiology and Community Health. 2015 Aug;69(8):775-81.*

Voortman T, Vitezova A, Bramer WM, Ars CL, Bautista PK, Buitrago-Lopez A, Felix JF, Leermakers ET, **Sajjad A**, Sedaghat S, Tharner A, Franco OH, van den Hooven EH. Effects of protein intake on blood pressure, insulin sensitivity and blood lipids in children: a systematic review. *British Journal of Nutrition. 2015 Feb 14;113(3):383-402.*

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Baena CP, Olandoski M, Younge JO, Buitrago-Lopez A, Darweesh SK, Campos N, Sedaghat S, **Sajjad A**, van Herpt TT, Freak-Poli R, van den Hooven E, Felix JF, Faria-Neto JR, Chowdhury R, Franco OH. Effects of lifestyle-related interventions on blood pressure in low and middle-income countries: systematic review and meta-analysis. *Journal of Hypertension. 2014 May;32(5):961-73.*

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Chowdhury R, Stevens S, Ward H, Chowdhury S, **Sajjad A**, Franco OH. Circulating vitamin D, calcium and risk of cerebrovascular disease: a systematic review and meta-analysis. *European Journal of Epidemiology. 2012 Aug;27(8):581-91.*

PhD PORTFOLIO

Name PhD student:	Ayesha Sajjad
Erasmus MC department:	Epidemiology
PhD period:	January 2012 – April 2016
Promotor:	Prof.dr. Henning Tiemeier
Copromotors:	Dr. M. Arfan Ikram, Dr. Sabine J. Roza

PhD Training	**Year**	**Workload (ECTS)**
Doctor of Science (DSs), Clinical Epidemiology NIHES, Erasmus University Rotterdam, the Netherlands	2013	
Courses		
Causal Inference	2012	0.7
History of Epidemiologic Ideas	2012	0.7
Advances in Epidemiologic Analysis	2012	0.4
Epidemiologic Research-an avant-garde introduction	2012	0.7
Bayesian Statistics	2012	1.1
Survival Analysis for Clinicians	2012	1.9
Principles of Epidemiologic Data-analysis	2012	0.7
Nutrition and Physical Activity	2012	1.4
Courses for the Quantitative Researcher	2012	1.4
Advances in Genome-Wide Association Studies	2012	1.4
Basic Course on SPSS	2012	1.0
An Introduction to the analysis of the next-generation sequencing data	2014	1.4
Conferences, Meetings, and Workshops		
European Society of Cardiology, Munich, Germany	2012	2.0
Developmental Origins of Health and Disease, Rotterdam	2012	0.3
Workshop Systematic Review and Meta-analysis, Erasmus MC	2012	0.3
Nutrimenthe Project Meeting, Rotterdam	2012	0.3
European Congress of Epidemiology – Healthy Living, Maastricht	2015	1.0
Workshop Media contacts for researchers	2015	0.3
Teaching		
- Lecturer – Stroke Surveillance in low an middle income countries - Global Health Course. Erasmus MC	2012-2015	2.0
- Supervising practical - 'Public Health in low and middle income countries"	2012	0.5
Supervising students		
- 'Post traumatic distress syndrome (PTSD) and serum lipid concentrations: a systematic review'. Department of Psychiatry, Erasmus MC	2012	0.5

- 'Celecoxib as an add-on treatment in patients with schizophrenia: a systematic review'. Department of Psychiatry, Erasmus MC	2013	0.5
- 'Loss of spouse and mortality'. Research mobility program ERAWEB Department of Epidemiology, Erasmus MC	2015	1.0

Peer Review for scientific journals	2012-2016	
British Medical Journal (BMJ)		0.4
European Journal of Epidemiology		0.4

1 ECTS (European Credit Transfer System equal to workload of 28 hrs

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Erica & Gabrielle, by now you must be able to recognize the look on my face from far when I approach you for arranging an appointment with Henning OR Arfan. Thank you for entertaining my phone calls when I was working from home. Gabrielle, thank you for arranging my hospitality agreements on time. Erica, thanks to you for taking care of the registration processes with pedel on my behalf. A sincere thanks to Hermine and Solange for giving the right HR advice and taking care of the administrative processes. A special thanks to Hetty for getting the last part of the administration regarding this thesis through. A big hug to all you ladies!

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ABOUT THE AUTHOR

Ayesha Sajjad was born on February 24th, 1982, in Lahore, Pakistan. She completed her primary education at Lahore Grammar School and her secondary education at University College Lahore in 2001. She then started medical school at Lahore Medical and Dental College, which is affiliated with the University of Health Sciences, Lahore, Pakistan. After graduating as a medical doctor in 2007, she worked as a research associate and teaching assistant at Aga Khan University, Karachi, Pakistan, where she conducted laboratory based research and supervised medical students till 2009. She then left for the United States where she passed the United States Medical Licensing Exams. During that time, she volunteered to be a part of a neuro-cognitive research group at Baylor College of Medicine, Houston, where she was introduced to clinical research. She then decided to pursue her career in the field of research. In order to gain further training in research, in 2010, she moved to the United Kingdom where she was admitted to a Master's program at the Institute of Public Health, Cambridge with a full scholarship awarded by the Cambridge Commonwealth Trust. She obtained a Master's (MPhil) degree in Public Health from the University of Cambridge in 2011.

In 2012, she was admitted to a Doctor of Science (DSc) program at the Netherlands Institute for Health Sciences in Rotterdam, The Netherlands. She obtained her DSc diploma in Clinical Epidemiology from Erasmus University in 2013. Thereafter, she continued her work at the department of Epidemiology at Erasmus MC culminating in this PhD thesis under the supervision of Prof. Dr. H. Tiemeier and co-supervision of Dr. M. A. Ikram and Dr. S. J. Roza. From March 2016, she started working as a post-doctoral scientific researcher in the field of Epidemiology at the Academic Medical Center, Amsterdam, The Netherlands.

Ayesha is married to Midas Tameris and they have one daughter, Ariel.