Early-life Nutrition and Cardiometabolic Health across the Life Course

Elisabeth Theodora Maria Leermakers

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I fell into a burning ring of fire,
I went down, down, down,
and the flames went higher.
And it burns, burns, burns,
the ring of fire.
The ring of fire.

~ Johnny Cash

Early-life Nutrition and Cardiometabolic Health across the Life Course

Voeding in het vroege leven en
cardiometabole gezondheid
gedurende het leven

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Manuscripts that form the basis of this thesis

Chapter 2

E.T.M. Leermakers, S.K.L. Darweesh, C.P. Baena, E.M. Moreira, D. Melo van Lent, M.J. Tielemans, T. Muka, A. Vitezova, R. Chowdhury, W.M. Bramer, J.C. Kiefte- de Jong, J.F. Felix*, O.H. Franco*. The effects of lutein on cardiometabolic health across the life course: a systematic review and meta-analysis.
The BMJ (under review)

E.T.M. Leermakers, J.C. Kiefte- de Jong*, E.M. Moreira*, S.K.L. Darweesh, T. Visser, T. Voortman, P.K. Bautista, R. Chowdhury, D. Gorman, W.M. Bramer, J.F. Felix, O.H. Franco. The effects of choline on health across the life course: a systematic review.
Nutrition Reviews (in press)

Chapter 3

E.T.M. Leermakers*, M. van den Broek*, V.W.V. Jaddoe, E.A.P. Steegers, F. Rivadeneira, H. Raat, A. Hofman, O.H. Franco, J.C. Kiefte- de Jong. Maternal dietary patterns during pregnancy and body composition of the child at the age of 6 years: the Generation R Study.
American Journal of Clinical Nutrition (under review)

E.T.M. Leermakers, M.J. Tielemans, M. van den Broek, V.W.V. Jaddoe, O.H. Franco, J.C. Kiefte- de Jong. Maternal dietary patterns and offspring cardiometabolic health at 6 years: the Generation R Study.
Journal of Hypertension (under review)

Chapter 4

E.T.M. Leermakers, J.C. Kiefte- de Jong, A. Hofman, V.W.V. Jaddoe, O.H. Franco. Lutein intake in toddlers and cardiometabolic health at the age of 6 years: the Generation R Study.
British Journal of Nutrition (in revision)

E.T.M. Leermakers, J.F. Felix*, N.S. Erler*, A. Ćerimagić, A.I. Wijtzes, A. Hofman, H. Raat, H.A. Moll, F. Rivadeneira, V.W.V. Jaddoe, O.H. Franco, J.C. Kiefte- de Jong. Sugar-containing beverage intake in toddlers and body composition up to age 6 years: the Generation R Study.
European Journal of Clinical Nutrition ***69****, 314-321*

E.T.M. Leermakers, J.F. Felix, H. Raat, V.W.V. Jaddoe, O.H. Franco, J.C. Kiefte- de Jong. Sugar-containing beverage intake at the age of 1 year and cardiometabolic health at the age of 6 years: the Generation R Study.
Internation Journal of Behavioral Nutrition and Physical Activity (under review)

E.T.M. Leermakers*, T. Voortman*, O.H. Franco, A. Hofman, H.A. Moll, V.W.V. Jaddoe, E.H. van den Hooven, J.C. Kiefte- de Jong. *A priori* and *a posteriori* dietary patterns at the age of 1 year and body composition at the age of 6 years: the Generation R Study.
American Journal of Clinical Nutrition (under review)

*Authors contributed equally

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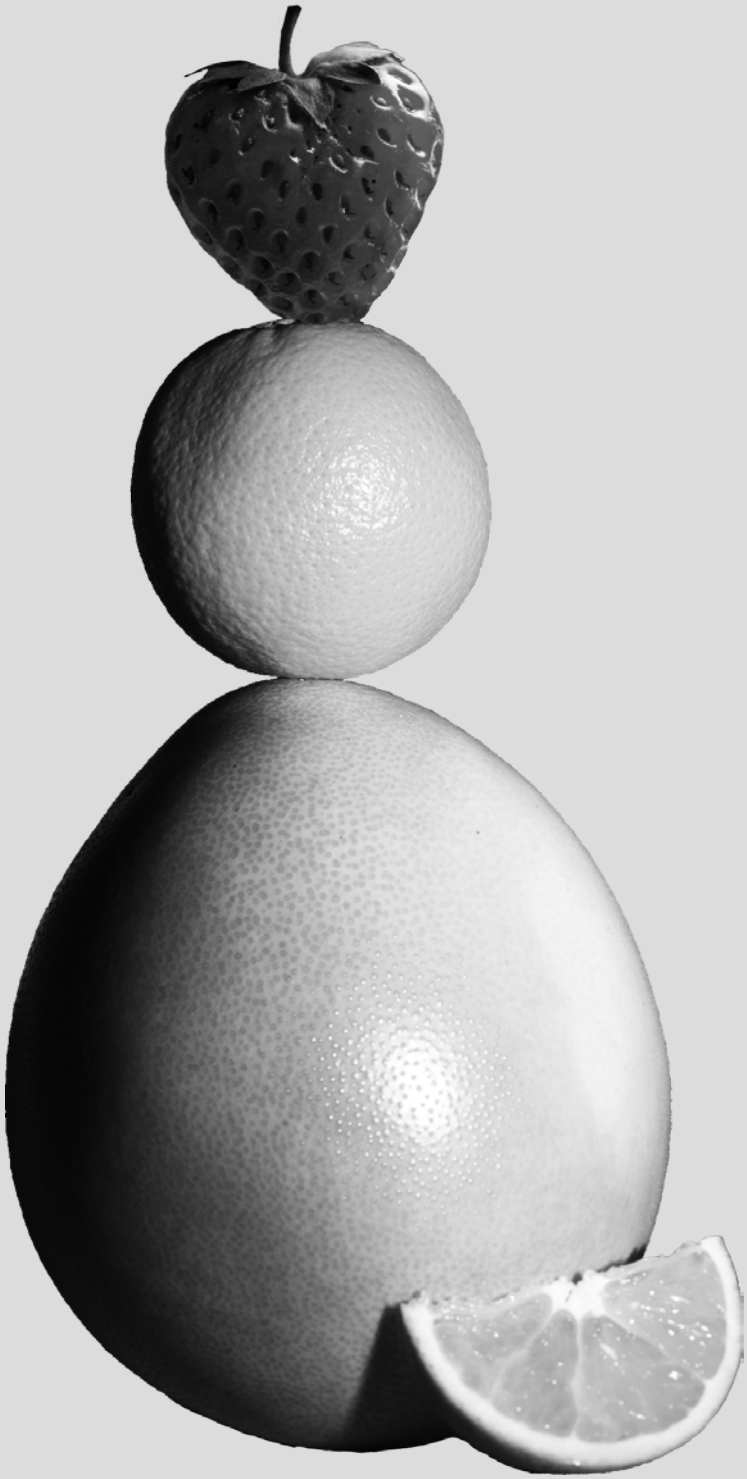


Chapter 1

General Introduction

1.1 Background

Cardiometabolic health

In the last decade, non-communicable diseases have become the leading cause of death worldwide.[1] Cardiovascular mortality is the main cause of death and was responsible for 30% of all deaths in 2012.[1] In addition, almost 10% of the world's adult population has type 2 diabetes mellitus, and most of them will die from cardiovascular diseases.[2]

Cardiovascular events and type 2 diabetes mellitus also have a high impact on quality of life besides their risk of premature death.[3,4] Therefore, research in the last decades aimed to identify the major risk factors for cardiometabolic diseases, to be able to detect disease processes already at an early stage.[5] This helps to identify persons at risks, and enables health care professionals to treat these risk factors already before cardiometabolic diseases develop.[6] Important risk factors are insulin resistance, hyperlipidemia, hypertension and obesity,[7] together known as the metabolic syndrome.[8] Besides that these risk factors could lead to mortality, they also affect quality of life much earlier.[9] Furthermore, treatment of these risk factors is often not effective and it is thus of paramount importance that these risk factors are prevented, in order to maintain optimal cardiometabolic health.[6]

Adiposity is one of these risk factors and is strongly related to risk of cardiometabolic diseases.[1] In addition to adverse effects on cardiovascular and metabolic health, adiposity has many other adverse short- and long-term consequences on health, such as infertility, musculoskeletal problems, sleep apnea, and higher risk of chronic obstructive pulmonary disease.[10] Thus, adiposity decreases the chance of healthy survival significantly, and maintaining a healthy weight is thus a key factor for healthy ageing.[11] Prevalence of obesity has rapidly increased over the last decades, also among children.[12] In the Netherlands, 12.2% of children between 4 and 12 years of age were classified as overweight or obese in 2013.[13] Similar to obesity in adults, obesity in children adversely affects cardiometabolic health and increases risk of disease in later life,[14] but also adversely affects development of the child, which cannot always be reversed.[14] Furthermore, childhood obesity has a large effect on emotional well-being of the child and its family.[14]

The role of nutrition in cardiometabolic health

Lifestyle is the most important modifiable risk factor for cardiovascular disease and type 2 diabetes mellitus.[15] According to the WHO, 80% of premature death from cardiometabolic diseases could be prevented by a healthy lifestyle.[1] Diet is a crucial factor of lifestyle, and having a healthy diet is thus important in maintaining cardiometabolic health.[16]

Differences in cardiometabolic disease prevalence between countries have been suggested to be in large part caused by differences in traditional dietary patterns, which has for example led to the research interest in the Mediterranean diet.[17] Besides studying the whole diet, focus can be on individual foods or food groups, or on specific nutrients.[18] Food groups that have received a lot of interest in relation to cardiometabolic

health are for example sugar-containing beverages, which may increase the risk of cardiometabolic diseases,[19-21] and vegetables, which are associated with decreased risks.[22-24] As the beneficial effects of vegetables seemed most pronounced for green leafy vegetables, research has aimed to identify the nutrients responsible for the observed effects, which is followed by an increased interest for the group of carotenoids.[25] Studying nutrition, individual nutrients, foods, or whole diet, can give further insights in etiology of disease and could also play a role in the prevention and treatment of diseases. The aim of this thesis was to identify etiological associations between nutrition and cardiometabolic health. Studies on dietary factors that are related to risk factors for cardiometabolic diseases, such as body composition, blood pressure, blood lipids and insulin, are needed for establishing the optimal diet and are important for the development of guidelines aiming to reduce the burden of cardiometabolic diseases. Studying this in early life creates the opportunity to evaluate the development of deviations from optimal health, and can help in preserving optimal cardiometabolic health.[26]

Life course perspective of cardiometabolic health

The risk of cardiometabolic diseases can be traced back to nutrition during early life.[26] Maternal nutrition during pregnancy can affect the developing fetus and thereby influence susceptibility of their offspring to obesity, diabetes and cardiovascular diseases in later life.[27] In addition, dietary behaviors that develop early in life can track throughout the life course[28] and exert its effects much later.[26] While most diseases become more apparent with ageing and prevalence of chronic diseases is highest in elderly, disease susceptibility may have already long been established at that stage.[26] Exposures during the process of ageing can reduce a person's resilience, which is the ability to maintain physical and psychological stability, and reduced resilience will increase the risk of chronic diseases.[29] Childhood may be a key period in which resilience is developed and important aspects for future health are established.[30] Detecting essential exposures in early life when children are still in good health, makes it possible to influence risk of diseases in later life, and early life is thus an excellent period to focus on maintaining this cardiometabolic health.

1.2 Objectives

The objective of this thesis was to study the role of nutrition in several stages of life on cardiometabolic health, with the use of diverse approaches to nutritional epidemiology, namely nutrients, foods and dietary patterns.

First, the aim was to perform a systematic review of the literature on the effects of nutrients (lutein and choline) on health across the life course. Additionally, the aim was to assess the role of nutrition in early life on cardiometabolic health in childhood, in a population-based prospective cohort study. Nutrition in fetal life focused on maternal dietary patterns. Nutrition in early childhood was assessed as nutrients (lutein), food groups (sugar-containing beverages) and overall diet (*a priori* and *a posteriori* dietary patterns).

1.3 Study designs

Systematic reviews

The research described in **chapter 2** of this thesis are systematic reviews of the literature. Details of the methods, such as the search strategy and article inclusion criteria, can be found in the specific chapters. Briefly, in collaboration with an experienced information specialist, the literature was systematically searched for publications on the topic of interest, and experts in the field were contacted. Two independent reviewers screened all articles for eligibility. Relevant information, including study characteristics, methods and results, were extracted from the articles. A quality score was applied to evaluate the quality of included studies. This quality score was based on previously used scoring systems[24,31] and was modified for the use of different study designs. A comprehensive overview of all included studies is given. In addition, a meta-analyses was performed where possible (**chapter 2.1**). Our focus was on the effects of lutein and choline on cardiometabolic health, but given the suggested beneficial effects of choline on cognition,[32,33] we additionally examined the associations of choline with neurological health (**chapter 2.2**).

Generation R Study

Study design

The studies presented in **chapters 3 and 4** of this thesis were part of the Generation R Study, a population based prospective cohort study from fetal life until young adulthood in Rotterdam, the second largest city of the Netherlands.[34] The Generation R Study is designed to identify early determinants of variations in growth, development and health during the life course. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for the study. For the studies described in this thesis, only women of Dutch origin were included. Assessments during pregnancy were planned in each trimester and included physical measurements and self-administered questionnaires. From birth until the child's age of 4 years, we obtained information on health of the children by questionnaires filled out by the primary caregiver and by measurements which were performed at regular routine visits to the child health centres. When the children were 6 years of age (median 5.9 years, 95% range 5.7 to 6.5), they visited our dedicated research facility in the Sophia's children hospital where different health outcomes were measured.

Dietary assessment

Maternal diet was assessed at enrollment in the study, with the use of a 293-item semi-quantitative self-administered food-frequency questionnaire (FFQ). Median gestational age at assessment was 13.4 weeks (95% range 9.9 to 22.8 weeks). The questionnaire asked about habitual diet over the last three months, thereby covering mostly diet in the first trimester of pregnancy. Child diet was assessed with a 211-item semi-quantitative food-frequency questionnaire (FFQ), which was filled in by the primary caregiver when the child was around 13 months of age (median 12.9, 95% range 12.2 to 19.2), and covered habitual diet of the last month.

Assessment of cardiometabolic health

Information on anthropometric measures of children from birth up to 4 years of age was obtained from the child health centres. Height and weight were measured by trained staff, and age- and sex-specific standard deviation scores were calculated based on Dutch reference growth curves (Growth Analyzer 3.0, Dutch Growth Research Foundation).

At the visit to our research centre at the child's age of 6 years, cardiometabolic health outcomes were measured. Measurements included child anthropometrics and blood pressure. Total and regional fat mass and fat-free mass were measured with the use of Dual-energy X-ray absorptiometry-scans. Blood was drawn and blood lipids and insulin levels were determined. Age- and sex-specific standard deviation scores were created based on the entire Generation R study population.

A combined cardiometabolic risk factor score was calculated by summing the age- and sex-specific standard deviation scores of body fat percentage, blood pressure, insulin levels, triglycerides and the inverse of HDL cholesterol.

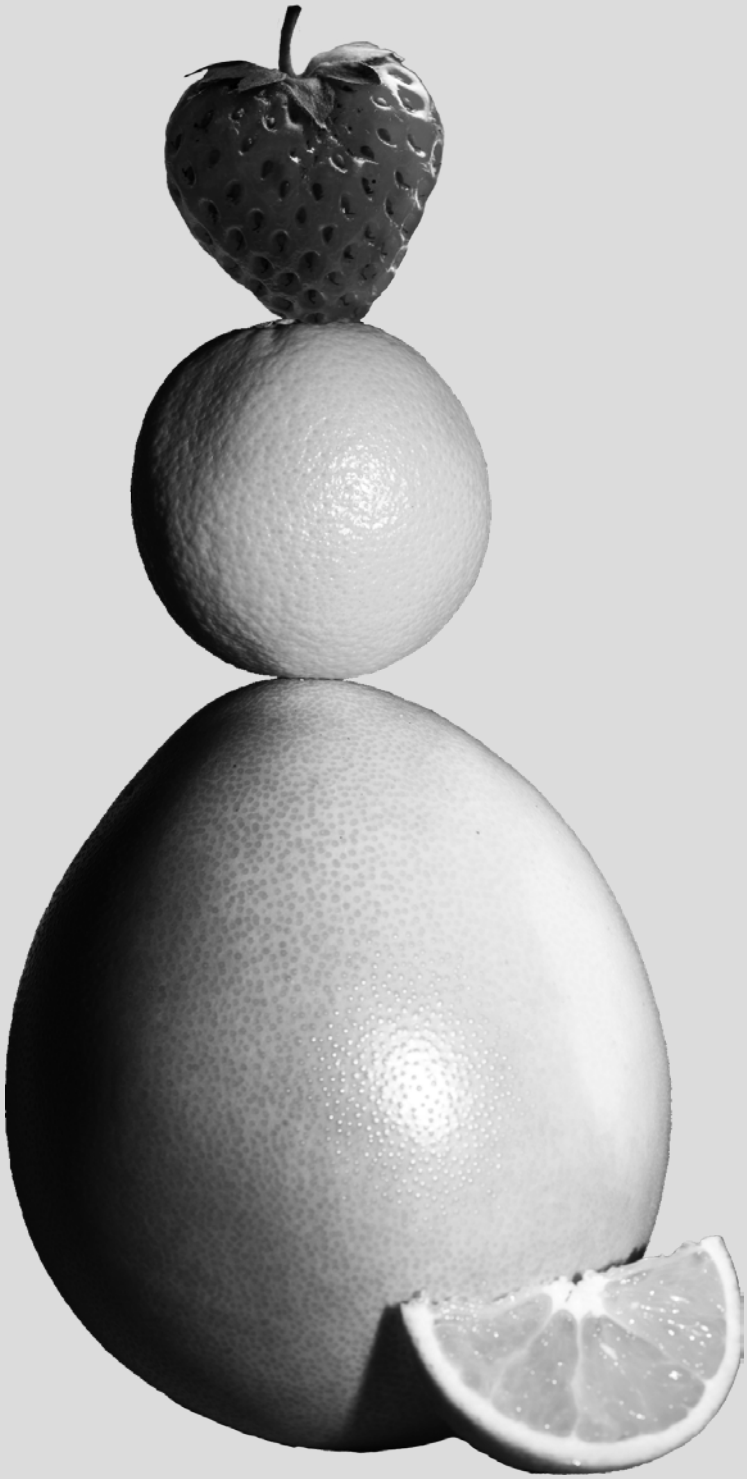
1.4 Thesis outline

Subsequent to this general introduction, **chapter 2** of this thesis describes the systematic reviews of the literature on the effects of lutein and choline on health across the life course. **Chapter 2.1** describes the literature on the effects of lutein on cardiometabolic health. Also, it includes a meta-analysis on the associations of lutein with risk of several cardiometabolic diseases. **Chapter 2.2** gives a comprehensive overview of studies that assessed choline in relation to health across the life course.

Chapter 3 is focused on the relation between overall diet during pregnancy with cardiometabolic health in offspring, within the Generation R Study. In **chapter 3.1**, the associations are reported for different maternal dietary patterns with offspring body composition in childhood, and **chapter 3.2** describes the relation between different dietary patterns of the mothers and measures of cardiometabolic health in their children.

In **chapter 4**, we evaluate the associations of nutrition of the child in early life with cardiometabolic health later in childhood, within the Generation R Study. In **chapter 4.1**, we focused on lutein intake of the child in infancy in relation to body composition and cardiometabolic health in childhood. **Chapter 4.2** describes the relation between sugar-containing beverage intake in infancy and body composition up to school-age. Subsequently, **chapter 4.3** describes the associations of sugar-containing beverage intake in infancy with measures of cardiometabolic health in childhood. **Chapter 4.4** reports on the associations of several dietary patterns in infancy with body composition later in childhood.

Lastly, **chapter 5** gives an overview of the main findings and conclusions from these studies. It also discusses the advantages and disadvantages of the different approaches to nutritional epidemiology. Furthermore, it gives recommendations for future research and implications for preventive medicine based on the results described in this thesis.



Chapter 2

Nutrition and Health across the Life Course

E.T.M. Leermakers, S.K.L. Darweesh, C.P. Baena, E.M. Moreira, D. Melo van Lent, M.J. Tielemans, T. Muka, A. Vitezova, R. Chowdhury, W.M. Bramer, J.C. Kiefte- de Jong, J.F. Felix, O.H.F. Franco

Abstract

Background The antioxidant lutein is suggested to be beneficial for cardiometabolic helath due to its protective effect against oxidative stress, but evidence has not systematically been evaluated.

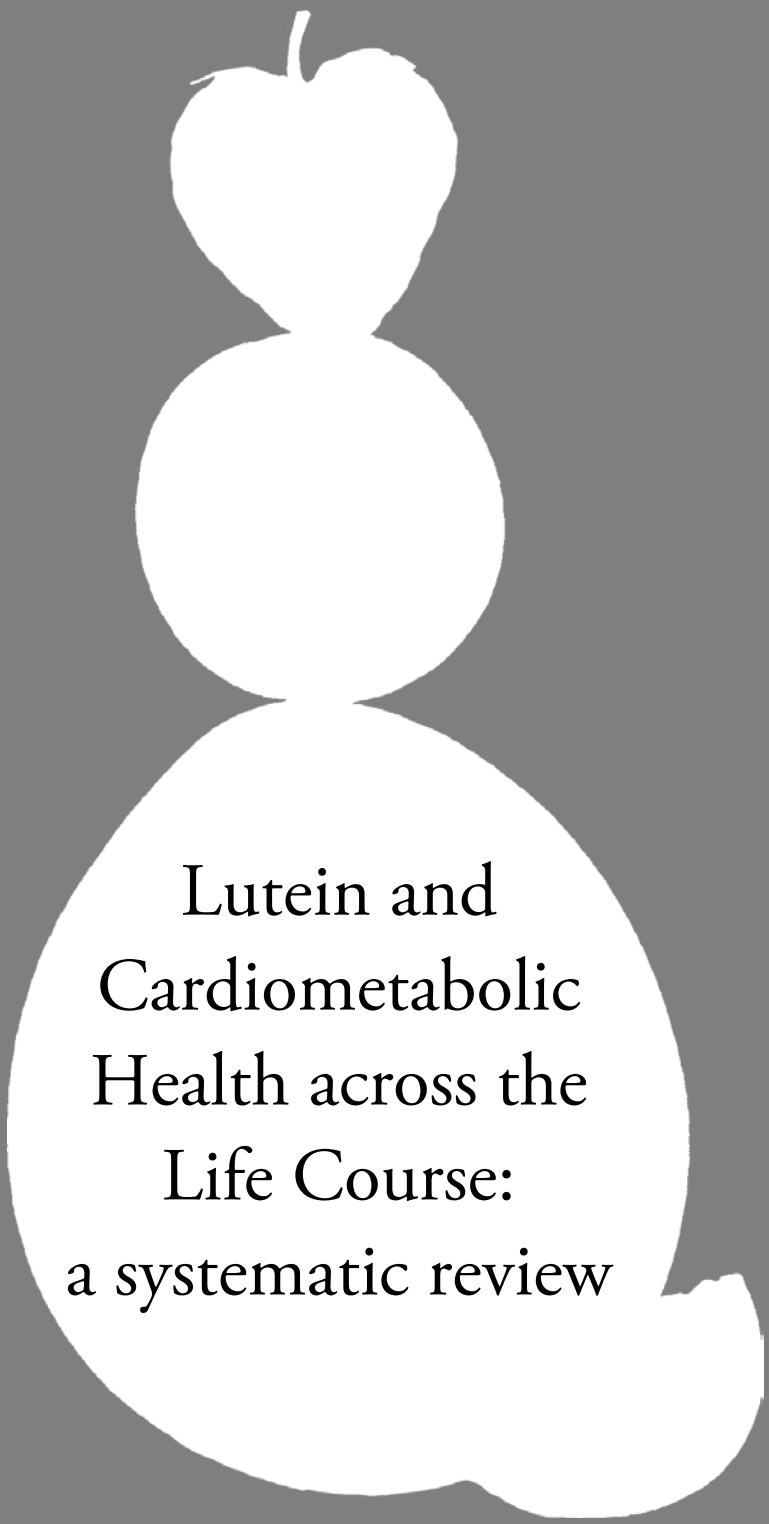
Objective We aimed to systematically evaluate the literature on the effects of lutein on cardiometabolic health in different life stages.

Design and data sources Systematic review and meta-analysis of literature published in Medline, Embase, Cochrane Central, Web of Science, PubMed and Google Scholar up to August 26th 2014.

Study selection Studies included were trials, cohort, case-control and cross-sectional studies in which the association of lutein levels, dietary intake or supplements with cardiometabolic outcomes was reported. Two independent investigators reviewed the articles.

Results Seventy-one relevant articles were identified, including a total of 387,569 participants. Only one paper investigated the effects of lutein during pregnancy and three studied the effects of lutein in children. Furthermore, 31 longitudinal studies, 33 cross-sectional and three intervention studies were done in adults. Meta-analysis showed a lower risk of coronary heart disease (pooled RR 0.88 (95%CI 0.80; 0.98)) and stroke (pooled RR 0.82 (95%CI 0.72; 0.93)), for the highest vs the lowest tertile of lutein (intake or level). There was no significant association with type 2 diabetes mellitus (pooled RR 0.97 (95%CI 0.77; 1.22)), but higher lutein was associated with lower risk of metabolic syndrome (pooled RR 0.75 (95%CI 0.60; 0.92)), for the highest vs the lowest tertile (intake or level). The literature on risk factors of cardiometabolic diseases showed that lutein might be beneficial for atherosclerosis and inflammatory markers, but there were inconsistent associations with blood pressure, adiposity, insulin resistance and blood lipids.

Conclusions Our findings suggest that higher dietary intake of lutein and higher blood levels of lutein are generally associated with better cardiometabolic health. However, evidence mainly comes from observational studies in adults, whereas large-scale intervention studies and studies of lutein during pregnancy and childhood are scarce.



Lutein and Cardiometabolic Health across the Life Course: a systematic review

Introduction

Lutein is a naturally occurring carotenoid, which is synthesized within dark green leafy vegetables such as spinach and kale. Lutein is mostly known for its effect on visual function and its preventive effect on cataract and macular degeneration,[35] potentially through protection against oxidative stress.[36] Given its antioxidant properties, it is hypothesized that lutein may also have beneficial effects in metabolic and cardiovascular diseases. Although the larger group of carotenoids has been associated with cardiometabolic protection,[37] research has mostly focused on other carotenoids, such as beta-carotene.[38] Nevertheless, interventional studies using supplements of beta-carotene have failed to reproduce the beneficial effects that were seen in observational studies.[39 40] Thus, it yet needs to be determined which is the substance behind the previously published beneficial effects of the carotenoids. The xanthophyll lutein is of particular interest since lutein may be a more active antioxidant than beta-carotene.[41]

Currently, there are no recommendations for dietary lutein intake, and it is important to study the effects of lutein in order to determine if such recommendations are required. Several studies have focused on the effects of lutein in the past decades, but the potential effects of lutein on cardiometabolic health at different life stages have not yet been systematically evaluated.

Therefore, our objective was to systematically search the available literature for articles describing associations of lutein (either from dietary intake, supplementation or blood levels) in relation to cardiometabolic health across the life course.

Our primary aim was to assess the association of lutein with risk of cardiometabolic diseases and mortality, including coronary heart disease, stroke, metabolic syndrome and type 2 diabetes mellitus.

Our secondary aim was to systematically evaluate the literature regarding the associations between lutein and risk factors for cardiometabolic diseases, such as adiposity, insulin resistance, hypertension, atherosclerosis, inflammatory markers and blood lipids to gain more insight into the mechanisms underlying possible associations between lutein and cardiometabolic diseases.

Methods

Search strategy

An experienced information specialist from the medical library, together with the first author, conducted a systematic search of the current literature from inception up to August 26th 2014 in four major electronic databases: Medline, Embase, Cochrane Central, Web of Science. Additionally we searched not yet indexed articles in PubMed and downloaded the most relevant references from Google Scholar. We searched for studies in which associations of lutein, carotenoids or xantophylls (either supplementation, dietary intake, or blood levels) with body composition, cardiovascular, inflammatory or metabolic outcomes were reported at any stage of life, ranging from fetal life, through childhood, to adulthood. The computer-based searches combined search terms related to the exposure

and outcomes of interest, with filters for epidemiological studies in humans without any restriction on language or age of subjects. Search terms were searched both as controlled vocabulary (MeSH for Medline and Emtree terms for Embase), and as free text words in title and/or abstract. Further details on the search strategy are shown in **Supplementary Material S2.1.1**.

Study identification and selection

Working in pairs, two reviewers independently reviewed title and abstract of each reference to determine whether the study should be included. Studies were included if they were interventional or observational (cohort, case-control or cross-sectional) studies; if they studied the effects of carotenoids, xantophylls or lutein (dietary intake, supplementation or blood levels) as exposure; and if the outcomes of interest were related to cardiometabolic (cardiovascular, metabolic, inflammatory, growth or adiposity) health.

Letters, abstracts, reviews, conference proceedings, case reports and studies not carried out in humans were excluded. To avoid bias due to selective reporting of significant results in the abstract, abstracts were included for full text screening if they reported that carotenoids or xantophylls were studied, even if lutein was not specifically mentioned. Any disagreements in article selection were resolved through discussion and a third reviewer was available to resolve any remaining disagreement. Full text articles were retrieved for the selected papers after initial appraisal and assessed once more by the same two independent reviewers to ensure that they satisfied the selection criteria. In case of multiple publications from the same study, only the most recent and/or complete report was included.

Data extraction

Detailed study-level characteristics were extracted including study design (such as sample size, duration of follow-up, country), population characteristics (such as age, sex, ethnicity), exposure assessment (such as biomarker, diet assessment method), outcome assessment (such as validity of the method), analysis (such as statistical method, measure of association), results (such as effect estimate, standard error/confidence interval) and covariates (such as key confounders, additional adjustments).

P-values were rounded to two decimals. If significant findings were reported without an effect estimate, we reported the direction of effect in the tables using a downward arrow ↓ to indicate a negative association and an upward arrow ↑ to indicate a positive association. From studies that reported multiple results, we included only results from the most extensive adjusted model and from the most powerful analysis (e.g. linear regression over correlation). From studies that repeatedly measured the exposure or the outcom with different follow-up periods, we included only results from the longest follow-up period. Data were extracted by one reviewer, and a random 10% of data extraction was checked by an independent reviewer. Detailed results of the included studies are presented in **Supplementary Material S2.1.2 and S2.1.3**.

Quality scoring

We used a predefined quality score (QS) to evaluate the quality of included studies. The QS is a modified version of previously used scoring systems.[24 31] A score of 0, 1 or 2 points was allocated to each of the following five items: study design, study size, exposure assessment, outcome assessment, and adjustment for potential confounders. This allowed a total score between 0 and 10 points, with 10 representing the highest quality. Details on the applied QS are presented in **Supplementary Material S2.1.4**. The assigned score for each item is presented in **Supplementary Material S2.1.5**. In case of multiple outcomes or study designs, the highest QS is presented in **Table 2.1.1**, but analysis specific QS is shown in the harvest plots (**Figures 2.1.3A-F** and **Figures 2.1.4A-D**) and in the footnote of **Supplementary Material S2.1.3.**

Meta-analysis

To enable a consistent approach for the meta-analysis, relative risk estimates for associations of lutein and several outcomes that were differently reported by each study were transformed, using methods previously described.[42 43] Estimates were transformed to tertiles, and the transformed estimates thus represent the risk in the highest tertile of lutein, as compared to the lowest tertile. In a normal distribution, the means of the highest and lowest tertile lie 2.18 SD apart, therefore the log relative risks per SD were multiplied by 2.18 to obtain risk for highest as compared to lowest tertile. The means in the extreme quintiles are 2.8 SD apart, thus for conversion from quintiles to tertiles the conversion factor 2.18/2.80 was used. Similarly, scaling factors of 2.18/2.54 were used to convert quartiles, and 2.18/1.59 to convert from higher versus lower halves. We calculated standard errors of the log relative risks using published confidence limits and transformed the standard errors in the same way. This method enabled us to meta-analyze the results of the articles on disease risk, namely risk of coronary heart disease, risk of stroke, risk of metabolic syndrome and risk of type 2 diabetes mellitus. For these outcomes, we contacted authors to retrieve additional information, if meta-analysis was not possible based on the published results.

Heterogeneity between studies was tested by using the I^2 statistic (0 to 100%), which describes the percentage of variation across studies that is due to heterogeneity rather than to chance.[44] Analyses were done using random and fixed effects methods and results of both methods are presented.

Subgroup analyses were done to assess if the results differed by exposure assessment (dietary intake or blood levels), or by study design (prospective, cross-sectional, case-control). Sensitivity analyses were done to assess the influence of each individual study on the pooled relative risks, by omitting each study one by one. Funnel plots were used to assess the possibility of publication bias. All analyses were performed using STATA SE.

Harvest plots

For the associations of lutein with cardiometabolic risk factors, we used harvest plots to summarize reported evidence in a graphical way.[45] The harvest plots shows from

all studies on the outcome of interest if they reported inverse associations, positive associations, or no significant associations. The height of the bar represents the quality score, while the filling of the bar represents the exposure assessment (i.e. blood levels, dietary intake or supplements). Some outcomes were combined in one figure. More specifically, we combined all measures of blood pressure, (i.e. systolic blood pressure, diastolic blood pressure, hypertension), all measures of atherosclerosis (i.e. atherosclerosis of carotid arteries, (ilio)femoral arteries and abdominal aorta), all measures of insulin resistance (i.e. glucose, insulin, HOMA and HbA1c) and all measures of adiposity (i.e. body mass index, body fat percentage and waist circumference). We only present harvest plots if more than 3 studies reported on the same outcome.

Results

Study selection

Overall, 4,377 unique references were identified (**Figure 2.1.1**), of which 4,045 papers were excluded based on title and/or abstract. Full texts of the remaining 332 articles were further screened and 71 articles were included for data extraction.

Characteristics of studies included

Table 2.1.1 shows the characteristics of the 71 studies included in this systematic review. These studies contained a total of 387,569 participants (range 10 to 112,348) with mean ages ranging from birth to 82 years. Since some studies were performed within the same cohort, the populations partially overlapped. Of the 71 included papers, the vast majority (67 articles) were published after the year 2000. In children, we identified one intervention study,[46] one longitudinal study,[47] and two cross-sectional studies.[48,49] of which one also studied maternal lutein levels.[49] The remaining 67 studies all focused on adults, predominantly on middle-aged and elderly, of which 31 longitudinally, 33 cross-sectionally and only three as intervention study .The assigned QS ranged from 1 to 10, with an average of 6.5. Of the 71 studies, 21 studies received a score of 8 or higher, and 18 had a score of 5 or lower. From the total 71 studies, 26 studies were performed in Europe and 25 in the USA. The other studies were from Japan (6), China (5), Australia (3), Korea, Singapore, Costa Rica, Thailand, Uganda and the Philippines (1 each).

Figure 2.1.1 Flow chart of study selection

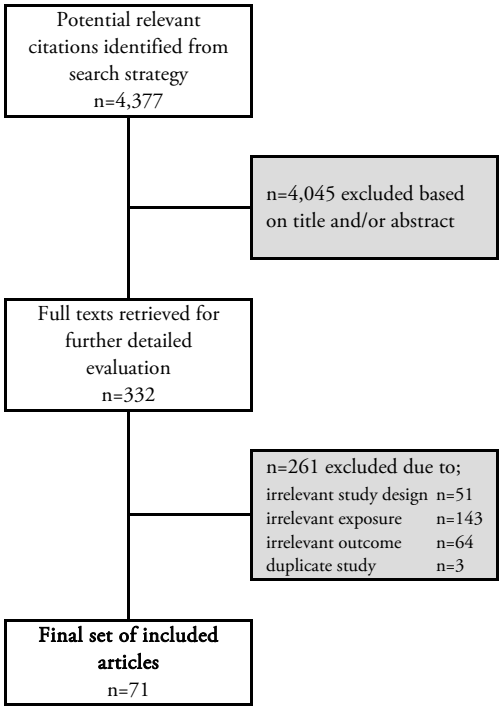


Table 2.1.1 Summary of the 71 studies included in this review

First author (year)	Country	Study design (FUP)	Total n	Age (y)	Population	QS
Fetal life and childhood						
Beydoun (2012)[48]	USA	Cross-sectional	1,339	16	General population	7
Capeding (2010)[46]	Philippines	Intervention (4mo)	230	10 d	Healthy term infants	10
Masters (2007)[49]	Poland	Cross-sectional	251	Birth	Term infants from healthy pregnant women . Nested in cohort.	5
Melikian (2001)[47]	Uganda	Longitudinal (5.5mo)	194	3.5 mo	HIV infected infants	5
Adults						
Almushatat (2006)[97]	UK	Cross-sectional	78	70§	Patients with prostate cancer	4
Ascherio (1999)[65]	USA	Longitudinal (8y)	43,738	40-75	Male healthcare professionals	8
Bates (2011)[66]	UK	Longitudinal (14y)	526*	77[1]	General population	6
Ben Amara (2014)[106]	France	Cross-sectional	108	41	Obese subjects	5
Beydoun (2011)[76]	USA	Cross-sectional	3,008	45[1]	General population	8
Blondin (2013)[111]	USA	Longitudinal (~2mo)	259	27	Premenopausal women	7
Brighenti (2005)[98]	Italy	Cross-sectional	243	60	(Ex)workers of a food company	3
Buijsse (2008)[69]	the Netherlands	Longitudinal (15y)	559	72	General population	6
Coyne (2005)[110]	Australia	Cross-sectional	1,570	>25	General population	6
Coyne (2009)[77]	Australia	Cross-sectional	1,523	>25	General population	7
D'Adamo (2012)[104]	USA	Longitudinal (1y)	148	82	Women with hip fracture	6
D'Odorico (2000)[82]	Italy	Longitudinal (5y)	392	54	General population	6
Dwyer (2001)[87]	USA	Longitudinal (1.5y)	477	50[1]	Employees of utility company	7
Ford (1999)[132]	USA	Cross-sectional	1,611	40-74	General population	8
Ford (2000)[50]	USA	Cross-sectional	10,163	54[1]	General population	8
Gey (2010)[51]	N. Ireland & France	Longitudinal (5y)	435	50-59	Cases with CHD, healthy controls, nested in cohort study	7
Granado-Lorencio (2006)[112]	Spain	Longitudinal (3mo)	40	21	Patients with type 1 diabetes	5
Graydon (2012)[99]	N. Ireland	Intervention (8w)	50	38	Healthy volunteers	8
Hak (2003)[53]	USA	Longitudinal (13y)	514	61[1]	Male physicians, cases with MI, healthy controls. Nested in cohort.	8
Hak (2004)[63]	USA	Longitudinal (13y)	312	61[1]	Male physicians, cases with stroke, healthy controls Nested in cohort.	7
Hirvonen (2000)[64]	Finland	Longitudinal (6.1y)	26,593	57	Male smokers in a trial of alpha-tocopherol and beta carotene	7
Hozawa (2006)[72]	USA	Longitudinal (15y)	4,493	25[1]	General population	9
Hozawa (2007)[94]	USA	Longitudinal (15y)	4,580	25	General population	9
Hozawa (2009)[81]	USA	Longitudinal (20y)	4,412	25[1]	General population	9
Iribarren (1997)[83]	USA	Cross-sectional	231	59	Cases with highest IMT, controls with lowest IMT Nested in cohort.	6
Ito (2006)[62]	Japan	Longitudinal (>8y)	3,254	39-85	General population	8
Kabagambe (2005)[56]	Costa Rica	Cross-sectional	2,912	58	Cases: first acute MI Controls: general population	7
Karppi (2011)[84]	Finland	Cross-sectional	1,212	72	General population	7

Table 2.1.1 (continued) Summary of the 71 studies included in this review

First author (Year)	Country	Study design (FUP)	Total n	Age (y)	Population	QS
Kataja-Tuomola (2011)[73]	Finland	Longitudinal (10y)§	25,505	57/58 [3]	Male smokers	8
Klipstein-Grobusch (2000)[85]	the Netherlands	Cross-sectional	217	67/66 [3]	Cases with atherosclerosis. Healthy controls, nested in cohort.	5
Knekt (2004)[58]	7 cohorts‡	Longitudinal (10y)	112,348	35-80[1]	General population	7
Koh (2011)[54]	Singapore	Longitudinal (1.85y)	840	69[2]	Cases with AMI, controls free of CVD. Nested in cohort.	8
Mayne (2004)[67]	USA	Longitudinal (4.3y)§	216	62[1]	Participants of a trial in patients with oropharyngeal cancer	6
Montonen (2004)[74]	Finland	Longitudinal (23y)	4,304	54/52 [3]	General population	7
Murr (2009)[59]	Austria	Cross-sectional	1,463	63§	Patients for angiography. Cases/ controls with/without stenosis	5
Nakamura (2006)[93]	Japan	Cross-sectional	876	55	General population	7
Nieto (2000)[86]	USA	Longitudinal (14y)	300	58/57[3]	Cases with highest IMT, controls with lowest IMT Nested in cohort.	6
Olea (2012)[113]	Spain	Cross-sectional	52	79	Patients with wet AMD	1
Osganian (2003)[57]	USA	Longitudinal (12y)	73,261	50[1]	General population (US Nurses)	8
Polidori (2007)[88]	Italy	Cross-sectional	92	69	Cases: atherosclerosis. Controls: matched volunteers	6
Renzi (2012)[115]	USA	Cross-sectional	66	23[1]	Participants of lutein supplementation trial	5
Rerksuppaphol (2010)[61]	Thailand	Cross-sectional	80	60	Cases: previous CHD Controls: self-reported healthy volunteers	3
Ribaya-Mercado (1995)[116]	USA	Cross-sectional	10	66	Participants of trial on beta-carotene supplementation	4
Rowley (2003)[100]	Australia	Cross-sectional	171	38	Aboriginals	6
Ruiz Rejon (2002)[60]	Spain	Cross-sectional	210	50	Cases: myocardial infarction controls: hospital visitors	4
Sesso (2004)[70]	USA	Longitudinal (4.8y)	966	59	Female participants of a trial of beta carotene, vit E, aspirin supplements.	8
Sesso (2005)[71]	USA	Longitudinal (2.1y)	998	70	Male participants of a trial of multivitamin, vit C, vit E supplements.	8
Shardell (2011)[68]	USA	Longitudinal (>12y)	13,293	43	General population	9
Sluijs (2009)[78]	the Netherlands	Cross-sectional	374	60	General population	5
Street (1994)[52]	USA	Longitudinal (7-14y)	226	23-58[1]	Cases: first MI. Controls: community and hospital. Cohort of blood donors	6
Sugiura (2006)[114]	Japan	Cross-sectional	812	55	General population	7
Sugiura (2008)[79]	Japan	Cross-sectional	958	55	General population	5
Sundl (2009)[101]	Austria	Cross-sectional	37	55	Patients on peritoneal dialysis	4
Suzuki (2010)[102]	Japan	Cross-sectional	437	61	General population	6
Suzuki (2011)[80]	Japan	Cross-sectional	931	59	General population	6
Tavani (2006)[55]	Italy	Cross-sectional	1,442	60§	Cases: first episode of AMI. Controls: admitted with no AMI	6
Tornwall (2000)[92]	Finland	Longitudinal (4y)§	26,872	58	Male smokers from a trial of alphatocopherol, beta carotene	8
van Herpen-Broekmans (2004)[103]	the Netherlands	Cross-sectional	379	42	General population	5
Wang (2006)[75]	USA	Longitudinal (<11y)	940	56	Participants of a trial of aspirin, vit E supplements	7
Wang (2013)[109]	China	Intervention (12w)	116	55	Healthy volunteers	10

Table 2.1.1 (continued) Summary of the 71 studies included in this review

First author (Year)	Country	Study design (FUP)	Total n	Age (y)	Population	QS
Wang (2014)[96]	USA	Cross-sectional	2,856	>20	General population	8
Waters (2007)[108]	USA	Cross-sectional	22	50-77	Postmenopausal women from a trial of eggs with cholesterol and lutein	2
Xu (2012)[a 89]	China	Cross-sectional	80	56	Cases: early atherosclerosis. Matched controls, nested in cohort.	5
Xu (2012)[b 105]	China	Intervention (3mo)	65	57	Patients with early atherosclerosis	9
Yeon (2012)[107]	Korea	Longitudinal (2w)	22	23	Overweight female participants in a trial of fruit and vegetable intake.	6
Zou (2011)[90]	China	Cross-sectional	232	56	General population	5
Zou (2014)[91]	China	Longitudinal (1y)	45	57	Participants of a trial (20mg lutein/d for 12 m)	6

§ median; † Participants from cities in Denmark, France, Italy, the Netherlands, Portugal, Spain, Switzerland, and the UK
ǂ Atherosclerosis Risk in Communities Study; Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (the placebo arm); Finnish Mobile Clinic Health Examination Survey; Health Professional Professionals Follow-Up Study; Iowa Women's Health Study; Nurses' Health Study
*Bates (2011): n=526 is maximum sample size. Unclear in what sample size lutein was available.
[1]Not based on total n for analysis (e.g. due to loss to FUP);Bates(2011) n=1,054. Beydoun(2011) n=3,203. Dwyer(2001) n=480. Ford(2000) n=11,210. Hak(2003) n=1,062. Hak(2004) n=594. Hozawa(2006) n=4,580. Hozawa(2009) n=4,712. Knekt(2004) n=129,244. Mayne(2004) n=259. Osganian(2003) n=73,261. Renzi(2012) n=108. Street(1994) n=369; [2]Mean age at diagnosis, [3]Cases/controls respectively

Associations between lutein and coronary heart disease

Ten studies on lutein and coronary heart disease were pooled, with 8,239 cases from in total 203,630 participants[50-59] (**Figure 2.1.2**). Six studies were longitudinal with a follow-up between 1.85 and 14 years, the other four were cross-sectional. The pooled results show that the higher lutein was associated with a lower risk of coronary heart disease (RR 0.88 (95%CI 0.80; 0.98)), highest vs. lowest tertile.

Two studies could not be included in the meta-analysis because they only compared lutein between cases and controls. One study (QS 4) found lower levels of lutein in the cases[60] while the other (QS 3) found no difference.[61] A third study that could not be pooled (QS 8) found no association between lutein and risk of mortality from CHD (HR 0.82 (95%CI 0.42; 1.58), per unit increase in lutein levels).[62]

Associations between lutein and stroke

Results of three longitudinal studies that reported on the associations between lutein and stroke were pooled.[63-65] The number of controls largely overlap, but the total number of unique cases was 1,398. Pooled results showed that higher lutein was associated with a lower risk of stroke (RR 0.82 (95%CI 0.72; 0.93)), highest vs. lowest tertile (**Figure 2.1.2**).

One study (QS 8) could not be included in the meta-analysis because it did not report information on the distribution of lutein but this study reported results in the same direction (stroke-specific mortality HR 0.72 (95%CI 0.37; 1.37) per unit increase of lutein levels).[62]

Associations between lutein and mortality from cardiovascular diseases

Five studies reported on lutein in relation to mortality from any cardiovascular disease (QS 6 to 9). Mean follow-up time ranged from 4.25 to 15 years and sample sizes ranged from 216 to 13,293. None of the studies found significant associations, but the effect estimates were mostly in the direction of higher lutein being associated with a lower risk of mortality from cardiovascular disease,[62,66-68] except for

one study.[69] Two studies from the same cohort (one in males, one in females) were not included in the meta-analyses because they reported only on a combined outcome of any cardiovascular disease. Both studies found no significant associations.[70 71]

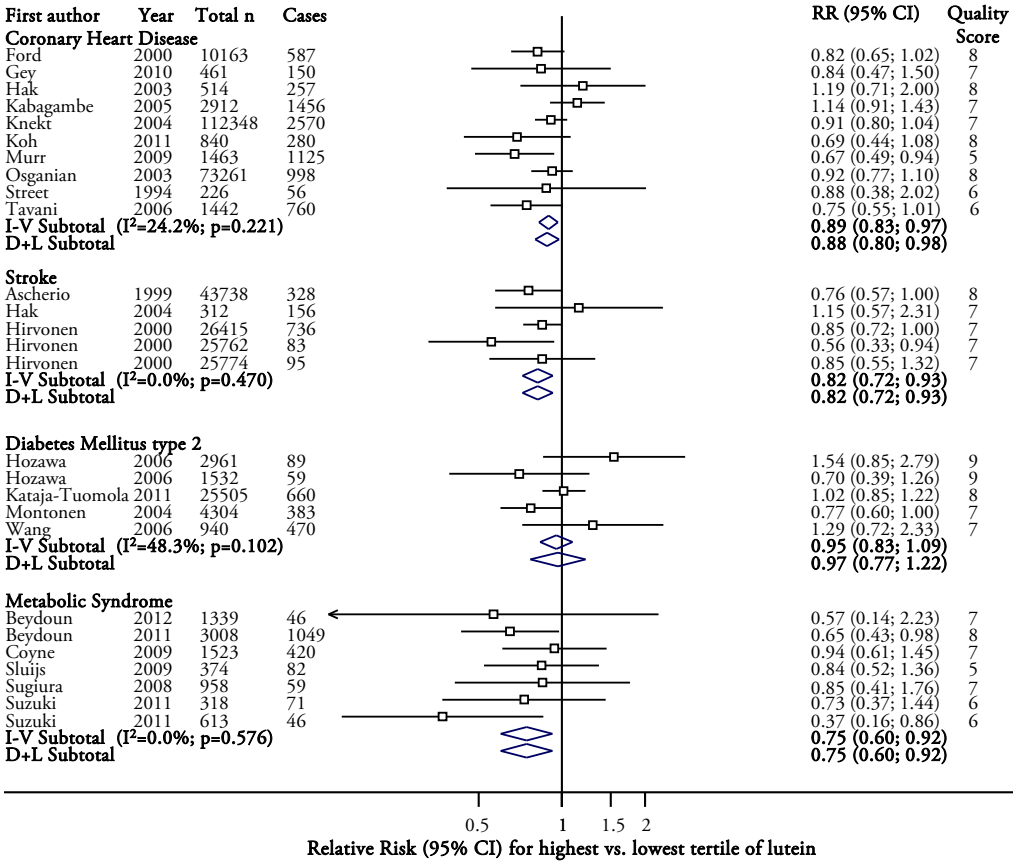
Associations between lutein and type 2 diabetes mellitus

Figure 2.1.2 presents the meta-analysis of the results of lutein in relation to type 2 diabetes. Four studies were included[72-75] which were all longitudinal observational studies with follow-up periods between 10 and 23 years, including in total 35,242 participants (of which 1,661 cases). The pooled results showed no significant association between lutein and risk of diabetes (RR 0.97 (95%CI 0.77; 1.22), highest versus lowest tertile of lutein).

Associations between lutein and metabolic syndrome

Of the six studies on metabolic syndrome, one was in adolescents[48] and the others were in adults.[76-80] All studies were cross-sectional, with in total 8,133 participants (of which 1,773 cases). The highest tertile of lutein was associated with a lower risk of metabolic syndrome (RR 0.75 (95%CI 0.60; 0.92)), as compared to the lowest tertile (**Figure 2.1.2**).

Figure 2.1.2 Meta-analysis of the associations between lutein and risk of CHD, stroke, DMT2 and metabolic syndrome.



I-V= Inverse Variance (fixed-effects model); D+L= DerSimonian and Laird (random-effects model)

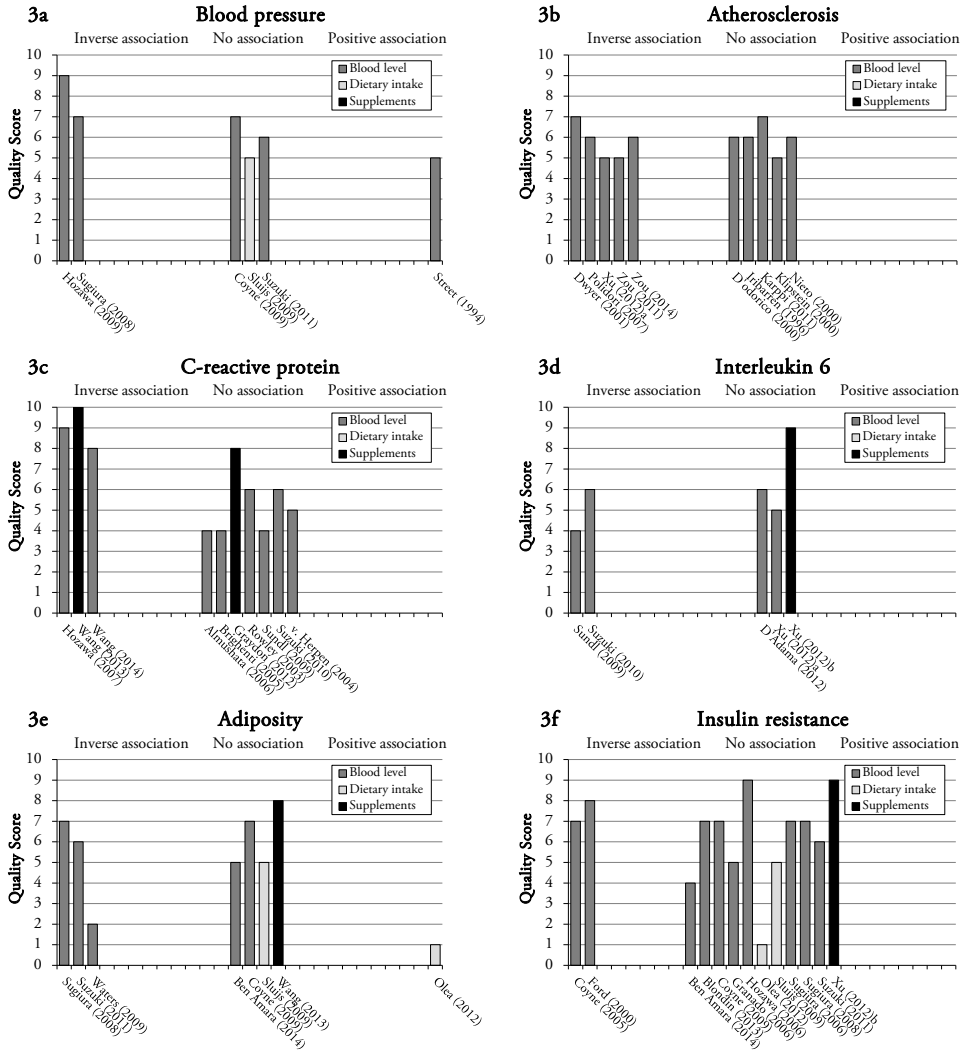
Additional analyses

There was no statistical evidence for heterogeneity between studies for any of the outcomes included in the meta-analyses. Subgroup analyses by exposure assessment (i.e. dietary intake or blood levels), or by study design (i.e. prospective, cross-sectional or case-control studies) also did not show differences in results in the subgroups.

To examine the influence of the individual studies on the overall risk estimate, sensitivity analyses were performed by omitting each study one by one, which showed that none of the individual studies significantly affected the results.

Funnel plots to assess the possibility of publication bias did not show any obvious asymmetry (data not shown).

Figure 2.1.3 Harvest plots of the evidence of an association between lutein and cardiometabolic risk factor



In the study of Suzuki (2010), the inverse association between lutein and interleukin-6 was significant in women only.
In the study of Suzuki (2011), the inverse association between lutein and adiposity was significant in women only.

Associations between lutein and cardiometabolic risk factors

Figure 2.1.3 shows harvest plots of the association between lutein and blood pressure, and between lutein and atherosclerosis. From the six studies on blood pressure, two studies observed a significant inverse association[79,81] and these studies were of somewhat higher quality than those reporting no significant association[77,78,80] or a positive association[52] (**Figure 2.1.3a**). Out of the 10 studies on atherosclerosis, five studies found no significant associations[82-86] and five studies with a similar quality found a significant inverse association[87-91] (**Figure 2.1.3b**). Three studies reported on other cardiovascular risk factors. The largest study (n=26,872, QS 8) observed that intake of lutein (assessed retrospectively, 2 years before the outcome) was significantly associated with a lower risk of intermittent claudication.[92] The other two studies found no significant associations between lutein levels and pulse wave velocity (QS 7)[93], and between lutein levels of arterial stiffness (QS 5).[90]

We identified 15 studies on inflammatory markers, which reported on C-reactive protein (10 papers), interleukine-6 (5 papers), leukocytes (3 papers), TNF-alpha (2 papers), fibrinogen (2 papers), IFN-gamma (1 paper) and interleukin-1 (1 paper). The harvest plot for C-reactive protein shows that of 10 studies, three found a significant inverse association[94-96] including one intervention study.[95] The other seven studies[97-103] (also including one intervention study[99]) found no significant associations (**Figure 2.1.3c**).

The harvest plot for interleukin-6 shows that two studies observed significant inverse associations[101,102] while three studies (including an intervention study) did not[89,104,105](**Figure 2.1.3d**).

Of three studies on leukocytes, two (QS 5 and 8) found significant inverse associations[94,106] and one study (QS 5) found a non-significant inverse association.[103] Of two studies on TNF-alpha (both QS 6), one study found an inverse association,[104] while the other did not.[102] The two studies on fibrinogen (QS 5 and 9) did not find significant associations[94,103] and neither did the study on IFN-gamma (QS 5).[89] The only study (QS 6) on IL-1, found a significant inverse association between lutein blood levels and IL-1 beta in lipopolysaccharide-activated peripheral blood mononuclear cells.[107]

Three studies reported on the relation between lutein and child body composition. One study (QS 6) found no significant associations of either maternal blood levels or cord blood levels of lutein with birth weight, birth length or head circumference.[49] An intervention study in the Philippines (QS 8) also did not find growth differences between children receiving lutein-fortified infant formula and those receiving regular formula,[46] but a study in HIV-infected infants (QS 5), found lutein levels at 3,5 months to be significantly associated with higher attained weight and height at 9 months, but not with weight or height velocity.[47]

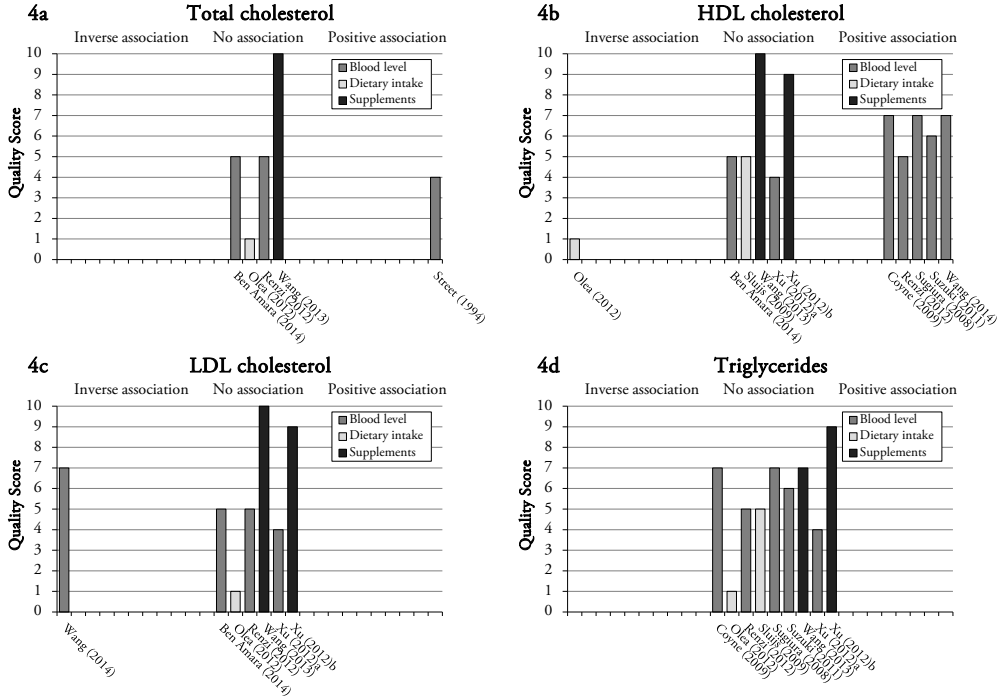
Figure 2.1.3e shows the harvest plot of the association between lutein and adiposity, measured as body fat percentage, body mass index or abdominal adiposity using waist circumference. Of a total of eight studies, three studies showed a significant inverse association[79,80,108] but four studies[77,78,106,109]

(including a high quality intervention study[109]) did not. However, one of the studies that did not find an association with waist circumference, BMI or visceral fat, did find that higher dietary intake of lutein was associated with lower levels of subcutaneous fat.[78] Also, one of the studies observed that the inverse association between lutein levels with waist circumference was only significant in women, but not in men.[80]

The harvest plot of insulin resistance (glucose, insulin, HOMA, HbA1c) is shown in **Figure 2.1.3f**. We identified 13 studies, of which two found a significant inverse association[50,110] and the others no associations.[72,77-80,105,106,111-114]

Harvest plots of the associations of lutein with blood lipids are shown in **Figure 2.1.4**. For total cholesterol, one study found a positive association[52], but one intervention study[109] and three observational studies[106,113,115] found no association (**Figure 2.1.4a**). Out of 11 studies on HDL-c, one found an inverse association[113] while five studies found higher lutein levels to be associated with higher HDL-c[77,79,80,96,115] However, also five studies[78,89,105,106,109], of which two interventional[105,109], found no significant associations (**Figure 2.1.4b**). For LDL-c, two interventional[105,109] and four observational studies[89,106,113,115] found no significant associations. Only one study found a significant association, with higher blood levels of lutein being associated with lower LDL-c[96] (**Figure 2.1.4c**). Nine studies on triglycerides (including also two intervention studies[105,109]) all found no significant associations[77-80,105,113,115] (**Figure 2.1.4d**). One study (QS 4) was not included in the harvest plots because it assessed total lipids (cholesterol and triglycerides combined). This study found no significant association.[116]

Figure 2.1.4 Harvest plots of the evidence of an association between lutein and blood lipids



Discussion

In this systematic review with meta-analysis, we showed that higher lutein was associated with a lower risk of coronary heart disease, stroke, cardiovascular mortality and metabolic syndrome, but not with risk of type 2 diabetes mellitus. The literature on risk factors of cardiometabolic diseases showed that lutein could possibly prevent atherosclerosis and reduce inflammatory markers, but there were inconsistent associations with blood pressure, adiposity, insulin resistance and blood lipids. The majority of the studies was observational and performed in adults, and the effects of lutein on cardiometabolic health in children remains largely unaddressed.

Several biological mechanisms have been proposed to underlie the potential beneficial effect of lutein on cardiometabolic health, including, antioxidant effects, effects on immune response and inflammation, and vascular changes. These combinatory mechanism suggest that lutein could act as a beneficial factor for overall health as well as for specific organ systems. Antioxidant effects may play a role in the possible beneficial effect of lutein on the risk of metabolic syndrome, because it has been suggested that oxidative stress (for example induced by smoking), can lead to insulin resistance.[117,118] Lower oxidative stress, either from low levels of oxidant stressors or from high levels of antioxidants, might thus decrease insulin resistance and reduce the risk of metabolic syndrome. Our meta-analysis indeed showed a significant inverse association between lutein and the risk of metabolic syndrome, but no significant association with the risk of type 2 diabetes mellitus. If the protective effect of lutein on metabolic syndrome is indeed through increasing insulin sensitivity, it is striking that there is no association with type 2 diabetes mellitus. Only two of 13 papers on insulin resistance showed a significant inverse association with lutein, hence this mechanism seems unlikely. It could be hypothesized that the association of lutein with metabolic syndrome arises because lutein may influence other components of the metabolic syndrome, such as blood pressure, triglycerides and adiposity. However, in our systematic review we found no consistent associations with any of these components. Since lutein is absorbed along with fat in the gastrointestinal tract and transported through chylomicrons, a relationship between lutein and lipid levels can be expected.[115] In this systematic review, several observational studies demonstrated an association between lutein and lipid levels, in particular higher HDL-levels,[77,79,80,115] but the two intervention studies did not demonstrate any effect of lutein supplementation on lipid levels.[105,109] Furthermore, none of the observational studies on triglycerides showed a significant association and results for adiposity were inconsistent, suggesting that it is unlikely that the association between lutein and metabolic syndrome is driven by these factors.

An important issue to consider when interpreting the meta-analysis of metabolic syndrome and diabetes, is the methodological quality of the studies. Most importantly, all studies in the meta-analysis for metabolic syndrome were cross-sectional. In cross-sectional studies, there is a higher risk of reverse

causation, which would mean that lower levels of lutein are a consequence of disease processes, rather than a cause. This might be of particular concern in diseases where oxidative stress can play a role, because blood levels of antioxidants are being used to counteract oxidative stress and depletion of lutein levels might thus occur in patients with obesity or metabolic syndrome, which has been suggested by several studies.[119-121] The studies on type 2 diabetes mellitus were all prospective studies with fairly long follow-up periods (over 10 years), which make them less susceptible to reverse causation.

Our meta-analysis also showed that higher lutein intake or levels were associated with a lower risk of coronary heart disease and stroke. The attention to the role of lutein in the prevention of age-related macular degeneration has shifted from a local effect in the eye towards a possible systemic anti-inflammatory function.[35] In our systematic review, we indeed observed that lutein was associated with lower levels of several inflammatory markers, with the strongest evidence for C-reactive protein. C-reactive protein has been consistently associated with risk of cardiovascular diseases[122] but it is unclear whether it is a causal risk factor or rather a marker of disease.[123] The mechanisms by which lutein affects the immune response may, however, differ from its antioxidant effects.[124] Animal studies have shown that lutein enhances the antibody response to T-cell dependent antigens[125] and stimulates both cellular and humoral immunity.[126-128] In addition, there is some evidence that lutein can reduce wall thickening of the small arteries.[129,130] We identified ten studies on atherosclerosis of which five showed that higher blood levels of lutein were associated with less atherosclerosis. Both inflammatory activity and atherosclerosis play a role in the development of cardiovascular diseases and might be the underlying mechanisms for the association between lutein and cardiovascular disease.

Although there was no significant heterogeneity in the meta-analysis, it should be noted that we included studies using blood levels of lutein as exposure as well as studies using dietary intake of lutein. When dietary intake is used, there is no absolute measure of how much is absorbed into the body and the correlation between dietary intake of lutein and blood levels has been reported to be low.[131] Blood levels may thus provide a more direct measurement since they may be closer to the physiological pathways and may be less prone to measurement error, which can occur when using self-reported dietary intake. On the other hand, the use of blood levels may lead to reverse causality in case the disease itself can deplete lutein levels in the body.[119-121] Furthermore, with both measurements, any association that is found might be confounded by other carotenoids that have similar effects, since the majority of studies was observational and most of them were not able to adjust for the intake or blood levels of other carotenoids. In addition, lutein might be just a marker of a healthier dietary and the associations may also be confounded by other factors associated with a healthier lifestyle, such as physical activity. Thus, given the limited number of intervention studies causality of the observed associations should be interpreted with caution. Overall, further research is required to elucidate this. Some caution should also be taken when

generalizing the results of this review. As exposure patterns and confounders may differ in low- or middle-income countries, the associations between lutein and cardiovascular diseases that we found might not be similar and therefore the generalizability of our results may be limited. Out of 71 included studies included in this review, 51 were set in Europe or the USA, and hardly any of the studies were performed in low- or middle-income countries.

This systematic review has several strengths. An important strength is the systematic search in multiple databases by an experienced biomedical information specialist. Second, full texts were reviewed of studies on any carotenoid, to identify articles that examined lutein but did not mention lutein in title or abstract, which could occur especially when no significant associations of lutein are found. Furthermore, we used different indices of lutein status, which allows a complete overview of the literature on lutein. In addition, we included intermediate markers and risk factors for cardiometabolic diseases as well as hard endpoints. This enabled us to perform a meta-analysis on risk of certain diseases, but also gives some insight into the potential mechanisms underlying the associations.

In conclusion, to date, lutein has mostly been investigated in observational studies. These studies showed that higher dietary intake of lutein and higher blood levels are associated with a lower risk of coronary heart disease, stroke and cardiovascular mortality, possibly through less atherosclerosis and lower inflammatory activity. However, further studies and randomized controlled trials are required to evaluate the causality of these associations. A potential inverse association of lutein with metabolic syndrome should be further studied in high quality longitudinal studies, as this has thus far only been shown cross-sectional. As few studies focused on the effect of lutein during pregnancy and childhood, conclusions on the effect of lutein along the full life course cannot be drawn. Hence, more research is needed on the effects of lutein during fetal life, infancy and childhood.

Supplementary Material can be found online: http://hdl.handle.net/1765/77768

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Abstract

Background Choline is a precursor of betain and a precursor of acetylcholine and might therefore influence cardiovascular and cognitive outcomes. However, concerns have also been raised that choline may influence dyslipidemia because it is also an essential component of very low density lipoproteins.

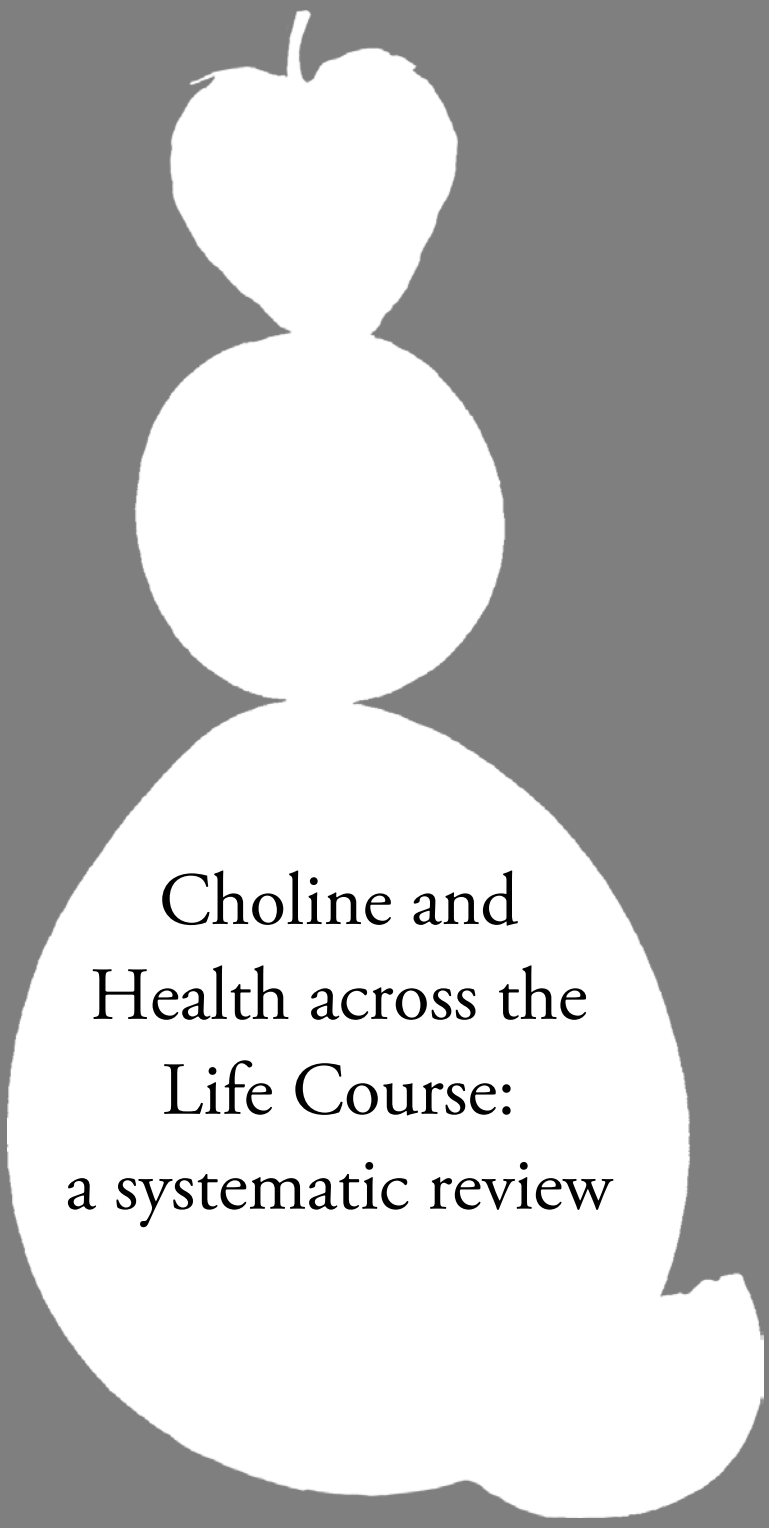
Objective We aimed to systematically evaluate the literature on the effect of choline on body composition, metabolic, cardiovascular, respiratory and neutological outcomes in different life stages.

Design and data sources Systematic review and meta-analysis of literature published in Medline, Embase, Cochrane Central, Web of Science, PubMed and Google Scholar up to July 25th 2014.

Study selection Studies included were trials, cohort, case-control and cross-sectional studies in which the association of of choline levels, dietary intake or supplements with body composition, metabolic, inflammatory, cardiovascular, respiratory and neurological outcomes during pregnancy, childhood or adulthood, was reported.

Results We included 50 relevant articles. There is some observational evidence that availaibility of choline during pregnancy may be beneficial for child neurological health. In adults, there may be a possible beneficial effect of choline on cognition and glucose metabolism, but high quality (intervention) studies are lacking. Results were inconsistent for body composition, blood lipids and cardiovascular health

Conclusions: Evidence to confirm the suggested effects of choline on health in different stages of life is scarce. Potential beneficial effects of choline need to be confirmed by intervention studies. Possible harmful effects on cardiometabolic health need careful evaluation.


Choline and
Health across the
Life Course:
a systematic review

Introduction

Choline is a water-soluble nutrient that is officially considered a required dietary nutrient by the US Institute of Medicine's Food and Nutrition Board since 1998. Choline is required for structural integrity and signaling functions of cell membranes, methyl group metabolism and neurotransmitter synthesis.[133] As a precursor of betaine, choline may enhance the remethylation of homocysteine into methionine.[134] Hyperhomocysteinemia has been suggested to be related to the development of cardiovascular disease, and therefore it is hypothesized that the effect of choline on lowering homocysteine levels might be beneficial for cardiovascular health.[134] In contrast, phosphatidylcholine, a phospholipid that contains choline, is an essential component of very low density lipoproteins. For that reason, it has been argued that high dietary choline intake might be related to hypercholesterolemia and associated increased risk of cardiovascular disease,[134] and betaine supplementation has been reported to increase plasma LDL cholesterol[135]and decrease HDL cholesterol.[136] Nevertheless, choline is mainly metabolized and stored in the liver and choline deficiency can induce abnormal phospholipid syntheses that may lead to the development of non-alcohol fatty liver disease (NAFLD).[137] This evidence is mainly from animal studies or selected patient populations,[138,139], but whether choline also could prevent NAFLD in the general population is not yet established.

As choline is also a precursor of the neurotransmitter acetylcholine, it may be related to cognitive function and brain development.[134] Demand for choline increases during pregnancy and lactation due to altered one-carbon metabolism, and it has been suggested that choline is important for the neurological development of the unborn child.[134,140] Animal studies suggest a causal relationship between dietary availability of choline during development and cognitive function,[33] but this has not been firmly established in humans yet. Choline has been extensively studied in stroke patients and patients with cognitive deficits,[32,141] but it is unknown if choline is also beneficial for cognition for the general population.

A full overview of the evidence on the associations of choline with health outcomes in different life stages is currently not available. Therefore our aim was to systematically search the available literature to evaluate associations of choline in relation to body composition (growth and adiposity), neurological (cognitive function, fetal neurodevelopment), cardiovascular (coronary heart disease and stroke), metabolic (type 2 diabetes mellitus, glucose metabolism, NAFLD), inflammatory (C-reactive protein, pro-inflammatory cytokines), and respiratory (chronic pulmonary diseases and lung function) outcomes across the life course, in participants free from diseases at baseline.

Methods

Search strategy

An experienced information specialist from the medical library, together with the first author, conducted a systematic review of the current literature from inception up to July 25th 2014

in four major electronic databases: Medline, Embase, Cochrane Central, Web of Science. Additionally not yet indexed articles in PubMed were searched and the most relevant references from Google Scholar were downloaded. We searched for studies in which the association of choline (blood levels, dietary intake or supplementation) with body composition, neurological, cardiovascular, metabolic, inflammatory, or respiratory outcomes at different life stages (i.e. during fetal life, in childhood and adulthood) were reported. The computer-based searches combined search terms related to the exposure and outcomes of interest, without any language restriction. Further details on the search strategy are shown in **Supplementary Material S2.2.1**, including the number of (unique) references identified per database.

Study identification and selection

Studies were eligible for inclusion if they were interventional or observational (cohort, case-control or cross-sectional) studies; if they studied the effects of choline (biomarker or dietary intake) in any form (including e.g. lecithin, citicoline, phosphatidylcholine) as exposure; and if the outcomes of interest were related to body composition, cardiovascular, metabolic, respiratory or neurolgocial health

We excluded letters, abstracts, reviews, conference proceedings, case reports, studies not carried out in humans, and those which were carried out in selected patient populations or participants with diseases at baseline unless they were compared with a control group of healthy individuals.Two independent reviewers screened the titles and abstracts of all initially identified studies according to the selection criteria. Any disagreements in article selection were resolved through discussion and a third author was available to resolve any disagreement. For all included abstracts, full text articles were retrieved and were assessed once more by two independent authors to ensure that they satisfied all predefined selection criteria. In case of multiple publications from the same study reporting on the same outcome, only the study with the longest follow-up, or the study with the most complete analyses was included. Of the 10% most recent articles, reference lists were screened and authors were contacted, as well as experts in the field, to retrieve additional relevant articles.

Data extraction and analyses

Data were extracted using a structured database created prior to the literature search. Detailed study-level characteristics were extracted, including study characteristics (such as design, sample size, duration of follow-up, inclusion criteria), population characteristics (such as age, sex, ethnicity), exposure measurement (such as blood level, diet assessment method, supplementation), outcome assessment (such as validity of the method, criteria), analysis (such as statistical method, measure of association, sensitivity analyses), results (such as effect estimate, standard error/confidence interval) and covariates (such as key confounders, additional adjustments). In case of repeatedly measured outcomes, only results from the longest follow-up are presented. Data were extracted by one author, and a random 10% of data were checked by an independent author.

Quality analysis

We used a predefined quality score (QS) to evaluate the quality of included studies. The QS is a modified version of previously used scoring systems.[24,31] A score of 0, 1, or 2 points was allocated to each of the following five items: study design, study size, exposure assessment, outcome assessment, and adjustment for potential confounders. This allowed a total score between 0 and 10 points, with 10 representing the highest quality. Details on the applied QS are presented in **Supplementary Material S2.2.2.** In case of multiple outcomes or study designs, the highest QS is presented in table 1. The assigned score for each item is presented in **Supplementary Material S2.2.3**.

Results

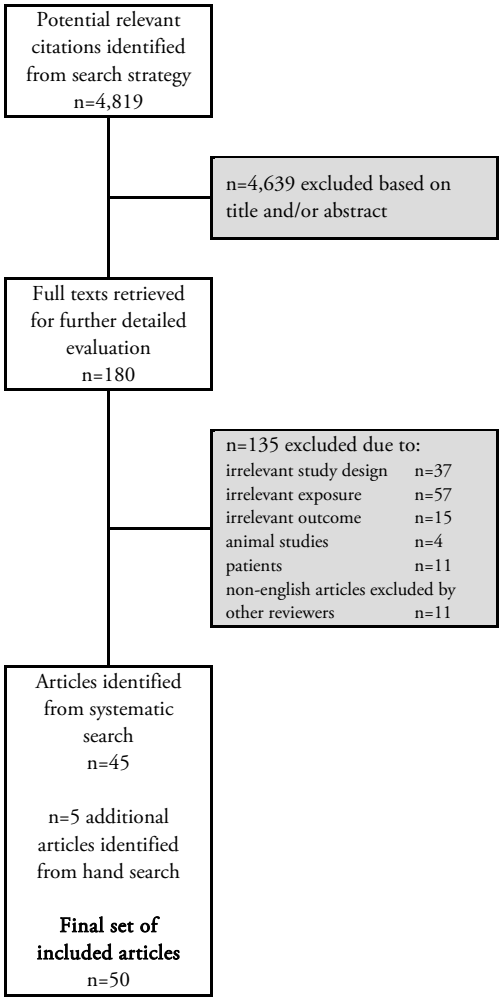
Study selection

In the search strategy 4,819 unique references were identified (**Figure 2.2.1**). Following initial screening, 4,639 papers were excluded based on title or abstract. Of the 180 remaining papers, the full texts were checked and 45 articles were included. Five articles were additionally identified by hand searching. Thus, the total number of paper included in this review was 50 articles.

Characteristics of studies included

Table 2.2.1 shows the characteristics of the 50 included articles involving a total number of 61,709 participants (range from 6 to 16,165 participants per study, but some study populations slightly overlap) with age at outcome measurement ranging from birth to 74 years of age. The articles were published between 1978 and 2014, with the majority (76%) being published after 2000. The overall QS of the included studies ranged from 2 to 10, with a mean score of 6.6. The scores for each item of the QS per study are shown in **Supplementary Material S2.2.3.** There were 31 observational studies, of which 14 were cross-sectional and 17 were longitudinal. Furthermore, we included 19 intervention studies.

Figure 2.2.1 Flow chart of study selection



We identified 11 articles that reported on choline during pregnancy, which subsequently assessed health outcomes in children from birth to 7.8 years old. Five papers reported on effects of choline intake or levels in childhood, and the remaining 33 articles focused on adults.

Of the 11 studies that investigated choline intake or levels during pregnancy, two were intervention studies, five studies assessed blood levels and the other four reported on dietary intake. The studies during pregnancy were, except for one study from the Netherlands and one in Ireland, all done in North America. One reported on congenital heart defects, two of them reported on birth weight, all other studies had child neurological health as their outcome.

We identified five articles that reported on the effects of choline in childhood, all of which were cross-sectional studies. A study from the Republic of Seychelles reported on child neurological outcomes. Three studies (two from Spain and one from the USA) examined cord blood choline in relation to birth weight. The fourth study was a German study published in1983 that assessed child overweight.

Of the 34 studies in adults, 18 were performed in the USA, 13 in Europe and the others in Japan, China and Australia. Half of the studies in adults were intervention studies, the other half were observational (7 longitudinal and 10 cross-sectional).

Table 2.2.1 Summary of the 50 studies included in this review

First author (year)	Country	Study design (FUP)	Total n	Age (y)	Outcomes	QS
Fetal life[1]						
Boeke (2013)[146]	USA	Longitudinal (7.8y)	813	7.8	Neurological	7
Carmichael (2010)[141]	USA	Longitudinal (CC)(1y) (RD)	955	Birth	Neurological	7
Cheatham (2012)[144]	USA	Interventional (1y)	99	1	Neurological	9
Hogeveen (2013)[164]	Netherlands	Longitudinal (NM)	3,66[2]	Birth	Body composition	5
Jiang (2012)[163]	USA	Interventional (12w)	24	Birth	Body composition	7
Mills (2014)[143]	Dublin	Longitudinal (CC) (NM)	769	Birth	Neurological	7
Shaw (2004)[142]	USA	Longitudinal (CC)	864	Birth	Neurological	7
Shaw (2014)[184]	USA	Longitudinal (CC) (NM)	414	Birth	Cardiovascular	6
Signore (2008)[147]	USA	Longitudinal (6y)	400	5.5	Neurological	7
Villamor (2012)[145]	USA	Longitudinal (4y)	1,210	3.3	Neurological	7
Wu (2012)[148]	Canada	Longitudinal (2y)	154	18mo	Neurological	7
Children						
Buchman (2001)[167]	USA	Cross-sectional	114	Birth	Body composition	2
Ivorra (2012)[165]	Spain	Cross-sectional	50	Birth	Body composition	3
Kupke (1983)[168]	Germany	Cross-sectional	357	5	Body composition	2
Sanz-Cortés (2013)[166]	Spain	Cross-sectional	154	Birth	Body composition	5
Strain (2013)[149]	Republic of Seychelles	Cross-sectional	210	5.6	Neurological	5
Adults						
Benton (2004)[156]	UK	Interventional (3d)	400	22	Neurological	10
Bertoia (2014)[185]	USA	Longitudinal (CC) (>20y)	1,138	63	Cardiovascular	7
Bidulescu (2007)[186]	USA	Longitudinal (14y)	14,430	54	Cardiovascular	8
Chen (2013)[175]	China	Cross-sectional (CC)	372	59	Metabolic	5
Dalmeijer (2008)[135]	the Netherlands	Longitudinal (8.1y)	16,165	57	Metabolic & Cardiovascular	8
Davis (1980)[157]	USA	Interventional (3d)	15	18-34	Neurological	8

Table 2.2.1 (continued) Summary of the 50 studies included in this review

First author (year)	Country	Study design (FUP)	Total n	Age (y)	Outcomes	QS
Detopoulou (2008)[181]	Greece	Cross-sectional	3,042	40	Metabolic	6
Deuster (2002)[154]	USA	Interventional (3h)	13	28	Neurological	8
Drachman (1982)[160]	USA	Interventional (5w)	16	70	Neurological	8
Evans (2007)[179]	USA	Interventional (4y)	22	61*	Metabolic	7
Ferrannini (2013)[174]	European countries[3]	Longitudinal (3[4]/9.5[5] y)	3,482	45	Metabolic	8
Floegel (2012)[172]	Germany	Longitudinal (CC) (7y)	3,082	51	Metabolic	8
Harris (1983)[155]	USA	Interventional (5h)	9	40	Neurological	8
Imajo (2012)[183]	Japan	Cross-sectional (CC)	135	47	Metabolic	4
Kalhan (2010)[182]	USA	Cross-sectional (CC)	60	43	Metabolic	5
Killgore (2009)[169]	USA	Interventional (6w)	16	47	Body composition	4
Ladd (1993)[153]	USA	Interventional (90min)	73	20*	Neurological	9
Mapstone (2014)[162]	USA	Longitudinal (CC)(5y)	101	81	Neurological	6
Meikle (2013)[173]	Australia	Cross-sectional	348	62*	Metabolic	5
	USA	Cross-sectional	987	35*	Metabolic	6
Mohs (1980)[158]	USA	Interventional (10d)	20	69	Neurological	8
Nurk (2012)[150]	Norway	Cross-sectional	2,195	70-74	Neurological	7
Olthof (2005)[134]	the Netherlands	Interventional (2w)	26	50-71	Metabolic	8
Poly (2011)[151]	USA	Longitudinal (3-10y)	1,391	611	Neurological	7
Ried (2013)[188]	Germany	Cross-sectional	2,925[6]	5[6]	Respiratory	7
Schriewer (1984)[170]	Germany	Cross-sectional	2,324	♂42/♀37	Metabolic, cardiovascular & Body composition	4
Simonsson (1982)[176]	Sweden	Interventional (6h)	6	24-39	Metabolic	3
Sitaram (1978)[152]	USA	Interventional (90 min)	10	24	Neurological	8
Stegemann (2014)[187]	Italy	Longitudinal (10y)	685	66	Cardiovascular	7
Sorgatz (1988)[159]	Germany	Interventional (6w)	65	44*	Neurological	9
Spiers (1996)[161]	USA	Interventional (3m)	90	67	Neurological	9
Veenema (2008)[180]	USA	Interventional (12w)	60	22	Metabolic	6
Wallace (2012)[177]	UK	Interventional (12w)	42	60	Metabolic	8
Yan (2012)[171]	USA	Cross-sectional	237	22ǂ	Body composition & Metabolic	3
Zeisel (1991)[178]	USA	Interventional (3w)	14	28	Metabolic	8

ǂmedian; *Mean age based on other population size (Evans n=25, Ladd n=80, Sorgatz n=68, Meikle Australian cohort n=251 USA cohort n=1076)
[1]Age at outcome measurement
[2]Study also reported on cord blood levels among 1,126 newborn,
[3]Austria, Denmark, Finland, France, Germany, Greece, Netherlands, Ireland, Italy, Sweden, Spain, Switzerland, United Kingdom,Serbia, Montenegro
[4]Risc Study, [5]Botnia study
[6]Analysis of COPD in n=1305

Neurological outcomes

Table 2.2.2 shows the results of the studies on choline in relation to neurological outcomes. Eight studies investigated the effects of choline exposure during pregnancy, one study was performed in children and two observational studies were performed in adults. Ten articles described intervention studies in adults using supplementation of choline.

Choline during pregnancy and neurological outcomes

Three studies (all with a QS of 7) reported on choline in early pregnancy (dietary intake[142 143] or blood level[144] and the risk of neurological birth defects in children. For spina bifida, two studies reported a lower risk of spina bifida in the highest quartile of intake, but only in the study comparing the highest with the lowest

quartile, this result was statistically significant (OR 0.45 (95%CI 0.22; 0.93)).[143] The study that assessed blood levels found no association of choline blood levels with risk of spina bifida.[144] For anencephaly, all three studies did not find significant associations.

The other studies in pregnancy focused on child cognition. One intervention study (QS 9) assessed the effects of phosphatidylcholine supplementation during pregnancy.[145] Among 99 pregnant women, 750 mg phosphatidylcholine per day from 18 weeks of gestation onwards did not have a significant effect on infant cognition at the age of 1 year. There was a potential adverse effect on long term memory (p=0.056).[145] Two studies (both with a QS of 7) assessed dietary intake during pregnancy in relation to child cognition at the ages 3 and 7.8 years, respectively.[146,147] At 3 years, none of the cognitive tests were associated with maternal choline intake.[146] The study with outcomes at 7.8 years also did not find any relationship between maternal choline intake and verbal intelligence, but did find a positive association between choline intake during second trimester and non-verbal intelligence and visual-spatial memory.[147] Two studies (both with a QS of 7) assessed maternal blood levels of choline during pregnancy and child cognition.[148,149] The first study assessed both free and total choline and found no association with child cognition at 6 years of age, in a sample of 400 children.[148] The second study (n=154) had a follow-up of up to 18 months, and described a positive association of choline levels during pregnancy with child cognition, but no association with motor skills, receptive and expressive language.[149]

Choline in children and neurological outcomes.

One cross-sectional study (QS 5) assessed levels of free choline in relation to various aspects of cognition among 210 children around the age of 5.6 years[150] and found no significant associations of choline blood levels.

Choline in adults and neurological outcomes

Thirteen articles described choline in relation to neurological outcomes in adults, of which 12 looked at cognition and one at Alzheimer's disease. Three were observational studies and ten were interventional. The first observational study found cross-sectional significant positive associations of blood levels of free choline with executive function, sensorimotor speed and global cognition, but not with visual-spatial skills, semantic or episodic memory.[151] The second observational study was longitudinal, and found no relationship between dietary choline intake and executive function, verbal memory, visual memory or verbal learning.[152] The ten intervention studies all had a QS of 7 or higher, with sample sizes ranging from 9 to 400. Interventions differed regarding type of choline, dosage and duration. In five studies, the intervention was a single dose of a supplement containing choline. Choline chloride (10g) improved serial learning and selective reminding, for low imageable words only,[153] and lecithin (containing 3.75g of choline) improved explicit memory,[154] which was not seen for 1.5g choline. Participants receiving a single dose of choline citrate (50mg/kg) did not perform better on cognitive tests as compared to those receiving placebo.[155] In another study, a single dose of lecithin (20g) did not improve cognitive outcomes, and there

was no relation between the increase in plasma choline after receiving the supplement and any of the outcomes.[156] The most recent and largest study (n=400) compared a single dose of lecithin (1.6g phosphatidylcholine) with a placebo and observed worse vigilance in the intervention group.[157] Of the studies assessing multiple days of intervention, a 3-day study found no effects of choline chloride supplements (16g/day) on various cognitive tests,[158] and another study with choline chloride (8g/day for 20 days) found no effects on memory.[159] One study assessing lecithin (5.4g/day for 6 weeks) found significantly better concentration and attention in the lecithin group as compared to placebo,[160] but a study with a much higher dose (26g/day for 5 weeks) found no effect on cognition.[161] Also the study with the longest follow-up of 3 months found no improvement in memory in subjects receiving 1g citicoline per day, as compared to placebo.[162] The study (QS 6) on Alzheimer's disease was a longitudinal study with 5 year follow-up, that had a metabolomics approach.[163] This study identified 8 choline-containing phospholipid species that were lower in patients who developed Alzheimer as compared to healthy controls, of which 4 remained significant in the validation.[163]

Body composition

The reported associations between choline and body composition are shown in **Table 2.2.3**. We identified two studies (one interventional and one observational) on the relation between maternal choline levels during pregnancy and child birth weight. The observational study also reported a relation between cord blood choline and birth weight. The latter association was also studied in three other studies. Except for the intervention study (QS 7), all studies had a QS of 5 or less. Three studies reported on choline and body composition in adults, these studies all had a QS of less than 4.

Choline during pregnancy and birth weight

An intervention study among 26 pregnant women compared choline supplementation of 100mg or 550mg per day for 12 weeks, and observed no differences between the groups in child birth weight.[164] An observational study assessed maternal choline levels among 366 pregnant women at 30-34 weeks of gestation, and found no significant association with birth weight.[165] The same study also assessed cord blood of 1,126 children, and found that higher choline levels were associated with lower birth weight.[165] In contrast, another (smaller) study examining this association observed that cord blood choline levels were significantly lower in children with a low birth weight, as compared to controls.[166] Similarly, the other Spanish study found lower cord blood choline levels in children small for gestational age as compared to appropriate for gestational age controls.[167] A study that assessed both free choline and phospholipid bound choline (PPLCH) in cord blood (both venous and arterial), found no association with birth weight.[168]

Choline in children and body composition

A German study from 1983 (QS 2), measured blood levels among 357 children, and found no significant differences in phosphatidylcholine distribution in overweight children as compared to those with normal weight.[169]

Choline in adults and body composition

Three studies on body composition were performed in adults, of which one was an intervention study. In this study (QS 4), 16 participants received low (500mg) or high (2000mg) doses of citicoline for 6 weeks. Weight change in this period did not differ between the two groups.[170] The first observational study (QS 4, n=2,324), published in 1984, found a significant correlation between HDL-phosphatidylcholine and Broca index (a measure of relative body weight), which was negative in men, but positive in women (correlation coefficients -0.10 and 0.13, respectively).[171] In contrast, the other, more recent study (QS 2) reported higher blood levels of free choline in overweight as compared to normal weight men, but this association was not significant.[172]

Metabolic health

The reported associations between choline and metabolic health are shown in **Table 2.2.4**. Five studies had a measure of insulin sensitivity as an outcome, nine studies described the relationship between choline and blood lipids, one study assessed choline in relation to inflammatory markers, and two studies had non-alcoholic fatty liver disease (NAFLD) as outcome.

Choline and insulin sensitivity

Four studies in adults assessed the association between choline and glucose metabolism and three studies investigated the association of choline and the risk of type 2 diabetes mellitus. Two studies used a targeted metabolomics approach to associate phosphatidyl-containing phospholipids (and many other metabolites) with risk of type 2 diabetes.[173 174] Both studies identified several metabolites to be significantly related to type 2 diabetes risk, where several species were associated with an increased risk, while others were associated with a decreased risk.[173,174] One of these studies also looked at blood glucose and found some species to be related with lower fasting plasma glucose and lower 2-hour postload glucose.[174]

A longitudinal study in cohorts from 14 European countries found that each SD increase of linoleoyl-glycerophosphocholine blood levels was associated with a significantly lower risk of type 2 diabetes and dysglycemia.[175] In addition, a cross-sectional Chinese study also found higher blood levels to be associated with a lower risk of Type 2 diabetes,[176] and a cross-sectional study among young adults from USA found a non-significant inverse correlation of blood levels of free choline with fasting glucose.[172]

Blood lipids

We identified nine papers that reported on the effects of choline on blood lipids. Six were intervention studies,[135,177-180] two observational studies assessed blood levels of choline[171,172] and one observational study assessed dietary intake of choline.[136]

Of the two studies on choline blood levels (both cross-sectional), one study (QS 5) found that HDL-phosphatidylcholine was correlated with higher HDL cholesterol and higher total cholesterol in both men and women, and with lower LDL cholesterol in women only and lower triglycerides in men only.[171] However, the other study (QS 3) found no significant

associations between blood levels of free choline and cholesterol among 237 Mexican-American men.[172] The one study that assessed dietary intake among 16,165 women (QS 6), found no significant associations with cholesterol after 8 years of follow-up.[136]

The intervention studies each had a QS of 6 or higher, but included a small number of participants (60 or fewer), who were either only male or only female. A study from 1982 assessed blood lipids at several time points within 6 hours after consuming a meal containing 8g of egg phosphatidylcholine or placebo, and observed no differences in blood lipids between the two groups.[177] One study (n=14) observed that total cholesterol was significantly lower after a 3 week choline deficient diet, but no significant results were found for triglycerides, HDL-c or LDL-c.[179] One study with controlled meals and supplements had participants consuming either 300mg, 550mg, 1100mg or 2200 mg choline per day for 12 weeks, and observed that total cholesterol was increased in 1100mg and 2200mg group, as compared to the 300mg group, but no significant results were found on HDL-c or LDL-c.[181] The other three intervention studies used supplements, but all used a different type of choline. An intervention study among 26 males reported that after 2 weeks of phosphatidylcholine supplementation, no difference was seen in total cholesterol, HDL-c, LDL-c or total/HDL-c between the supplemented and the placebo groups[135]. However, triglycerides were 0.14 mmol/L higher (95%CI 0.06; 0.21) in the supplemented group. A study among 22 postmenopausal women also found slightly higher triglyceride levels after 4 weeks of soy lecithin supplementation, but this was not significant.[180] However, this study did find significantly lower LDL-c levels in the supplemented group, but observed no significant effects on total cholesterol, HDL-c or the HDL/LDL ratio.[180] The last intervention study had the highest number of participants (n=42), who were all male, and this study observed no significant differences in blood lipids after 12 weeks of supplementation with choline bitartrate (containing 1g choline per day), as compared to placebo.[178]

Inflammatory markers

We identified only one study that reported on choline in relation to inflammatory markers in a healthy population. This cross-sectional study (QS 6, n=3,042) reported an inverse association between dietary intake of choline and several inflammatory markers (22% lower levels of C-reactive protein (p=0.05), 26% lower levels of interleukin-6 (p=0.02) and 6% lower levels of TNF-alpha (p=0.006), in the highest versus the lowest tertile of intake of choline).[182]

Non-alcoholic fatty liver disease

One cross-sectional study (QS 5) found several metabolites of choline to be lower in cases with non-alcoholic steatotohepatitis, as compared to healthy controls,[183] whereas another cross-sectional study (QS 4) found higher levels of free choline in patients with borderline steatohepatitis and steatohepatitis, as compared to healthy controls.[184] Both studies found less significant results in patients with simple steatosis, as compared to healthy controls.

Cardiovascular health

Six studies reported associations of choline with cardiovascular health in populations free of diseases at baseline, of which one assessed choline during fetal life, and the other five studied choline in adulthood (**Table 2.2.5**).

Choline during pregnancy and child cardiovascular health

One study (QS 2) related maternal choline levels during pregnancy with risk of conotruncal heart defects in the children, and this study found no association.[185]

Choline in adults and cardiovascular health.

One study (QS 7) analyzed dietary intake of choline in relation to peripheral artery disease more than 20 years later. The 4th quintile of choline intake was associated with an increased risk of peripheral artery disease in men (OR 1.46 (95%CI 1.08; 1.98), as compared to the lowest quintile), but no significant results in the other quintiles, nor a significant dose response. Also, there were no significant associations found in women.[186] A cross-sectional study from 1984 (QS of 4 and n=2,324), observed no significant correlations between levels of HDL-phosphatidylcholine and blood pressure.[171] Two studies (QS 8) assessed dietary intake of choline in relation to coronary heart disease and stroke[136,187] and one study (QS 7) used blood levels.[188] One study found that dietary choline intake was not associated with the risk of coronary heart disease after 14 years of follow up.[187] The second study among 16,165 post-menopausal women also found no significant associations between choline intake and risk of coronary heart disease and stroke 8 years later, but effect estimates of the highest choline intake tended towards a higher risk of coronary heart disease, and a lower risk of stroke.[136] The study that used blood levels assessed many choline-containing phospholipids and found some phosphatidylcholine species to be associated with a higher risk of cardiovascular events after 10 years of follow-up.[188]

Respiratory health

We identified only one study that assessed dietary intake, supplement intake or blood levels of choline in relation to respiratory health in populations free from diseases at baseline (**Table 2.2.6**). This cross-sectional study from Germany (QS 7) had a metabolomics approach and observed an increased risk of asthma per SD increase in two species of acyl-alkyl phosphatidylcholine, but not in 39 other acyl-alkyl phosphatidylcholine species, or any of the diacyl or lyso phosphatidylcholines.[189] Also, none of the choline-containing phospholipids were associated with COPD.[189]

Discussion

Main findings

To the best of our knowledge, this is the first systematic review that evaluates whether choline (supplementation, intake or status) was associated with body composition, cardiovascular, metabolic, respiratory and neurological health across in healthy populations the life course. Observational studies suggest that choline may be beneficial for neurological health, in particular cognition, as well as for insulin sensitivity. Although some

studies found effects of choline on body composition, lipid levels and cardiovascular outcomes, these results were inconsistent. Overall, the amount or quality of the available evidence for each outcome was low to moderate and evidence from randomized controlled trials is scarce

Mechanisms of action

Choline in food is mostly present in the form of phosphatidylcholine. Other dietary forms of choline include glycerophosphocholine, phosphocholine or sphingomyelin. Usually, choline is evaluated as total choline intake, including all dietary forms. In food, the highest total choline concentrations (mg/100g) are found in beef liver (418), chicken liver (290), eggs (251), wheat germ (152), bacon (125), dried soybeans (116) and pork (103).[190] Choline metabolites are hydrolyzed throughout the small intestine and, once absorbed, free choline enters the portal circulation. Choline can continue to circulate in the body in its free from, or be used in different pathways, including binding to phospholipids again to form phosphatidylcholine, formation of acetylcholine for neurotransmission, and formation of betaine to be used as a methyl donor.[134]

Only since 1998, choline is recognized as essential nutrient, when experimental studies had shown organ dysfunction in healthy men after they were deprived of choline.[179] At first, choline was not considered essential, since the body can derive choline from de novo synthesis of phosphatidylcholine, catalyzed by phosphatidylethanolamine-N-methyltransferase (PEMT).[137,191] PEMT activity is increased by estrogen, and therefore premenopausal women are more resistant to a low choline diet.[191,192] In addition, genes that are related to PEMT activity contribute to the inter-individual differences in choline requirement.[192] The differences in choline requirement could also contribute to the inconsistency between studies, and should be considered in future studies.

Choline and brain development and cognitive function

Choline has a role in central nervous system development. It is required for the structural integrity of cell membranes and for myelin sheets, in the form of the phospholipids phosphatidylcholine and sphingomyelin, the forms in which choline is mostly present in the body.[134] Demand for choline increases during pregnancy and lactation.[140] Choline is transferred from mother to child via the placenta and through breast milk.[140] Human milk is rich in choline compounds, but soy-derived infant formulas have much lower total choline concentrations.[193] It has been hypothesized that this may partly explain why infants fed breast milk have higher IQs than formula-fed children.[194] Evidence from animal studies is compelling and suggest a causal relationship between availability of choline during pregnancy and lactation and cognitive function in offspring.[33] However, in our systematic review, only one intervention study addressed this topic and this study failed to show the beneficial effects of maternal choline intake on child cognition that had been suggested in some observational studies. The possible effects might depend on the dose of

choline, or a specific susceptible time window in fetal life. In the intervention study, choline supplements were given from 18 weeks of gestation onwards, but as brain development mostly occurs in the first trimester of pregnancy, exposure to choline at earlier gestational ages might be more relevant to cognitive outcomes in the offspring. In contrast, the study from Project Viva showed stronger effects of dietary intake in 2nd trimester, as compared to 1st trimester.[147] There currently is not enough evidence to draw firm conclusions on the effects of choline in early life an cognition.

In our review, the scarce evidence available suggests that choline intake in the periconceptional period might be related to a lower risk of spina bifida, but more research is needed. The mechanisms underlying the potential beneficial effect of choline might be explained by epigenetic mechanisms through the one-carbon pathway.[134] Choline can provide methyl donors in the methylation of homocysteine to methionine and enhanced methyl-donor supply and DNA methylation status has been suggested to be associated with neural tube defects.[133,195] In addition, the same mechanism might apply to other birth defects, such as orofacial malformations.[196,197] Besides a role of choline in child neurological health, choline might also have a positive effect on cognitive function later in life, as choline serves as a precursor for the neurotransmitter acetylcholine. Also, choline might contribute to cognitive function in the elderly by serving as a phospholipid precursor in the form of phosphatidylcholine. As the brain ages, there is a decrease in phospholipids in neuronal membranes, leading to decreased structural integrity. Choline has been used as treatment for stroke under the hypothesis that increasing blood levels of choline through supplementation, could increase the availability of phosphatidylcholine in the brain, which in turn could have a positive effect on membrane structure and potentially result in improved memory function.[32] Choline levels have previously been found to be lower in patients with Alzheimer's disease, and in our systematic review, we identified a study that also found lower choline-containing phospholipids to be prospectively associated with risk of developing Alzheimer's disease.[163] The depletion of choline in Alzheimer patients has led to the hypothesis that supplementation with lecithin, a major source of choline, could improve outcome of patients suffering from dementia. However, a Cochrane review of twelve randomized trials concluded that there is no evidence for the use of lecithin in the treatment of dementia.[198] There is some evidence that supplementation of CDP-choline has a positive effect on memory and behavior in at least the short to medium term, but only in elderly people with cognitive deficits associated with chronic cerebral disorders of the brain.[141] In this systematic review, we also do not see clear beneficial effects of choline supplements on cognition in the healthy population, but differences between studies makes it difficult to draw firm conclusions. The interventions that were performed ranged from a single dose to 3 months of supplementation, studies used different types of choline and dosages differed largely, which makes it impossible to meta-analyze the results.

Choline and body composition, metabolic and cardiovascular outcomes

Research on the effects of choline on body composition showed inconsistent results. Two studies assessed maternal blood levels during pregnancy,[164,165] and four assessed cord blood levels.[165-168,199] Three studies found significant results on birth weight, but in opposite directions.[165-167] One study suggested that there is a relationship between choline and body weight in adults, with the direction opposite for men and women.[171] Specific metabolites of choline might be related to risk of type 2 diabetes,[173-175] but more evidence is needed. So far, intervention and observational studies assessing choline in relation to blood lipid levels show inconsistent results . Research is needed on the effects of choline intake during pregnancy and in childhood on metabolic outcomes. To our knowledge, there have been no studies on the effects of choline in fetal life or childhood on metabolic health. In adults, a large number of articles have related choline to increased risk of cardiovascular disease in selected patient populations.[200-203] Five studies were identified that examined this effect in a healthy population,[136,171,186-188] which showed inconsistent results, but some suggested higher choline to be associated with higher risks of cardiovascular diseases.[136,186,188]

Several animal and patient-based studies has suggested a role for choline in the etiology of NAFLD. For example, studies in rodents have shown that a choline-deficient diet increased the risk of developing NAFLD.[204] Also, patients on long-term parenteral nutrition with steatohepatitis who are choline deficient show reversal of NAFLD with supplementation of choline.[199] In addition, individuals with specific polymorphisms in PEMT genes have increased risk of developing NAFLD.[205] Although many studies have showed increased liver enzymes in relation to a low-choline diet, we only identified two studies among humans that compared patients with a diagnosis of NAFLD with healthy controls. One of these studies showed that choline levels were higher in patients as compared to controls, which is likely to reflect metabolic changes in patients with NAFLD, instead of an increased risk of NAFLD with increasing choline intake, but this relationship needs to be further studies in non-selected populations.

Choline was suggested to have an effect on cardiometabolic outcomes through several mechanisms. First, it may be related to its role in one-carbon metabolism.[134] Choline is a precursor of betaine, which serves as a methyldonor to convert homocysteine to methionine. Accordingly, high choline levels decrease homocysteine levels.[135] As such, choline may be associated with a decreased risk of cardiovascular disease, assuming that homocysteine is causally related to the development of cardiovascular disease, which is a topic of discussion in current literature.[206] Hyper-homocystenemia is associated with metabolic disturbances such as hyperlipidemia and abdominal obesity.[207,208] Therefore, underlying mechanisms for an effect of choline on cardiovascular health might also be attributable to other cardiometabolic risk factors.

Second, gut flora metabolism has been suggested to play a role in the relation between choline and cardiovascular disease.[209] Gut

bacteria can use choline-containing nutrients as fuel, producing trimethylamine, which is further oxidized to trimetyhlamine-N-oxide (TMAO).[210] Increased levels of TMAO have been associated with increased risk of cardiovascular events,[210] possibly due to increased atherosclerosis.[209] This hypothesis also fits with our findings, namely a tendency towards a higher risk of cardiovascular events end peripheral artery disease, instead of the lower risk that would be expected based on the homocysteine hypothesis.

Third, it may be related to lipid metabolism since phosphatidylcholine is an essential component of very low density lipoproteins. There is some concern that choline may affect lipid metabolism unfavorably but this has not been fully elucidated. On the one hand, the presence of choline in the form of phosphatidylcholine is essential for cholesterol and triglycerides to be transported by VLDL from the liver to the vessels.[211] On the other hand, the enzyme BHMT (betaine-homocysteine-methyltransferase) can increase VLDL secretion[211] and betaine intake has been found to be associated with higher LDL-c[135] and lower HDL-c.[136] The evidence of intervention studies in this review is not compelling enough to draw a final conclusion, and larger high quality trials are necessary.

Methodological considerations

One of our study's strengths is the detailed systematic search in several electronic databases without any language restriction. Furthermore, we used data from over 50,000 participants which were not selected on the basis of pre-existing disease, which makes our results generalizable to the larger general population. However, the current review was limited by the moderate amount of high quality articles. Therefore, our findings intensify the need for prospective cohort studies and intervention studies with large sample sizes to study the effects of choline. Until a decade ago, it was not possible to analyse dietary intake of choline. Since information on the choline content of most food products has become available, research in this area has increased rapidly and dietary intake of choline can be calculated from widely used instruments such as food-frequency questionnaires. Furthermore, evidence from animal studies and small population-based observations has led to clinical trials on choline supplementation. These recent developments will help to further elucidate the role of choline in human health.

Conclusions

In conclusion, observational evidence suggest a possible beneficial effect of choline during pregnancy on neurological health, in particular cognition. Studies on the effect of choline in children are lacking. Choline may be related to metabolic health such as insulin sensitivity and NAFLD but high quality intervention studies are scarce. Results were inconsistent for body composition, lipid levels and cardiovascular outcomes to draw final conclusions. Further intervention studies and large prospective cohort studies are needed to complement the existing evidence and to clarify potential effects of choline along the life-course.

Supplementary Material can be found online: http://hdl.handle.net/1765/77768

Table 2.2.2 Reported associations between choline and neurological health

First author (year)	Choline	Specific outcome	Statistical analysis	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Birth defects (fetal life)										
Carmichael (2010) [142]	Dietary intake of choline	Spina Bifida	Logistic regression		OR for birth defect per quartile of maternal choline intake (Q2/3=ref)[b]	Q1: 1.2	0.7	2.1	NS	+++
						Q4: 0.7	0.4	1.3	NS	+++
						Q1: 1.1	0.6	2.0	NS	
						Q4: 1.1	0.6	2.0	NS	+++
Mills (2014) [144]	Blood level of choline	Total NTDs	Kruskal-Wallis test		Difference in maternal choline levels in cases vs controls				NS	+
		Spina Bifida							NS	+
		Anencephaly							NS	+
Shaw (2004) [143]	Dietary intake of choline	Total NTDs	Logistic regression		OR for birth defect per quartile of maternal choline intake (Q1=ref)[b]	Q2: 0.63	0.42	0.99	<0.05	+++
						Q3: 0.65	0.39	1.07	NS	+++
						Q4: 0.51	0.25	1.07	NS	+++
		Spina Bifida				Q2: 0.66	0.42	1.04	NS	+
						Q3: 0.68	0.41	1.14	NS	+
						Q4: 0.45	0.22	0.93	0.005	+
		Anencephaly				Q2: 0.64	0.39	1.07	NS	+
						Q3: 0.56	0.31	1.02	NS	+
						Q4: 0.52	0.24	1.17	NS	+
Cognition (fetal life)										
Boeke (2013) [147]‡	Dietary intake of choline	Visual-spatial memory	Linear regression	1st trimester		Q2: 0.3	-0.6	1.1	NS	+++
						Q3: 0.8	0.0	1.7	NS	+++
						Q4: 0.7	-0.2	1.5	NS	+++
									0.08 (P trend)	+++
				2nd trimester		Q2: 0.6	-0.3	1.4	NS	+++
						Q3: 0.7	-0.2	1.5	NS	+++
						Q4: 1.4	0.5	2.4	<0.05	+++
									0.003 (P trend)	+++
		Verbal intelligence	Linear regression	1st trimester		Q2: 1.4	-0.9	3.7	NS	+++
						Q3: 1.4	-1.0	3.7	NS	+++
						Q4: 1.3	-1.1	3.7	NS	+++
					Difference in score per quartile of intake (Q1=ref)[2]				0.32 (P trend)	+++
				2nd trimester		Q2: 0.4	-2.0	2.9	NS	+++
						Q3: 1.1	-1.3	3.6	NS	+++
						Q4: 0.9	-1.7	3.5	NS	+++
									0.43 (P trend)	+++
		Non-verbal intelligence	Linear regression	1st trimester		Q2: 0.3	-2.8	3.4	NS	+++
						Q3: -0.6	-3.7	2.5	NS	+++
						Q4: 1.0	-2.2	4.2	NS	+++
									0.63 (P trend)	+++
				2nd trimester		Q2: 1.6	-1.6	4.8	NS	+++
						Q3: 1.3	-1.8	4.5	NS	+++
						Q4: 3.5	0.1	6.9	NS	+++
									0.06 (P trend)	+++

Table 2.2.2 (continued) Reported associations between choline and neurological health

First Author (year)	Choline	Specific outcome	Statistical Analysis	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Cognition										
Fetal life										
Cheatham (2012) [145]	Supplement intake of PC	Visuospatial memory	ANOVA		Mean percentage correct on cognitive task in supplement (750 mg choline from 18w GA) vs. placebo				0.327	randomized
		Long term memory							0.056	randomized
		Expressive vocabulary							0.368	randomized
		Global index							0.412	randomized
Signore (2008) [148]	Blood level of choline	Full scale IQ	Linear regression	free choline	Change in score per z-score of choline	-0.12			0.85	++
				total choline		0.49			0.44	++
		Verbal IQ		free choline		-0.42			0.5	++
				total choline		0.003			1	++
		Performance IQ		free choline		0.42			0.54	++
				total choline		0.77			0.28	++
		Block Design subtest		free choline		0.19			0.17	++
				total choline		0.20			0.14	++
		Information subtest		free choline		-0.16			0.25	++
				total choline		-0.02			0.9	++
Villamor (2012) [146]‡	Dietary intake of choline	Receptive language	Linear regression		Change in score per 450 mg increase in choline	-3.1	-11	4.8	NS	+++
		Visual-motor, visual-spatial and fine motor abilities[c]				-2.8	-9.9	4.3	NS	+++
Wu (2012) [149]	Blood level of free choline	Cognitive score	Linear regression	16w GA	Increase in neurodevelopment score per unit increase in (ln)choline	6.05	1.58	10.53 [d]	0.009	+++
				36w GA					NS	NM
		Receptive language		16w GA		0.67	-2.21	3.54 [d]	0.65	+++
				36w GA					NS	NM
		Expressive language		16w GA		-0.519	-3.78	2.76 [d]	0.76	+++
				36w GA					NS	NM
		Fine motor		16w GA		-0.28	-2.58	2.02 [d]	0.814	+++
				36w GA					NS	NM
		Gross motor		16w GA		2.86	-0.03	5.74 [d]	0.055	+++
				36w GA					NS	NM
Children										
Strain (2013) [150]	Bloodlevel of free choline	Finger tapping	Linear regression		Point difference in outcome per SD increase of choline	0.310	-0.34	0.96 [d]	NS	++
		Auditory comprehension				0.030	-0.13	0.19 [d]	NS	++
		Verbal knowledge				-0.030	-0.23	0.17 [d]	NS	++
		Applied problems				-0.210	-0.45	0.03 [d]	NS	++
		Letter-word recognition				0.035	-0.28	0.35 [d]	NS	++
		Verbal intelligence				0.072	-0.08	0.23 [d]	NS	++
		Non-verbal intelligence				0.044	-0.03	0.12 [d]	NS	++

Table 2.2.2 (continued) Reported associations between choline and neurological health

First author (year)	Choline	Specific outcome	Statistical analysis	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Cognition Adults										
Benton (2004) [157]	Lecithin supplement intake	Reaction time	ANOVA		Performance on test after 3 days of lecithin (containing 1.6 g phospohatidylcholine per day) vs. placebo				NS	randomized
		Vigilance				-			<0.03	randomized
		Memory							NS	randomized
Davis (1980) [158]	Choline chloride supplement intake	Short term memory	NM		Peformance on test after 3 days choline chloride supplements (16 g/day)				NS	randomized
		Long term memory (retrieval)							NS	randomized
		Long term memory (selective reminding)							NS	randomized
Deuster (2002) [155]	Choline-citraat supplement intake	Choice reaction time, logical resaoning, visual vigilance, serial addition and substraction, working memory, spatial memory, decoding	MANOVA		Performance on test after single dose choline citrate (50mg/kg) vs placebo				NS	randomized
Drachman (1982) [161]	Lecithin supplement intake	Memory test, digit span, word span, supraspan learning, subjective memory	ANOVA		Performance on test after 5 weeks lecithin (26 g/day, containing 95% phosphatidylcholine) vs. placebo				NS	randomized
Harris (1983) [156]	Lecithin supplement intake	Word list memorization, retrieval paired associates, word recognition	Student's t tests		Performance on test after single dose of lecithin (20 g) vs. placebo				NS	randomized
	Blood level of choline				Performance on test in relation to increase in plasma choline (after lecithin supplement)				NS	-
Ladd (1993) [154]	Lecithin supplement intake	Explicit memory	MANOVA	1.50 g choline	Performance on test after a single dose lecithin (two doses) vs. placebo				0.092	randomized
				3.75 g choline		+			0.018	randomized
Mohs (1980) [159]	Choline chloride Supplement intake	Memory retrieval	NM		Performance on test after 20 days choline chloride (8 g/day) vs. placebo				NS	randomized
		Memory storage							NS	
Nurk (2012) [151]	Blood level of free choline	Episodic memory	ANOVA		Mean test score in high vs. low choline (above/below 8,36 µmol/l)				0.58	+++
		Sensorimotor speed				+			0.004	+++
		Perceptual speed and executive function				+			0.005	+++
		Visuospatial skills							0.83	+++
		Global cognition				+			0.010	+++
		Semantic memory							0.25	+++

Table 2.2.2 (continued). Reported associations between choline and neurological health

First Author (year)	Choline	Specific outcome	Statistical Analysis	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Cognition										
Adults										
Poly (2011)[152]	Dietary intake of choline	Verbal memory, visual memory, verbal learning, executive function	Linear regression		Change in test score per 1 unit change in log choline				NS	+[e]
Sitaram (1978)[153]	Choline chloride supplement intake	Serial learning	T-test		Performance on test after a single dose of choline chloride (10 g) vs. placebo	+			<0.05	randomized
		Selective reminding (low imageable words)				+			<0.01	randomized
		Selective reminding (high imageable words0							NS	randomized
Sorgatz (1988)[160]	Lecithin supplement intake	Concentration	MANOVA	performance	Performance on test after 6 week lecithin (5.4 g/day) vs. placebo	+			<0.05	randomized
				accuracy		+			<0.05	randomized
		Attention	MANOVA	performance		+			<0.05	randomized
				accuracy		+			<0.05	randomized
Spiers (1996)[162]	Citicoline supplement intake	Immediate and delayed logical memory	ANCOVA		Performance after 3 months citicoline (1g/day) vs. placebo				NS	randomized
Alzheimer										
Mapstone (2014)[163]	Blood level of phospholipids[f]	Alzheimer's disease	Mann-Whitney U test	Diacyl-PC	Levels of phospholipids in patients who developed Alzheimer vs. healthy controls (discovery sample, n=71)	C36:6 -			p<0.05	+
						C38:0 -			p<0.05	+
						C38:6 -			p<0.05	+
						C40:1 -			p<0.05	+
						C40:2 -			p<0.05	+
						C40:6 -			p<0.05	+
				Acylalkyl-PC		C40:6 -			p<0.05	+
				Lyso-PC		C18:2 -			p<0.05	+
				Diacyl-PC	Levels of phospholipids in patients who developed Alzheimer vs. healthy controls (validation sample (n=30)	C36:6 -			<0.05	+
						C38:0			NS	+
						C38:6			NS	+
						C40:1			NS	+
						C40:2 -			<0.05	+
						C40:6 -			<0.05	+
				Acylalkyl-PC		C40:6 -			<0.05	+
				Lyso-PC		C18:2			NS	+

[a]Adjustment level was categorized as follows; unadjusted -; 4 covariates or less +; 5 to 8 covariates ++; 9 or more covariates +++; Effect estimate: negative association (-), positive association (+)
[b]Quartiles based on controls; [c]Visual motor subtest, visual-spatial subtest and fine motor subtest were also analyzed separately, all non-significant. [d]95%CI calculated based on reported SE. [e]not mentioned if multivariable model was also tested; [f]Choline containing phospholipids, ‡ both studies are from Project Viva

Table 2.2.3 Reported associations between choline and body composition

First author (year)	Exposure	Type of choline	Statistical analysis	Outcome	Measure of association	Effect estimate (95% CI)	p-value	Adjustment level[a]
Fetal life								
Hogeveen (2013) [165]	Blood level	Choline	Linear regression	Birth weight	Change in standardized BW per SD increase in maternal choline blood level	27 (-25; 80)	NS	++
Jiang (2012) [164]	Supplement intake	Choline	Student's t test	Birth weight	BW after 12 weeks maternal supplements (550 mg vs. 100 mg choline/day)		>0.11	randomized
Children								
Buchman (2001) [168]	Blood level	Free choline PPLCH	Pearson's correlation	Birth weight	Correlation coefficients		NS NS	- -
Hogeveen (2013) [165]	Blood level	Choline	Linear regression	Birth weight	Change in standardized BW per SD increase in cord blood choline	-60 (-89; -31)	<0.05	++
Ivorra (2012) [166]	Blood level	Free choline	Kruskal-Wallis test	Birth weight	Cord blood choline in LBW vs. control	-	<0.01	+
Kupke (1983) [169]	Blood level	PC	Mann-Whitney U test	Overweight	Distribution in overweight vs. normal weight		NS	-
Sanz-Cortés (2013) [167]	Blood level	Choline	Mann-Whitney U test	Small for gestational age	Choline levels in cases vs. controls	-	<0.05	+
Adults								
Killgore (2009) [170]	Supplement intake	Citicoline	NM	Weight	Change in weight of 2000 mg vs. 500 mg citicoline/day for 6 weeks	-	NS	randomized
Schriewer (1984) [171]	Blood level	HDL-PC	Spearman correlation	Broca index	Correlation coefficient (men) Correlation coefficient (women)	-0.1015 0.1291	<0.001 <0.001	- -
Yan (2012) [172]	Blood level	Free choline	ANOVA	Overweight	Mean choline in overweight vs. normal weight	+	0.15	-

[a]Adjustment level was categorized as followed; unadjusted -; 4 covariates or less +; 5 to 8 covariates ++; 9 or more covariates +++. Effect estimate: negative association (-), positive association (+)

Table 2.2.4 Reported associations between choline and metabolic health

First author (year)	Exposure	Type of choline	Statistical analysis	Outcome	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Insulin sensitivity Adults											
Chen (2013) [176]	Blood level	Choline	Logistic regression	Type 2 diabetes		OR for DMT2 per tertile of choline level (T1=ref)	T2: 0.02	0.01	0.06	<0.05	+
							T3: 0.01	0.00	0.02	<0.05	+
										<0.001 (P trend)	+
Ferrannini (2013) [175]	Blood level	Lineoleoyl-GPC	Logistic regression	Dysglycemia		OR per SD increase of L-GPC	0.64	0.48	0.85	<0.05	++
				Type 2 diabetes			0.67	0.54	0.84	<0.05	++
Floegel (2012) [173]	Blood level	Phospholipids[b]	Cox regression	Type 2 diabetes	Diacyl-PC	RR for DMT2 per SD increase	C32:1 1. 15	1.03	1.29	<0.05	+++
							C36:1 1. 25	1.10	1.41	<0.05	+++
							C38:3 1. 25	1.22	1.55	<0.05	+++
							C40:5 1. 19	1.06	1.32	<0.05	+++
					Acyl-alkyl-PC		C34:3 0. 92	0.78	1.07	NS	+++
							C40:6 0. 94	0.82	1.08	NS	+++
							C42:5 0. 96	0.83	1.12	NS	+++
							C44:4 0. 97	0.84	1.12	NS	+++
							C44:5 0. 95	0.82	1.09	NS	+++
					Lyso-PC		C18:2 0. 84	0.73	0.96	<0.05	+++
Meikle (2013) [174]											
Australian cohort	Blood level	Phospholipids[b]	Logistic regression	Type 2 diabetes	PC	OR for DMT2 per IQR increase in phospholipids	1.12	0.81	1.55	0.71[c]	+
					Alkyl-PC		0.67	0.49	0.93	0.0394[c]	+
					Alkenyl-PC		0.67	0.45	1.01	0.0108[c]	+
					Lyso-PC		1.00	0.72	1.40	0.978[c]	+
					Lysoalkyl-PC		0.74	0.52	1.07	0.181[c]	+
			Logistic regression	Prediabetes	PC	OR for prediabetes per IQR increase in phospholipids	1.20	0.84	1.70	0.465[c]	+
					Alkyl-PC		0.62	0.41	0.92	0.0418[c]	+
					Alkenyl-PC		0.65	0.39	1.06	0.141[c]	+
					Lyso-PC		1.52	1.03	2.26	0.0746[c]	+
					Lysoalkyl-PC		1.00	0.68	1.47	0.994[c]	+
			Linear regression	FPG	PC	Change in FPG per IQR increase in phospholipds	0.03	-0.10	0.17	0.675[c]	+
					Alkyl-PC		-0.08	-0.22	0.05	0.356[c]	+
					Alkenyl-PC		-0.11	-0.28	0.06	0.356[c]	+
					Lyso-PC		-0.14	-0.28	0.00	0.124[c]	+
					Lysoalkyl-PC		-0.23	-0.38	-0.09	0.011[c]	+
			Linear regression	2 hour PLG	PC	Change in 2h-PLG per IQR increase in phospholipds	-0.07	-0.69	0.15	0.32[c]	+
					Alkyl-PC		-0.53	-0.95	-0.11	0.0409[c]	+
					Alkenyl-PC		-0.66	-1.20	-0.13	0.0409[c]	+
					Lyso-PC		-0.34	-0.78	0.10	0.267[c]	+
					Lysoalkyl-PC		-0.27	-0.74	0.19	0.347[c]	+

Table 2.2.4 (continued) Reported associations between choline and metabolic health

First author (year)	Exposure	Type of choline	Statistical analysis	Outcome	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Insulin sensitivity											
Adults											
Meikle (2013) [174]											
Australian cohort	Blood level	Phospholipids[2]	Logistic regression	Type 2 diabetes	PC	OR for DMT2 per IQR increase in phospholipids	1.76	1.27	2.45	0.00179 [c]	+
					Alkyl-PC		0.75	0.53	1.08	0.176 [c]	+
					Alkenyl-PC		0.59	0.42	0.83	0.00523 [c]	+
					Lyso-PC		1.03	0.78	1.38	0.818 [c]	+
					Lysoalkyl-PC		0.68	0.48	0.97	0.0616 [c]	+
			Logistic regression	Prediabetes	PC	OR for prediabetes per IQR increase in phospholipids	1.69	1.27	2.25	0.00097 [c]	+
					Alkyl-PC		0.83	0.63	1.11	0.308 [c]	+
					Alkenyl-PC		0.64	0.49	0.85	0.00625 [c]	+
					Lyso-PC		0.84	0.64	1.11	0.308 [c]	+
					Lysoalkyl-PC		0.73	0.54	0.97	0.0671 [c]	+
			Linear regression	FPG	PC	Change in FPG per IQR increase in phospholipds	0.16	-0.03	0.35	0.139 [c]	+
					Alkyl-PC		-0.01	-0.20	0.17	0.937 [c]	+
					Alkenyl-PC		-0.18	-0.35	0.00	0.0675 [c]	+
					Lyso-PC		0.02	-0.16	0.19	0.937 [c]	+
					Lysoalkyl-PC		-0.36	-0.54	-0.18	0.00025 [c]	+
			Linear regression	2-hour PLG	PC	Change in 2h PLG per IQR increase in phospholipds	0.64	0.26	1.03	0.0021 [c]	+
					Alkyl-PC		-0.30	-0.67	0.07	0.14 [c]	+
					Alkenyl-PC		-0.69	-1.03	-0.35	0.000 20 [c]	+
					Lyso-PC		-0.08	-0.43	0.27	0.653 [c]	+
					Lysoalkyl-PC		-0.79	-1.15	-0.43	0.00006 [c]	+
Yan (2012) [172]	Blood level	Free choline	Correlation	Fasting glucose		Correlation coefficient	-			NS	+
Blood lipids											
Adults											
Dalmeijer (2008) [136]	Dietary intake	Choline	Linear regression	Total-c		Difference in mean lipid levels (mmol/L) (Q1=ref)	Q2: 0.11	-0.03	0.25	NS	++
							Q3: 0.04	-0.10	0.17	NS	++
							Q4: 0.11	-0.03	0.25	NS	++
				HDL-c			Q2: -0.03	-0.08	0.02	NS	++
							Q3: -0.02	-0.08	0.03	NS	++
							Q4: -0.03	-0.09	0.03	NS	++
				LDL-c			Q2: 0.14	0.01	0.27	<0.05	++
							Q3: 0.06	-0.07	0.19	NS	++
							Q4: 0.11	-0.02	0.24	NS	++
Evans (2007) [180]	Supplement intake	Soy Lecithin	ANOVA (repeated measures)	Total-c		Difference in blood lipids after 4 week treatment of 20 g soy lecithin/day vs. placebo	-			NS	randomized
				HDL-c			+			NS	randomized
				LDL-c			-			<0.05	randomized
				HDL/LDL			+			NS	randomized
				Triglycerides			+			NS	randomized

Table 2.2.4 (continued) Reported associations between choline and metabolic health

First author (year)	Exposure	Type of choline	Statistical analysis	Outcome	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Blood lipids Adults											
Olthof (2005) [135]	Supplement intake	PC	Paired t-test	Total-c		Change in blood levels (mmol/l)[d] (2.6 gram choline/day for 2 weeks vs. placebo)	0.1	-0.1	0.3	NS	randomized
				HDL-c			-0.0	-0.1	0.0	NS	randomized
				LDL-c			-0.0	-0.2	0.2	NS	randomized
				Triglycerides			0.14	0.06	0.21	<0.05	randomized
				Total/HDL-c			0.2	-0.0	0.3	NS	randomized
Schriewer (1984) [171]	Blood level	HDL-PC	Spearman correlation	Total-c	men		0.08			<0.001	-
					women		0.10			<0.01	-
				HDL-c	men		0.59			<0.001	-
					women	Correlation coefficients	0.61			<0.001	-
				LDL-c	men		0.00			NS	-
					women		-0.10			<0.01	-
				Triglycerides	men		-0.19			<0.001	-
					women		-0.04			NS	-
Simonsson (1982) [177]	Test meal	Egg PC	Paired t-test	HDL-c		Change in blood lipids 0, 2, 4 and 6 hours after consuming meal (8gr PC vs control)				NS	randomized
				LDL-c						NS	randomized
				VLDL-c						NS	randomized
Veenema (2008) [181]	Test meal + supplement	Choline chloride	ANOVA (repeated measures)	Total cholesterol		Difference in blood lipid response between choline intake groups (550mg, 1100mg or 2200mg vs 300mg choline/day for 12 weeks)	550mg			NS	randomized
							1100mg +			<0.05	randomized
							2200mg +			<0.05	randomized
				HDL-c						all NS	randomized
				LDL-c						all NS	randomized
Wallace (2012) [178]	Supplement intake	Choline bitartrate	ANOVA (repeated measures)	Total cholesterol		Difference in blood lipids (1gr choline/day for 12 weeks vs placebo)				NS	randomized
				HDL-c						NS	randomized
				LDL-c						NS	randomized
				Triglycerides						NS	randomized
Yan (2012) [172]	Blood level	Free choline	correlation	Total-c		Correlation coefficients	+			NS	+
				HDL-c			+			NS	+
				LDL-c			+			NS	+
				Total/HDL-c			-			NS	+
				Triglycerides			+			NS	+

Table 2.2.4 (continued) Reported associations between choline and metabolic health

First author (year)	Exposure	Type of choline	Statistical analysis	Outcome	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Blood lipids											
Adults											
Zeisel (1991) [179]	Supplement intake	Choline chloride	Two-sample t test	Total-c		Difference in levels (mmol/l) after choline deficient vs choline containing diet	-			<0.01	randomized
				HDL-c			-			NS	randomized
				LDL-c			-			NS	randomized
				Triglycerides (mg/dl)			-			NS	randomized
Inflammatory markers											
Adults											
Detopoulou (2008)[182]	Dietary intake	Choline	generalized linear models	C-reactive protein		% change of outcome in highest vs. lowest tertile	-22%			<0.05	++
				Interleukin-6			-26%			0.02	++
				TNF-alpha[e]			-6%			0.006	++
Fatty liver disease											
Adults											
Imajo (2012) [184]	Blood level	Free choline	t-test	Steatosis		Difference in free choline levels in cases vs controls				NS	-
				Borderline NASH			+			<0.05	-
				NASH			+			<0.05	-
Kalhan (2010) [183]	Blood level	Phospholipids[b,f]	t-test	NASH	GPC		-			<0.05	+
					1-oleoyl-GPC		-			<0.05	+
					1-lineleoyl-GPC	Difference in phospholipids in cases vs controls	-			<0.05	+
					1-arachidonoyl-GPC		-			<0.05	+
				Steatosis	1-oleoyl-GPC		-			<0.05	+

Effect estimate: negative association (-), positive association (+)

[a]Adjustment level was categorized as followed; unadjusted -; 4 covariates or less +; 5 to 8 covariates ++; 9 or more covariates +++.

[b]Choline containing phospholipids;

[c]Benjamini-Hochberg corrected p-value, rounded to 5 decimals;

[d]Triglycerides were log-transformed;

[e]Analysis of TNF-alpha was performed in a subgroup n=2540

[f]Several GPCs were tested (GPC, 1-palmitoyl-GPC, 2-palmitoyl-GPC, 1-stearoyl-GPC, 1-oleoyl-GPC, 1-linoleoyl-GPC, 1-eicosatrienoyl-GPC, 1-arachidonoy-GPC), the ones not reported in the table were not significant.

Table 2.2.5 Reported associations between choline and cardiovascular health

First author (year)	Exposure	Statistical analysis	Specific outcome	Sub-group	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	P-value	Adjustment level[a]
Fetal life										
Shaw (2014) [185]	Blood level of choline	Logistic regression	Conotruncal heart defects		OR for birth defect per quartile of choline levels (Q2/3=ref) [b]	Q1: 0.9	0.5	1.6	NS	+
						Q4: 1.0	0.6	1.6	NS	+
									0.92 (P trend)	+
Adults										
Bertoia (2014) [186]	Dietary intake of choline	Cox regression	Peripheral artery disease			Q2: 1.14	0.82	1.59	NS	+++
					HR for peripheral artery disease per quintile of choline intake (Q1=ref)	Q3: 1.33	0.97	1.83	NS	+++
				men		Q4: 1.46	1.08	1.98	<0.05	+++
						Q5: 1.24	0.91	1.68	NS	+++
									0.16 (P trend)	+++
						Q2: 0.91	0.59	1.38	NS	+++
					HR for peripheral artery disease per quintile of choline intake (Q1=ref)	Q3: 1.30	0.88	1.91	NS	+++
				women		Q4: 1.40	0.95	2.05	NS	+++
						Q5: 1.07	0.72	1.60	NS	+++
									0.16 (P trend)	+++
Bidulescu (2007) [187]	Dietary intake of choline	Cox regression	Non-fatal and fatal CHD		OR for non-fatal and fatal CHD (Q1=ref)	Q2: 0.93	0.76	1.13	NS	+++
						Q3: 1.10	0.87	1.37	NS	+++
						Q4: 1.09	0.79	1.50	NS	+++
Dalmeijer (2008) [136]	Dietary intake of choline	Cox regression	CHD and stroke			Q2: 0.78	0.58	1.03	NS	+++
						Q3: 0.99	0.73	1.34	NS	+++
						Q4: 1.04	0.71	1.53	NS	+++
			CHD			Q2: 0.93	0.69	1.25	NS	+++
					HR (Q1=ref)	Q3: 1.14	0.83	1.56	NS	+++
						Q4: 1.28	0.86	1.91	NS	+++
			Stroke			Q2: 0.95	0.64	1.43	NS	+++
						Q3: 0.94	0.60	1.48	NS	+++
						Q4: 0.61	0.33	1.13	NS	+++
Schriewer (1984) [171]	Blood level of HDL-PC	Spearman correlation	Systolic blood pressure	men	Correlation coefficients	-0.03			NS	-
				women		0.03			NS	-
			Diastolic blood pressure	men		-0.05			NS	-
				women		0.01			NS	-
Stegemann (2014) [188]	Blood level of phospholipids[3]	Cox regression		PC	HR for CVD including fatal and non-fatal MI, ischemic stroke and sudden cardiac death)	32:1 +			0.0011	+
						38:2 +			0.0049	+
			CVD			38:3 +			0.0041	+
						Other[c]			NS	+
				Lyso-PC					NS	

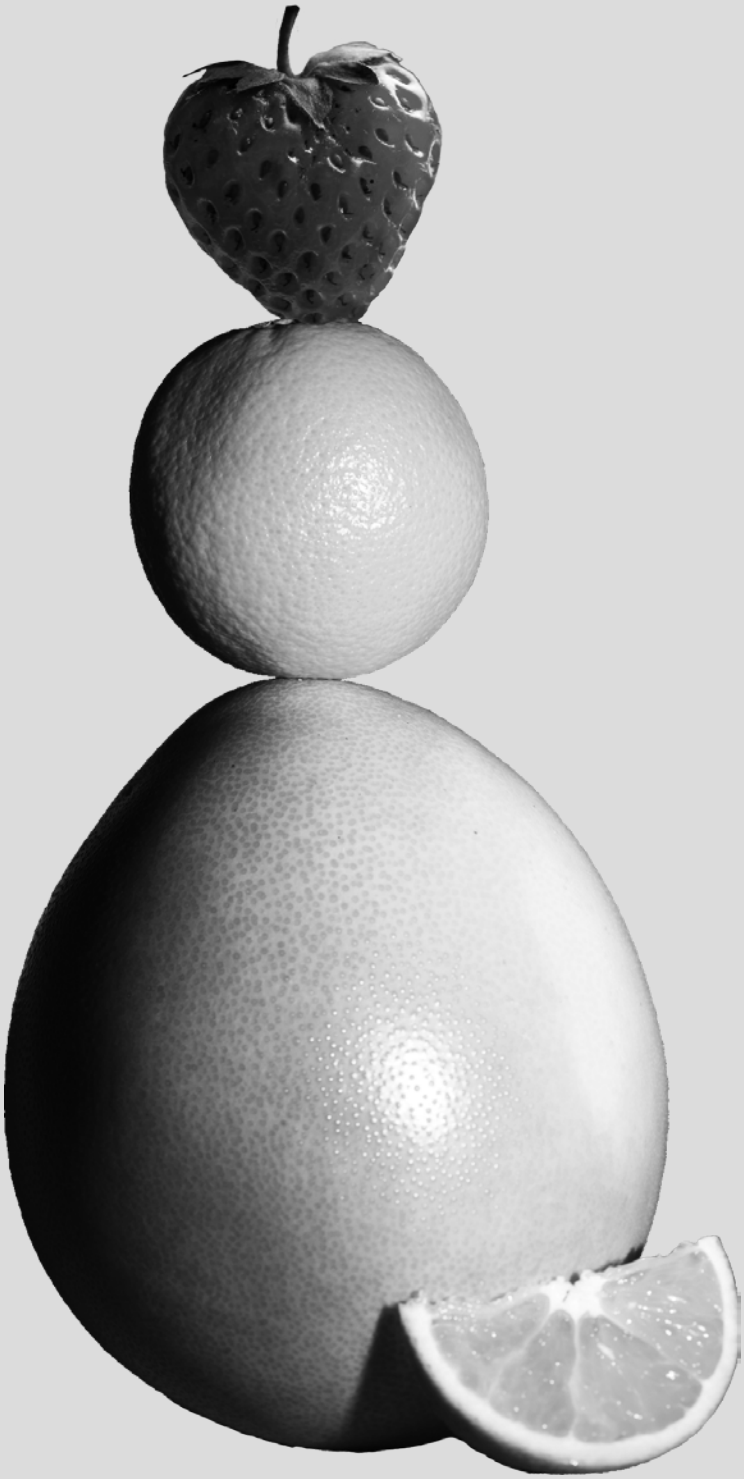
[a]Adjustment level was categorized as followed; unadjusted -; 4 covariates or less +; 5 to 8 covariates ++; 9 or more covariates +++. Effect estimate: negative association (-), positive association (+) [b]Quartiles based on controls. [c]Choline containing phospholipids, Other phospholipids were not significant after accounting for multiple testing (PCs 32:0, 32:2; 34.1, 34:2, 34:3, 36:1, 36:2, 36:3, 36:4, 36:5, 38:4, 38:5, 38:6, 40:2, 40:3, 40:4, 40:5, 40:6, O-32:0, O-32:1, O-34:1, O-34:2, O-34:3, O-38:3, O-38:4, O-38:5, O-40:0, O-40:1, O-40:2, O-40:3, O-40:4, O-40:5, O-40:6, O-42:5, O-42:6, lyso-PCs 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 20:3, 20:4, 20:5, 22:5, 22:6, O-16:0, O-18:0)

Table 2.2.6 Reported associations between choline and respiratory health

First author (year)	Exposure	Type of choline	Statistical analysis	Outcome	Subgroup	Measure of association	Effect estimate	LL 95%CI	UL 95%CI	p-value	Adjustment level[a]
Adults											
Ried (2013) [189]	Blood level	Phospholipids[b]	Logistic regression	Current asthma	Acyl-alkyl PC	OR for asthma per SD increase of phospholipid	C42:5; 1.39			0.0000735	++
							C42:1; 1.38			0.0000816	++
							Other[b]			NS	++
	Blood level	Phospholipids[b]	Logistic regression	COPD		OR for COPD per SD increase of phospholipid	Other[b]			NS	++

[a]Adjustment level was categorized as followed; unadjusted -; 4 covariates or less +; 5 to 8 covariates ++; 9 or more covariates +++. Effect estimate: negative association (-), positive association (+)

[b]Choline containing phospholipids, Other phospholipids were not significant after accounting for multiple testing (Diacyl PCs 24:0,C26:0,C28:1,C30:0,C30:2,C32:0,C32:1,C32:2,C32:3,C34:1,C34:2,C34:3,C34.4, C36:0,C36:1,C36:2,C36:3,C36:4,C36:5,C36:6,C38:0,C38:1,C38:3,C38:4,C38:5,C38:6,C40:1,C40:2,C40:3,C40:4,C40:5,C40:6,C42:0,C42:1,C42:2,C42:4,C42:5,C42:6, Acyl-alkyl PCs C30:0,C30:1,C30:2,C32:1,C32:2,C34:0, C34:1,C34:2,C34:3,C36:0,C36:1,C36:2,C36:3,C36:4,C36:5,C38:0,C38:1,C38:2,C38:3, C38:4,C38:5,C38:6, C40:0,C40:1,C40:2,C40:3,C40:4,C40:5,C40:6,C42:0,C42:1,C42:2,C42:3,C42:4,C42:5,C44:3,C44:4,C44:5,C44:6 and Lyso PCs C14:0,C16:0,C16:1,C17:0,C18:0,C18:1,C18:2,C18:2,C6:0,C20:3,C20:4,C24:0,C26:0,C26:1,C28:0,C28:1) (For COPD also Acyl-alkyl PCs C42:5 and C42:1 were NS).



Chapter 3

Maternal Nutrition and Offspring Cardiometabolic Health

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Abstract

Background Maternal diet during pregnancy may affect body composition of the offspring later in life, but evidence is still scarce.

Objective We aimed to examine whether maternal dietary patterns during pregnancy are associated with body composition of the child at the age of 6 years.

Methods This study was performed among 2,695 Dutch mother-childpairs from a population-based prospective cohort study from fetal life onwards. Maternal diet was assessed in early pregnancy by a 293-item semi-quantitative food-frequency questionnaire. A 'Vegetable, fish and oil', a 'Nuts, soy and high fiber cereals' and a 'Margarine, snacks and sugar' dietary pattern was derived from Principal Component Analysis. We measured weight and height of the child at age 6 at the research center. Total body fat and regional fat mass percentages of the child were assessed with dual-energy X-ray absorptiometry.

Results In the crude models, significant associations were found for higher adherence to the 'Vegetable, fish and oil' dietary pattern and the 'Nuts, soy and high fiber cereals' dietary patterns with lower BMI, lower body fat percentage, lower android/gynoid ratio and lower risk of being overweight, but these associations disappeared after adjustment for socio-demographic and lifestyle factors. The 'Margarine, snacks and sugar' dietary pattern was not associated with any of the outcomes.

Conclusion: Our results suggest that the associations between maternal dietary patterns during pregnancy and body composition of the child at age 6 years are explained by socio-demographic and lifestyle variables of mother and child.

Introduction

Childhood overweight and obesity are a major public health problem, and in 2010, the prevalence was estimated to be 11.7% in developed countries.[12] Overweight and obesity during childhood can cause several health complications, including insulin resistance and high blood pressure.[212] In addition, overweight and obese children are more likely to become overweight adults[213] and have a greater risk to develop chronic diseases, such as type 2 diabetes mellitus and cardiovascular diseases.[214]
Maternal lifestyle during pregnancy has been suggested to influence the risk of obesity in childhood. Barker (1986) was one of the first that proposed this phenomenon of fetal programming, also known as 'the Barker Hypothesis'.[215] Maternal diet during pregnancy is one of the lifestyle factors that may have an effect on fetal programming. Besides a direct effect of maternal diet on fetal growth, epigenetic alterations in the fetus may change fetal metabolism, which in its turn could alter growth or body composition unfavorably later in life.[216] Furthermore, specific groups of mothers might have different nutritional requirements and therefore may respond differently to the effects of diet, which could result in an altered body composition of the children within these subgroups.
Some studies indeed have shown an association between nutrition during pregnancy and body composition of the offspring. Higher maternal blood levels of n-6 fatty acids[217] and folate[218] have been associated with higher fat mass in the offspring at age 6. However, other studies did not replicate these findings.[219,220] Only one study studied food group intake during pregnancy, which showed that a higher maternal meat intake was associated with a higher fat mass in adolescents, but no associations with vegetables, fish, fruit and milk were found.[221] All of these studies investigated single nutrients or foods, while people do not eat isolated nutrients or foods, but, whole diets instead. The approach of analyzing dietary pattern accounts for synergy in whole diets and also considers the degree of interaction among nutrients.[222] To our knowledge, only one study investigated the association between maternal diet during pregnancy and infant body composition by using dietary pattern analysis and this study looked at body composition when the infant was up to 6 months of age.[223] The latter study found no association, raising the question if the children in the study population were too young to see the effects of fetal programming.
Hence, the purpose of this study was to investigate the influence of different maternal dietary patterns during pregnancy on multiple indices of body composition in the offspring at the age of 6 years.

Methods

Study design

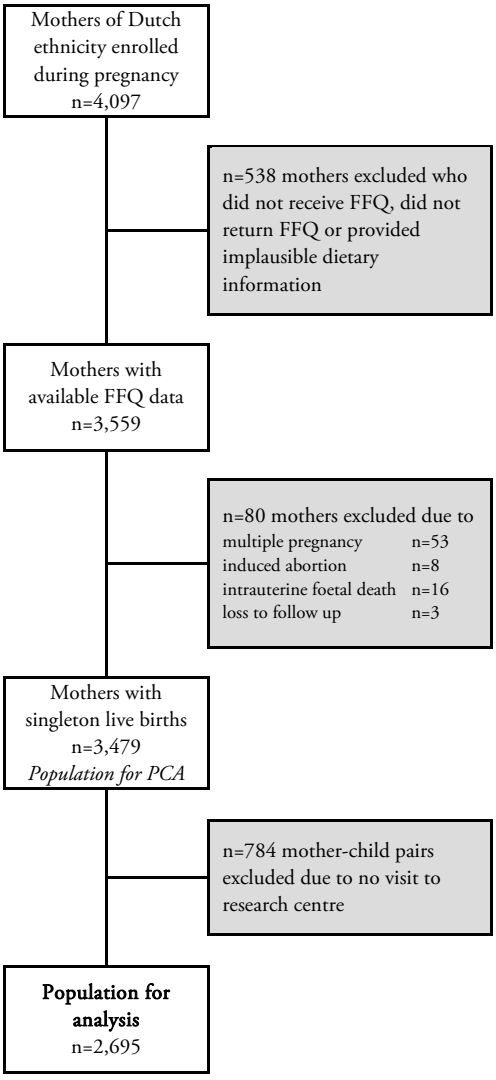
The present study was embedded within the Generation R Study, an ongoing population-based cohort study from fetal life onwards that has previously been described in detail.[34] The study was conducted following the World Medical Association Declaration of Helsinki and was approved by the Medical Ethics Committee at Erasmus Medical Centre,

Rotterdam, the Netherlands. Written consent was obtained from all participants.

Population for analysis

A flowchart of the selection process of the study population is shown in **Figure 3.1.1**. Since cultural differences could influence dietary patterns and the FFQ was designed for a Dutch population, only mothers of Dutch national origin were eligible for this study.

Figure 3.1.1 Flow chart of study population



Dietary patterns were determined in the 3,479 mothers with a singleton live birth and dietary data available. At the child's age of 6 years, 784 mother-child pairs did not visit the research centre, leading to a population for analysis of 2,695. Data on body fat mass was available in 96% of these children (n=2,585).

Dietary assessment

Information about dietary intake in early pregnancy (median 13.4 weeks of gestation, 95% range 9.9 to 22.8) was obtained with the use of a self-administered 293-item semi-quantitative food-frequency questionnaire (FFQ). This FFQ was validated in a Dutch elderly population and was adapted for use during pregnancy.[224] The FFQ was validated with three 24-hour recalls in a group of Dutch pregnant women in Rotterdam (n=71), who were visiting the midwifery. The intraclass correlation coefficients for energy-adjusted macronutrient intake were between 0.48 and 0.68 (unpublished data). The FFQ comprised of questions about consumption frequency, portion size, preparation method and additions to the dish. Dutch household measures and photographs were used to make approximations about portion size.[225] Average daily nutrient intake was calculated with the use of the Dutch food composition table 2006.[226]

Dietary patterns

Principal Component Analysis (PCA)[222] was used to determine dietary patterns. First, the 293 food items were reduced to 23 food groups (**Table 3.1.1** and **Supplementary Material S3.1.1**). This division was based on the

National Food Consumption Survey[227] but some adjustments were made in order to better capture specific nutrients (eg. dividing cereals into low and high-fiber cereals).

Table 3.1.1 Factor loadings of food groups in dietary patterns of the mothers during pregnancy (n=3,479)

Food group	Vegetables, fish & oil	Nuts, soy & high-fiber cereals	Margarine, snacks & sugar
Potatoes and other tubers	0.05	**-0.53**	**0.21**
Vegetables	**0.78***	0.17	-0.03
Fruits	0.13	**0.37***	0.02
High-fat dairy	**0.26**	**-0.26**	**0.29**
Low-fat dairy	-0.15	**0.29**	0.16
High-fiber cereals	**0.24**	**0.43***	**0.36**
Low-fiber cereals	**0.23**	-0.16	**0.25**
Meat	0.09	**-0.54**	**0.33**
Fish and shellfish	**0.45***	**0.24**	-0.11
Eggs	0.27	0.05	0.19
Vegetable oils	**0.74***	0.08	-0.12
Margarine and butter	-0.06	-0.03	**0.62***
Sugar and confections	-0.11	0.13	**0.56***
Snacks	0.05	0.08	**0.40***
Coffee and tea	**0.28**	**0.35**	0.10
Sugary drinks	-0.14	**-0.28**	**0.29**
Light drinks and water	0.13	**0.28**	-0.02
Alcoholic beverages	**0.35**	-0.00	-0.04
Condiments and sauces	0.05	-0.09	**0.39**
Soups and bouillon	**0.20**	-0.02	0.15
Nuts, seeds and olives	0.03	**0.64***	**0.30**
Soy products	0.00	**0.39***	-0.10
Legumes	**0.44**	-0.02	0.07

Food groups with bold numbers are considered to have a strong association (factor loading ≥ 0.2 or ≤ -0.2) with a dietary pattern.
The three highest factor loadings per dietary pattern are shown with an asterisk (*) and are used to label the pattern

Factors (i.e. dietary patterns) with an eigenvalue of ≥1.5 were extracted. Varimax rotation was used to improve interpretation of the dietary patterns. Subsequently, a factor loading was calculated for each food group, which illustrates the extent to which each food group is correlated with the specific dietary pattern. The three highest factor loadings were used to label the dietary pattern. For each mother, regression-based scores were extracted, which corresponds with the similarity of one's diet with one of the dietary patterns and were used as adherence scores for these dietary patterns. Subsequently, the adherence scores of the mothers of the population for analysis (n=2,695) were categorized into quartiles.

Child body composition

At the age of 6 years (median 72 months, 95% range 68 to 89 months), children were invited to visit our dedicated research facility in Sophia's children hospital, Rotterdam, the Netherlands, where measures of body composition were obtained by well-trained research staff. Weight was measured to the nearest gram using an electronic scale (SECA) and height was measured in standing position to the nearest 0.1 cm by a stadiometer (Holtain Limited). BMI was calculated (kg/m^2). Children were classified as overweight or obese according to the age- and sex-specific cut-off points from the International Obesity Task Force.[228] We used Dual-energy X-ray absorptiometry (DXA) scans (iDXA scanner, GE Healthcare, Madison, WI)[229] to assess total body fat and regional fat mass percentages. The iDXA scanner can measure total body fat with an accuracy of less than 0.25% coefficient of

variation.[229] Children were scanned in a supine position with their feet together in a neutral position and hands flat and pronated by their sides. Percentage body fat was calculated as 100% x [total body fat mass (kg)]/ [fat mass + lean mass + bone mass (kg)]. The android/gynoid fat mass ratio was calculated by dividing the abdominal fat mass by the fat mass around the hips, thighs and buttocks. The fat free mass index was calculated as [lean mass (kg) + bone mass (kg)]/ [height^2 (m)]. The fat mass index was calculated as [fat mass (kg)]/ [height^2 (m)]. Age- and sex-specific SD scores were calculated based on the total population.

Covariates

Several sociodemographic, medical and behavioral characteristics were considered as possible confounders. Information regarding maternal age, pre-pregnancy BMI (self-reported pre-pregnancy weight divided by height measured at intake (squared)), education (low vs. high), net monthly family income (<2200 vs. >2200 euro) parity (nulliparous vs. multiparous), maternal smoking during pregnancy (never, until pregnancy was known, or continued throughout pregnancy), maternal alcohol use during pregnancy (never, until pregnancy was known, or continued throughout pregnancy), folic acid supplementation (not used, started during first 10 weeks, or started periconceptional), stress during pregnancy (Global Severity Index), vomiting and feeling nauseous (once a week or less vs. daily or a few days a week) was obtained from prenatal questionnaires send in different trimesters. Information on sex of the child, gestational age and birth weight was available from obstetric records assessed in hospital registries and mid-wife practices [34]. Information regarding breast feeding of the child (never, partially breastfed in the first 4 months, or exclusively breastfed for at least 4 months) was collected by a combination of delivery reports and postnatal questionnaires. Other postnatal questionnaires completed by the mother included information on TV watching (hours/day) at 2 years and participation in sports (yes vs. no) at 6 years of age.

Statistical analyses

Models

All associations were first assessed in a crude model. Additionally, we used multivariable linear regression to determine the association between different dietary patterns and child BMI, FMI, FFMI total body fat percentage or android/gynoid fat mass ratio. Multivariable logistic regression was used to assess the association between the different dietary patterns and the risk of being overweight. The dietary patterns were analyzed in quartiles of adherence and trend tests were performed using adherence score as a continuous variable. Potential confounders were entered individually into a linear regression model of dietary patterns and child BMI, total body fat percentage or android/gynoid fat mass ratio at age 6 years. Covariates were considered as confounders when they induced a change in effect estimate of at least 5% for one of the outcomes, and included for all outcomes. Hence, the same multivariable models were used for all outcomes. Furthermore, we created a multivariable model with and without

maternal total energy intake to assess whether the association between the dietary patterns and child body composition was explained by total energy intake, because an association found between a dietary pattern that consists mainly of high energy foods and a disease outcome may be an effect of a high calorie intake instead of being determined by the specific foods of a pattern.

Missing data

To prevent bias due to missing data, we used multiple imputation[230] (m=10, for details and results: **Supplementary Material S3.1.2** and **S3.1.3**) to replace missing values on covariates. Analyses were performed in each of the 10 imputed data sets separately, and final results were pooled.

Additional analyses

Some variables were expected to be effect modifiers, because the effects of the different dietary patterns on child body composition might differ within strata of these variables. Therefore, we tested for possible interactions between dietary patterns and maternal pre-pregnancy BMI, maternal folic acid supplement use, maternal smoking during pregnancy, vomiting during pregnancy, nausea during pregnancy and maternal energy intake by adding an interaction term (i.e. adherence score x stratum) to the multivariable model. Also, based on the hypothesis that growth patterns might differ between girls and boys,[231] we stratified our analyses for sex of the child. All statistical analyses were performed using SPSS Statistics 22.0 (IBM Corp., Armonk, NY).

Results

Study population

Characteristics of the mothers in the study population are presented in **Table 3.1.2.**

Table 3.1.2 Characteristics of the participants (n=2,695)

Mother	
Age (y)	31.7 ± 4.2
Pre-pregnancy BMI (kg/m^2)	23.3 ± 3.9
Gestational age at enrollment (wk)	13.6 (9.9; 21.5)
Education level	
Primary or secondary	1,019 (37.8%)
Higher	1,640 (61.7%)
Missing	*36 (1.3%)*
Household income	
<2200 €/mo	591 (23.8%)
>2200 €/mo	1,893 (76.2%)
Missing	*211 (7.8%)*
Parity	
0	1,665 (61.9%)
≥1	1,026 (38.1%)
Missing	*4 (0.1%)*
Smoking	
Never during pregnancy	1,886 (75.9%)
Until pregnancy was known	236 (9.5%)
Continued during pregnancy	363 (14.6%)
Missing	*210 (7.8%)*
Alcohol	
Never during pregnancy	776 (28.8%)
Until pregnancy was known	413 (15.3%)
Continued during pregnancy	1,278 (51.8%)
Missing	*228 (8.5%)*
Folic acid supplement use	
No	203 (9.2%)
Started first 10 wks	734 (33.1%)
Started periconceptional	1,281 (57.8%)
Missing	*477 (17.7%)*
Total energy intake (kcal/d)	2,153 ± 503
Stress during pregnancy	0.12 (0.00; 0.77)

Values are means ± SD, absolute numbers (valid percentages) or medians (95% range).
Missing values for continuous variables were 373 (13.8%) for pre-pregnancy BMI and 284 (10.5%) for stress during pregnancy.

We identified a 'Vegetable, fish and oils', a 'Nuts, soy, and high-fiber cereals' and a 'Margarine, snacks and sugar' dietary pattern (**Table 3.1.1**), which together explained for 25.8% of the variance in food consumption.

Most mothers followed higher education (61.7%) and were also mostly nulliparous (61.9%). In addition, most mothers (75.9%) did not smoke during pregnancy, but approximately half of the mothers in the study population continued with alcohol drinking while they were pregnant (51.8%). Characteristics of the mothers and children who were excluded during the selection of the study population are shown in **Supplementary Material S3.1.4**.

Associations of dietary patterns with child body composition

Body Mass Index

Higher adherence to the 'Vegetable, fish and oil' dietary pattern was associated with lower BMI in the offspring in the crude model (**Table 3.1.3**). After adjustment for maternal and child sociodemographic and lifestyle factors, results were no longer significant. There were no associations between 'Nuts, soy and high-fiber cereals' and the 'Margarine, snacks and sugar' dietary pattern and child BMI.

From the analysis of FFMI and FMI it was seen that the crude associations were driven by a decrease in FMI, but no consistent associations remained after adjustment for sociodemographic and lifestyle variables (**Supplementary Material S3.1.5**). Additional adjustment for total energy intake did not alter the results (data not shown).

Total body fat percentage

The 'Vegetable, fish and oil' and the 'Nuts, soy and high-fiber cereals' dietary pattern were associated with lower body fat percentage in the offspring in crude models (**Table 3.1.3**). However, the associations disappeared after adjustment for socio-demographic and lifestyle variables. We observed no significant associations between the 'Margarine, snacks and sugar' dietary pattern and child body fat percentage. Additional adjustment for total energy intake did not alter the results (data not shown).

Android/gynoid fat mass ratio

Quartile 2 of the 'Vegetable, fish and oil' dietary pattern was significantly associated with a lower android/gynoid fat mass ratio in the crude model, but quartiles 3 and 4 of this dietary pattern and the trend test did not show significant associations (**Table 3.1.3**). A higher adherence to the 'Nuts, soy and high-fiber cereals' dietary pattern was significantly associated with lower android/gynoid fat mass ratio in the crude model. After adjustment for sociodemographic and lifestyle variables the associations did not remain significant.

There was no significant association between the 'Margarine, snacks and sugar' dietary pattern and android/gynoid fat mass ratio in the offspring. Additional adjustment for total energy intake did not alter these results (data not shown).

Child overweight/obesity

As compared to the lowest quartile, quartiles 3 and 4 of the 'Vegetable, fish and oil' dietary pattern and quartiles 2, 3 and 4 of the 'Nuts,

soy and high-fiber cereals' dietary pattern were significantly associated with a lower risk of the child being overweight, in the crude model (**Figure 3.1.2**). After adjustment for sociodemographic and lifestyle variables, these associations were no longer significant.

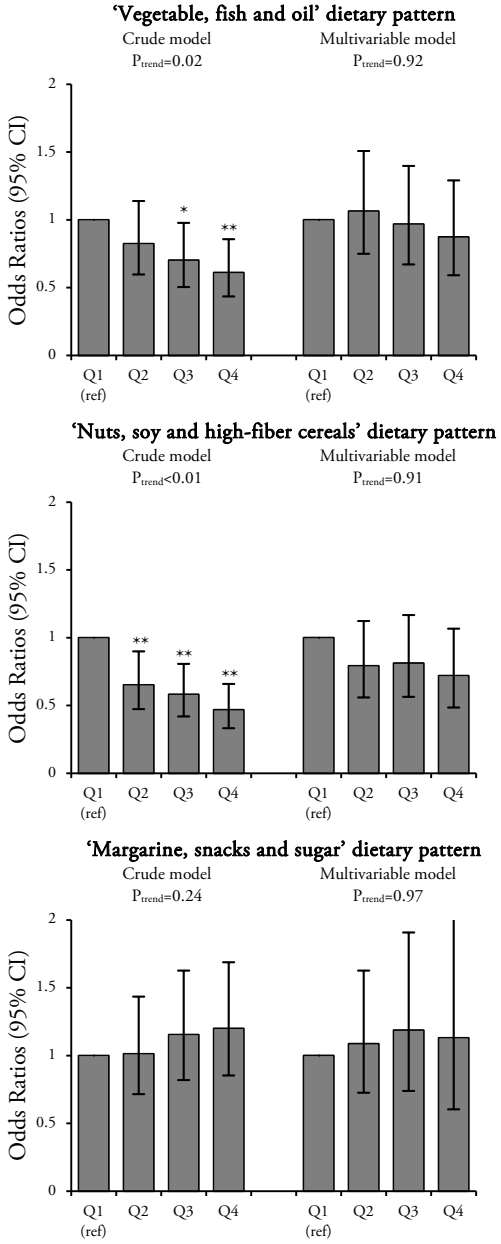
There was no significant association between the 'Margarine, snacks and sugar' dietary pattern and the child's risk of being overweight. Additional adjustment for total energy intake did not alter these results (data not shown).

Table 3.1.3 Associations between maternal dietary patterns and offspring body composition at the age of 6 years

	'Vegetable, fish and oil' dietary pattern					
	BMI n=2,689		**Body fat %** n=2,520		**Android/gynoid ratio** n=2,520	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.09 (-0.18, -0.00)*	-0.04 (-0.13, 0.05)	-0.20 (-0.29, -0.11)**	-0.08 (-0.17, 0.01)	-0.11 (-0.21, -0.01)*	-0.04 (-0.13, 0.06)
Q3	-0.15 (-0.23, -0.06)**	-0.07 (-0.16, 0.02)	-0.27 (-0.33, -0.15)**	-0.08 (-0.17, 0.02)	-0.08 (-0.17, 0.02)	0.03 (-0.07, 0.13)
Q4 high	-0.15 (-0.23, -0.06)**	-0.07 (-0.16, 0.02)	-0.29 (-0.37, -0.19)**	-0.09 (-0.18, 0.01)	-0.09 (-0.19, 0.00)	0.03 (-0.08, 0.13)
Per SD	p<0.01	p=0.21	p<0.01	p=0.46	p=0.39	p=0.15
	'Nuts, soy and high-fiber cereals' dietary pattern					
	BMI n=2,689		**Body fat %** n=2,520		**Android/gynoid ratio** n=2,520	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.08 (-0.17, 0.01)	-0.03 (-0.12, 0.06)	-0.18 (-0.27, -0.09)**	-0.07 (-0.16, 0.02)	-0.12 (-0.22, -0.02)**	-0.05 (-0.15. 0.01)
Q3	-0.06 (-0.20, 0.02)	0.03 (-0.07, 0.12)	-0.19 (-0.28, -0.09)**	-0.01 (-0.10, 0.08)	-0.20 (-0.30, -0.10)**	-0.09 (-0.19, 0.02)
Q4 high	-0.05 (-0.14, 0.03)	0.07 (-0.02, 0.17)	-0.22 (-0.31, -0.13)**	0.01 (-0.09 0.11)	-0.17 (-0.26, -0.07)**	-0.03 (-0.13, 0.03)
Per SD	p=0.44	p=0.03	p<0.01	p=0.76	p<0.01	p=0.16
	'Margarine, snacks and sugar' dietary pattern					
	BMI n=2,689		**Body fat %** n=2,520		**Android/gynoid ratio** n=2,520	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.04 (-0.05, 0.12)	0.04 (-0.06, 0.13)	-0.03 (-0.12, 0.06)	-0.03 (-0.07, 0.12)	0.00 (-0.10, 0.10)	0.01 (-0.09, 0.11)
Q3	-0.01 (-0.10, 0.08)	-0.03 (-0.14, 0.09)	-0.02 (-0.11, 0.07)	-0.05 (-0.06, 0.17)	0.02 (-0.08, 0.12)	0.02 (-0.11, 0.14)
Q4 high	-0.02 (-0.07, 0.10)	-0.02 (-0.17, 0.13)	-0.04 (-0.13, 0.06)	0.08 (-0.08, 0.23)	0.04 (-0.06, 0.14)	0.03 (-0.13, 0.20)
Per SD	p=0.98	p=0.46	p=0.75	p=0.05	p=0.26	p=0.47

Values (regression coefficients with 95%CI) reflect differences in outcomes (age- and sex-specific SD scores) for quartiles 2 to 4, as compared to the lowest quartile. Trend tests were performed by using adherence score (SD scores) as a continuous variable in the model.
Crude model is adjusted for height of the child at 6y
Multivariable model is additionally adjusted for maternal age, gestational age at dietary assessment, smoking, alcohol and folic acid use during pregnancy, educational level, family income, parity, pre-pregnancy BMI, stress during pregnancy, and child sex, breast feeding, TV watching at 2y , and participation in sports at 6y.
* p<0.05;** p<0.01.

Figure 3.1.2 Associations between dietary patterns and offspring's risk of being overweight at 6 years (n=2,625)



Odds Ratios (with 95%CI) reflect the risk of being overweight or obese for quartile 2 to 4, as compared to the lowest quartile. Trend tests were performed by using adherence score (SD scores) as a continuous variable Multivariable model is adjusted for maternal age at intake, gestational age at dietary assessment, smoking, alcohol and folic acid supplement use during pregnancy, educational level, family income, parity, pre-pregnancy BMI, stress during pregnancy, sex of the child, breastfeeding, watching TV at 2y and playing sports at 6y.* p<0.05, ** p<0.01

Additional analyses

We stratified for sex of the child. Results did not differ within strata of sex of the child (data not shown). Significant interactions were found between pre-pregnancy BMI and the dietary pattern 'Nuts, soy and high-fiber cereals', on the outcomes total body fat percentage and android/gynoid fat mass ratio of the child (p=0.02 and p<0.01, respectively), but there were no significant interactions with the other outcomes or dietary patterns (all p>0.12). Stratification by maternal pre-pregnancy BMI showed that the significant trend of the 'Nuts, soy and high-fiber cereals' pattern with child BMI was only present in the group of mothers with a pre-pregnancy BMI below 25 kg/m^2 (p for trend 0.04), while for android/gynoid ratio of the child, the stratification showed a significant trend of the 'Nuts, soy and high-fiber cereals' pattern with lower android/gynoid ratio of the child only in the group of mothers with pre-pregnancy BMI above 25 kg/m^2 (p for trend <0.01)(data not shown).

We found also a significant interaction between maternal folic acid use and the 'Nuts, soy and high-fiber cereals' dietary pattern, on BMI of the child (p<0.01) and the 'Vegetable, fish and oil' dietary patterns with body fat percentage of the child (p=0.04), but there were no significant interactions with other dietary patterns or outcomes (all p>0.07). Stratification for maternal folic acid use showed a significant association (p for trend <0.01) between the 'Nuts, soy and high-fiber cereals' dietary pattern with BMI of the child only in the group of mothers who started folic acid periconceptionally, but not in either of the

other groups (data not shown). We observed no significant associations between the 'Vegetable, fish and oil' dietary pattern with body fat percentage of the child, after stratification for maternal folic acid intake.

We observed a significant interaction of the 'Nuts, soy and high-fiber cereals' dietary pattern with maternal smoking during pregnancy on android/gynoid ratio of the child (p=0.02), but stratified analysis showed no significant associations in either strata of maternal smoking.

No significant interactions were found for maternal energy intake (all p>0.07), vomiting during pregnancy (all p>0.09) or feeling nauseous during pregnancy (all p>0.32).

Discussion

In a population-based prospective birth cohort, we showed that adherence to a 'Vegetable, fish and oil', 'Nuts, soy and high-fiber cereals or 'Margarine, snacks and sugar' dietary pattern during pregnancy was not independently associated with child body composition at age 6 years. All of the associations in the crude models of the 'Vegetable, fish and oil' and the 'Nuts, soy and high-fiber cereals' dietary patterns were explained by lifestyle and sociodemographic factors.

Maternal diet is one of the components of the intrauterine environment that is critical to fetal development.[216] In previous studies, a maternal 'Western diet'[232] and 'Wheat-products diet'[233] during pregnancy were shown to be associated with a lower birth weight, which in its turn could alter later body composition.[234,235] Based on the phenomena of fetal programming, which emphasizes prenatal nutrition as a key determinant for the increased risk of diseases later in life,[215] we hypothesized that maternal diet during pregnancy could have a substantial influence on a child's body composition.

There might be several explanations why the present findings do not correspond with the hypothesis. Dietary patterns derived from PCA are data-driven, and thus differ across populations. In our population, the most pronounced dietary patterns were characterized by high intake of healthy components . A more unhealthy diet, such as a Western diet, which is characterized by an excessive amount of saturated fat and carbohydrates, may have a larger impact on a child's body composition, beyond other lifestyle and sociodemographic factors. An animal study demonstrated that 10 week-old rats of mothers fed a junk food diet during gestation and lactation had an increased body weight.[236] In addition, previous studies on the influence of maternal nutrient intake on child body composition showed that food components that are seen as more unhealthy, such as high intake of n-6 fatty acids or glucose, are associated with a higher BMI at age 3 years[237] or fat mass at age 6 years[217], respectively. The 'Margarine, snacks and sugar' dietary pattern identified in our study has some similarities with a Western diet, but the pattern is also characterized by some relative 'healthier' foods, such as a higher intake of nuts, seeds and olives and high-fiber cereals, which may explain the discrepancy between our results and those from other studies.

Another explanation for our null-findings could be that previously reported associations between maternal nutrient intake during

pregnancy and child body composition may not be independent. Observational studies on diet and body composition are very susceptible to bias, since sedentary behavior, physical activity and other factors that are related to child body composition are often clustered with specific dietary patterns. In our study, all of the significant associations in the crude models were explained by sociodemographic and lifestyle factors. Other studies that did find associations between maternal diet during pregnancy and child body composition did not always adjust for maternal covariates, such as maternal folic acid use[217,221] or pre-pregnancy BMI,[218,221] which were important confounders in our analyses. Additionally, the study that reported the association between a high meat intake and a high fat mass in 16-year old adolescents[221] may have the benefits of the longer follow-up period, but this also increases the risk of residual confounding. More confounding factors could be involved and some of these factors may need to be measured more frequently. For example, information on sociodemographic factors were collected at the time of birth of these children, and could have changed during the follow-up.

To our knowledge, only one previous study investigated the association between maternal diet during pregnancy and child body composition using dietary pattern analysis.[223] This study did not find a significant association between maternal dietary patterns during the third trimester of pregnancy and infant weight at 4-6 months. They did not use PCA to derive a posteriori dietary patterns, but, instead of that, dietary patterns were evaluated by *a priori* diet scores, i.e. Alternative Healthy Eating Index for Pregnancy and Mediterranean diet, and therefore are likely to reflect optimal patterns. Thus, the focus of that study was more on a healthy diet, while an unhealthy diet during pregnancy might be more likely to unfavorably influence child body composition.

Our study has several strengths. The large sample size, prospective data collection and information on a large number of potential confounders are important strengths. Also, the comprehensive measures of the body composition of the children enabled us to investigate different aspects of body composition. Additionally, the use of dietary patterns has the advantage that it reflects the entire diet of the mothers, thereby considering the interactions among nutrients and synergy in diets.[222]

This study also has some limitations to consider. The definition of the food groups for identifying the dietary patterns was restricted by choices made in the design of the FFQ. For example, in one answer in the FFQ both vegetables with a high and a low folic acid content were combined, while it might be interesting to make a division between them based on an earlier study that reported that a higher maternal folate concentration during pregnancy was associated with a higher adiposity in the offspring at age 6 years.[218] Nevertheless, for other food groups it was possible to make a division, such as the dairy products food group, which we divided into high fat vs. low fat. Another limitation of the study is that a self-reported diet is prone to measurement error, because it relies on memory and people are likely to underestimate their nutritional intake.[238] Usually this leads to

an underestimation of any true association. We corrected for this by adjusting for total energy intake in an additional model, which reduces potential systematic measurement error,[239] and this did not alter our results.

In addition, as in many prospective studies, we had missing data on our covariates which may lead to attrition bias. However, we used multiple imputation to impute missing values, which has shown to be a reliable method to minimize attrition bias.[240] Furthermore, during the selection of the study population, 34.2% of the participants were excluded. The 1,402 mothers that were excluded were on average lower educated (50.7% only followed primary or secondary education, compared to 37.8% of the 2,695 mothers that were included), were more likely to continue smoking during pregnancy (24.1%, compared to 14.6% in the study population) and were less likely to start folic acid supplements periconceptionally (49.2%, compared to 57.8% in the study population). Thus, our study population has a selection towards a more healthy population. In a population where unhealthy dietary patterns are more clearly present, an association between maternal diet and child's body composition might be easier to detect.

In conclusion, we observed that maternal dietary patterns during pregnancy were not associated with body composition of the offspring at the age of 6 years after taking into account sociodemographic and lifestyle factors of both mothers and their children. Our results are not in line with previous hypothesis It might be worthwhile to further investigate te influence of maternal dietary patterns during pregnancy on child body composition in other populations or subgroups, since dietary patterns may differ between populations, and considering the limited number of studies on this topic.

Supplementary Material can be found online: http://hdl.handle.net/1765/77768

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Abstract

Background Maternal nutrition might be an important factor in influencing cardiometabolic health in offspring. However, research has focused mostly on specific nutrients or total energy, and possible effects of whole diet are unclear.

Objective We examined the associations between different dietary patterns during pregnancy and offspring cardiometabolic health at the age of 6 years.

Methods This study was performed among 2,695 Dutch mother-child pairs from a population-based prospective cohort study from fetal life onwards. Maternal diet was assessed in early pregnancy with the use of a 293-item semi-quantitative food-frequency questionnaire. We identified three a posteriori-dietary patterns using Principal Component Analysis, namely a 'Vegetable, fish and oil', 'Nuts, soy and high-fiber cereals' and 'Margarine, snacks and sugar'-dietary pattern. An a priori-dietary pattern was created based on the 'Dutch Healthy Diet Index'. At the child's age of 6 years, cardiometabolic health was measured, including insulin, HDL-cholesterol, triglycerides, systolic and diastolic blood pressure and pulse wave velocity.

Results In the crude models, significant associations were found for higher adherence to the 'Vegetable, fish and oil', 'Nuts, soy and high-fiber cereals' and 'Dutch Healthy Diet Index'-dietary patterns with lower blood pressure and lower pulse wave velocity. After adjustment for other socio-demographic and lifestyle factors, most associations disappeared, except for lower pulse wave velocity with the 'Vegetable, fish and oil'-dietary pattern (-0.20 SD (95%CI-0.33;-0.06), highest vs. lowest quartile). The 'Margarine, snacks and sugar'- pattern was not associated with any of the outcomes. No associations were found between maternal dietary patterns and blood lipids or insulin levels of the offspring.

Conclusion Our results suggest that there are no consistent independent associations of different dietary patterns during pregnancy with offspring cardiometabolic health at age 6. A possible association with pulse wave velocity needs to be further explored.

Introduction

Cardiometabolic diseases in adults have been linked to exposures during early life.[215] One of the consequences of malnutrition during pregnancy is low birth weight, and this may predispose higher risk of cardiometabolic diseases later in life.[241] However, maternal malnutrition has also been associated with offspring health without affecting size at birth.[242] In addition, micronutrient status during pregnancy has been related to cardiometabolic outcomes in the offspring, also independent of child's birth weight.[218] This suggests that energy intake and fetal growth restriction are not the only pathways in predisposing these children to a higher risk of chronic disease, but that a direct effect of maternal diet might exist.[216, 243]

Severe energy restriction during pregnancy is suggested to influence offspring health.[241] However, what the optimal diet is during pregnancy for adequate child health is still an unresolved question.[244] In addition, human studies on intrauterine exposures and later cardiometabolic health mainly focused on birth weight, and studies on the role of maternal diet are scarce and inconsistent.[244, 245]

Last decades, research in nutritional epidemiology started focusing on overall diet instead of only examining individual nutrients or foods, to take the interactions within diet into account.[222,246] Furthermore, studies based on dietary patterns are helpful in translating results from nutritional epidemiology to food-based dietary guidelines.[247]

A priori dietary patterns are usually defined based on dietary guidelines and expert advice, and thus generally reflect a diet that is related to health outcomes.[246] *A posteriori* dietary patterns are data-driven and thus reflect actual dietary patterns within specific study populations.

We aimed to examine the associations of different types of *a priori*- and *a posteriori*-defined dietary patterns during pregnancy with cardiometabolic health in offspring at the age of 6 years.

Methods

Study design

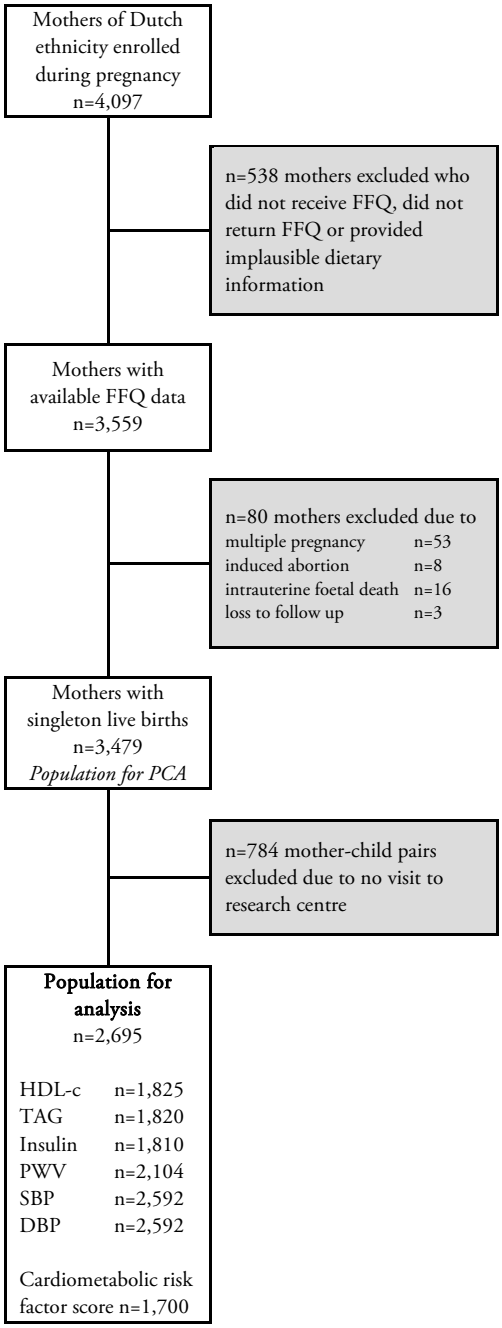
The present study was embedded within the Generation R Study, an ongoing population-based prospective cohort study from fetal life onwards that has been previously described in detail.[34] The study was conducted following the World Medical Association Declaration of Helsinki and was approved by the Medical Ethics Committee at Erasmus Medical Centre, Rotterdam, the Netherlands. Written consent was obtained from all participants.

Population for analysis

A flowchart of the selection process of the study population is shown in **Figure 3.2.1**. Since cultural differences could influence the definition of dietary patterns and the FFQ was designed for a Dutch population, we included only the subset of mothers with Dutch national origin. Dietary patterns were determined in the 3,479 mothers who had dietary data available and had a singleton live birth. At the child's age of 6 years, 784 children did not visit the research centre, leading to a population of 2,695 mother-child pairs eligible for this study.

Since not all children had all measurements done, population for analysis ranged from 1,700 to 2,592 (**Figure 3.2.1**).

Figure 3.2.1 Flow chart of study population



Dietary assessment

Diet in early pregnancy (median 13.4 weeks of gestation, 95% range 9.9 to 22.8) was assessed with a self-administered semi-quantitative food-frequency questionnaire (FFQ).[224] This FFQ was validated against three 24-hour recalls in Dutch pregnant women in Rotterdam (n=71), who were visiting the midwifery. The intraclass correlation coefficients for energy-adjusted macronutrient intake were between 0.48 and 0.68 (unpublished data). The FFQ consists of 293 items, and included questions about consumption frequency, portion size, preparation method and additions to the dish. Dutch household measures and photographs were used to approximate portion size.[225] Daily nutrient intake was calculated with the Dutch food composition table 2006. [226]

A priori dietary pattern

An *a priori* dietary pattern was defined based on the previously constructed 'Dutch Healthy Diet Index' (DHD-index) developed by van Lee et al.[248] The DHD-index consists of ten components: physical activity, vegetable, fruit, dietary fiber, fish, saturated fat (SFA), transfat (TFA), consumption occasions with acidic drinks and foods, sodium and alcohol, which represent the 2006 Dutch dietary guidelines. For the purpose of this study, we omitted the components 'physical activity', 'consumption of acidic drinks and foods' and 'TFA' since this data was not collected. Additionally, since alcohol consumption during pregnancy has known adverse effects on the fetus[249] and is therefore not recommended during pregnancy, we also excluded the alcohol component. The scores for the remaining six components ranged

from 0 to 10 points, resulting in a total score between 0 and 60. Higher scores correspond to a higher level of adherence to the Dutch dietary guidelines and therefore a healthier diet.

Table 3.2.1 Factor loadings of food groups in dietary patterns of the mothers during pregnancy (n=3,479)

Food group	Vegetables, fish & oil	Nuts, soy & high-fiber cereals	Margarine, snacks & sugar
Potatoes and other tubers	0.05	**-0.53**	**0.21**
Vegetables	**0.78***	0.17	-0.03
Fruits	0.13	**0.37***	0.02
High-fat dairy	**0.26**	**-0.26**	**0.29**
Low-fat dairy	-0.15	**0.29**	0.16
High-fiber cereals	**0.24**	**0.43***	**0.36**
Low-fiber cereals	**0.23**	-0.16	**0.25**
Meat	0.09	**-0.54**	**0.33**
Fish and shellfish	**0.45***	**0.24**	-0.11
Eggs	0.27	0.05	0.19
Vegetable oils	**0.74***	0.08	-0.12
Margarine and butter	-0.06	-0.03	**0.62***
Sugar and confections	-0.11	0.13	**0.56***
Snacks	0.05	0.08	**0.40***
Coffee and tea	**0.28**	**0.35**	0.10
Sugary drinks	-0.14	**-0.28**	**0.29**
Light drinks and water	0.13	**0.28**	-0.02
Alcoholic beverages	**0.35**	-0.00	-0.04
Condiments and sauces	0.05	-0.09	**0.39**
Soups and bouillon	**0.20**	-0.02	0.15
Nuts, seeds and olives	0.03	**0.64***	**0.30**
Soy products	0.00	**0.39***	-0.10
Legumes	**0.44**	-0.02	0.07

Food groups with bold numbers are considered to have a strong association (factor loading ≥ 0.2 or ≤ -0.2) with a dietary pattern.
The three highest factor loadings per dietary pattern are shown with an asterisk (*) and are used to label the pattern

A posteriori dietary patterns

Principal Component Analysis (PCA)[222] was used in order to determine *A posteriori* dietary patterns. First, the 293 individual food items were reduced to 23 food groups (**Table 3.2.1**). This division was based on the Dutch National Food Consumption Survey[227] but some adjustments towards this division have been made in order to better capture specific nutrients (eg. dividing cereals into low and high-fiber cereals). Factors (i.e. dietary patterns) with an eigenvalue of ≥1.5 were extracted. To improve interpretation of the dietary patterns, the Varimax rotation was used.[250] Subsequently, a factor loading was calculated for each single food group, which illustrates the extent to which each food group is correlated with the specific dietary pattern. The three highest factor loadings per dietary pattern were used to label the dietary pattern (**Table 3.2.1**). For each mother, regression-based scores were extracted and used as adherence scores for these dietary patterns. Subsequently, the adherence scores of the mothers of the population for analysis (n=2,695) were categorized into quartiles.

Cardiometabolic risk factors

At age 6 years, all children were invited to our dedicated research facility at the Erasmus Medical Centre, Sophia's Children Hospital. While the children were lying, systolic and diastolic blood pressure (SBP and DBP) were measured at the right brachial artery for four times with one-minute intervals, using the validated automatic phygmomanometer Datascope Accutor Plus TM (Paramus, NJ, USA).

Mean SBP and DBP were calculated, with exclusion of the first measurement. Carotid-femoral pulse wave velocity (PWV) was assessed using the automatic Complior SP device (Complior; Artech Medical, Pantin, France) with participants in supine position. Non-fasting blood samples were drawn by antecubital venipuncture. Insulin, HDL-c and triglyceride concentrations were measured with enzymatic methods (using a Cobas 8000 analyser, Roche, Almere, The Netherlands). Quality control samples demonstrated intra-assay and inter-assay coefficients of variation ranging from 0.69 to 1.57%. Body fat was measured by Dual-energy X-ray absorptiometry (DXA) scans (iDXA; General Electric, 2008, Madison, WI, USA). Percentage body fat was calculated as 100%*[total body fat mass(g)] /[total body mass (fat mass + lean mass + bone mass)(g)]. Results of body fat percentage are presented in **chapter 3.1**.

In addition to the individual cardiometabolic outcomes, we calculated a continuous cardiometabolic risk factor score. Following examples of previously defined metabolic syndrome scores for children,[251] we included five components: BF%, blood pressure (including DBP and SBP), and serum levels of HDL-c, triglycerides, and insulin. We summed the age- and sex-specific SD scores of these five variables, as proposed previously for pediatric populations.[251] The SD scores for HDL-c were multiplied by -1 since a higher HDL-c represents a better cardiometabolic profile. Hence, the cardiometabolic risk factor score was calculated as: SDS BF% + 0.5*SDS SBP + 0.5*SDS DBP + SDS TAG + (-1*SDS HDL-c) + SDS insulin.

Covariates

Information regarding paternal age, maternal age, pre-pregnancy BMI (self-reported pre-pregnancy weight divided by height measured at intake (squared)), education (low vs. high), family income (<2200 vs. >2200 euro per month), parity (nulliparous vs. multiparous), maternal smoking during pregnancy (never, until pregnancy was known, or continued throughout pregnancy), maternal alcohol use during pregnancy (never, until pregnancy was known, or continued throughout pregnancy), folic acid supplementation (not used, started during first 10 weeks, or started periconceptional) and stress during pregnancy (Global Severity Index) was obtained from prenatal questionnaires sent in different trimesters. Information regarding breastfeeding of the child (never, partially breastfed in the first 4 months, or exclusively breastfed for at least 4 months) was collected by a combination of delivery reports and postnatal questionnaires. Other postnatal questionnaires completed by the mother included information on child's TV watching (hours/day) at 2 years of age and participation in sports (yes vs. no) at 6 years of age. In a subgroup of children (60%) diet at one year of age was assessed with an FFQ, and a diet quality score was created.[252]

Statistical analyses

The dietary patterns were analyzed in quartiles and trend tests were performed using the SD score as a continuous variable. All associations were first assessed in a crude model. Additionally, potential confounders were entered individually into a linear regression model of dietary patterns and the

cardiometabolic risk factor score and were included in all modes when they induced a change in effect estimate of at least 5% for any dietary pattern. Hence, the same multivariable models were used for all exposures and outcomes. To prevent bias due to missing data, multiple imputation[230] was used to replace missing values on covariates. Analyses were performed in each of the 10 imputed data sets separately, and final results were pooled.

We performed several sensitivity analyses. We repeated the analysis excluding mothers with pre-pregnancy comorbidities (hyperlipidemia, diabetes mellitus or hypertension) (n=48), and mothers who vomited daily or a few days per week (n=321), as this might alter the effect of maternal diet. Furthermore, we additionally adjusted for maternal pregnancy complications (pregnancy induced hypertension, preeclampsia or gestational diabetes), maternal total energy intake and child weight at age 6 years, as they could be possible intermediates in the association of maternal diet with offspring cardiometabolic health. Also, we additionally adjusted for child diet quality at one year of age in a subgroup in which diet quality of the child was assessed (n=1,591). Additional analyses were also performed with the DHD-index including the component of alcohol intake.

We tested for possible interactions between dietary patterns and maternal pre-pregnancy BMI, child birth weight (SDS) and child sex by adding an interaction term to the multivariable model, because we considered these variables as potential effect modifiers. Stratified analyses were performed when a significant interaction was found. To avoid chance findings (type I errors) due to multiple testing, we corrected all p-values for the number of independent tests (i.e. number of dietary patterns). Thus, we used a p-value of 0.05/4=0.0125 as significance level. All statistical analyses were performed using SPSS Statistics 21.0 (IBM Corp., Armonk, NY).

Table 3.2.2 Characteristics of the participants (n=2,695)

Mother	
Age (y)	31.7 ± 4.2
Pre-pregnancy BMI (kg/m²)	23.3 ± 3.9
Gestational age at enrollment (wk)	13.6 (9.9; 21.5)
Education level	
Primary or secondary	1,019 (37.8%)
Higher	1,640 (61.7%)
Missing	*36 (1.3%)*
Household income	
<2200 €/mo	591 (23.8%)
>2200 €/mo	1,893 (76.2%)
Missing	*211 (7.8%)*
Parity	
0	1,665 (61.9%)
≥1	1,026 (38.1%)
Missing	*4 (0.1%)*
Smoking	
Never during pregnancy	1,886 (75.9%)
Until pregnancy was known	236 (9.5%)
Continued during pregnancy	363 (14.6%)
Missing	*210 (7.8%)*
Alcohol	
Never during pregnancy	776 (28.8%)
Until pregnancy was known	413 (15.3%)
Continued during pregnancy	1,278 (51.8%)
Missing	*228 (8.5%)*
Folic acid supplement use	
No	203 (9.2%)
Started in first 10 wks	734 (33.1%)
Started periconceptional	1,281 (57.8%)
Missing	*477 (17.7%)*
Total energy intake (kcal/d)	2,153 ± 503
Stress during pregnancy	0.12 (0.00; 0.77)

Values are means ± SD, absolute numbers (valid percentages) or medians (95% range).
Missing values for continuous variables were 373 (13.8%) for pre-pregnancy BMI and 284 (10.5%) for stress during pregnancy.

Results

Study population and dietary patterns

Table 3.2.2 shows the characteristics of the study population. Mothers were on average 31.7 years old (SD 4.2y) at enrollment, and most mothers were nulliparous (61.9%). Of the total population, 75.9% never smoked during pregnancy and most mothers used folic acid supplements during pregnancy (57.8% periconceptional, 33.1% started in first weeks), but 51.8% of the mothers continued alcohol drinking when pregnancy was known.

A posteriori dietary patterns were a 'Vegetable, fish and oil' dietary pattern, a 'Nuts, soy and high-fiber cereals' dietary pattern and a 'Margarine, snacks and sugar' dietary pattern (**Table 3.2.1**). Mean score on the *a priori* 'Dutch Healthy Diet Index' dietary pattern was 31.8 (SD 7.7), on a theoretical scale from 0 to 60. None of the mothers received the maximum score.

Cardiometabolic risk factor score

Table 3.2.3 shows the associations between the different maternal dietary patterns during pregnancy with the combined cardiometabolic risk factor score in the offspring at the age of 6 years. In the crude model, only the third quartile of the *a posteriori* 'Vegetable, fish and oil' dietary pattern was significantly associated with a lower cardiometabolic risk factor score. In the multivariable model, there were no significant associations between any of the *a posteriori* dietary patterns or the *a priori* dietary pattern during pregnancy with the cardiometabolic risk factor score in offspring at the age of 6 years.

Table 3.2.3 Associations of maternal dietary patterns during pregnancy with offspring cardiometabolic risk factor score at the age of 6 years

	Cardiometabolic risk factor score n=1,700	
	Crude	Multivariable
	'Vegetable, fish and oil' dietary pattern	
Q1 low	Reference	Reference
Q2	-0.15 (-0.28; -0.01)	-0.07 (-0.20; 0.07)
Q3	-0.21 (-0.34; -0.08)*	-0.09 (-0.22; 0.05)
Q4 high	-0.16 (-0.29; -0.03)	-0.01 (-0.14; 0.14)
Per SD	p=0.12	p=0.37
	'Nuts, soy and high-fiber cereals' dietary pattern	
Q1 low	Reference	Reference
Q2	0.11 (-0.03; 0.24)	0.17 (0.03; 0.30)
Q3	0.02 (-0.11; 0.15)	0.11 (-0.03; 0.25)
Q4 high	-0.04 (0.17; 0.09)	0.08 (-0.06; 0.23)
Per SD	p=0.21	p=0.65
	'Margarine, snacks and sugar' dietary pattern	
Q1 low	Reference	Reference
Q2	-0.08 (-0.21; 0.05)	-0.02 (-0.16; 0.12)
Q3	-0.09 (-0.22; 0.04)	-0.00 (-0.17; 0.17)
Q4 high	0.01 (-0.12; 0.14)	0.14 (-0.09; 0.37)
Per SD	p=0.91	p=0.26
	'Dutch Healthy Diet Index' dietary pattern	
Q1 low	Reference	Reference
Q2	0.03 (-0.10; 0.16)	0.06 (-0.07; 0.19)
Q3	0.00 (-0.13; 0.13)	0.07 (-0.06; 0.20)
Q4 high	0.03 (-0.10; 0.16)	0.09 (-0.04; 0.23)
Per SD	p=0.76	p=0.44

Values (regression coefficients with 95%CI) reflect differences in outcome (age- and sex-specific SD scores) for quartiles 2 to 4, as compared to the lowest quartile. Trend tests were performed by using adherence score (SD scores) as a continuous variable in the model. Multivariable model is adjusted for maternal age, gestational age at dietary assessment, smoking, alcohol and folic acid supplement use during pregnancy, educational level, family income, parity, pre-pregnancy BMI, stress during pregnancy, and child sex, breast feeding, TV watching at 2y, participation in sports at 6y and height at 6 y.
* p<0.0125

Table 3.2.4 Associations of maternal dietary patterns with offspring metabolic outcomes at the age of 6 years

	'Vegetable, fish and oil' dietary pattern					
	HDL cholesterol n=1,825		**Triglycerides** n=1,820		**Insulin** n=1,810	
	Crude	Multivariable	Crude	Multivariable	Adjusted	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.09 (-0.04; 0.22)	0.05 (-0.08; 0.18)	-0.01 (-0.15; 0.12)	0.02 (-0.12; 0.16)	0.00 (-0.13; 0.13)	-0.02 (-0.15; 0.12)
Q3	0.07 (-0.06; 0.20)	0.02 (-0.11; 0.16)	-0.10 (-0.23; 0.04)	-0.06 (-0.20; 0.08)	0.01 (-0.12; 0.14)	0.02 (-0.12; 0.16)
Q4 high	-0.00 (-0.13; 0.12)	-0.07 (-0.20; 0.07)	-0.07 (-0.20; 0.06)	-0.00 (-0.15; 0.14)	0.04 (-0.09; 0.17)	0.05 (-0.09; 0.19)
Per SD	p=0.48	p=0.10	p=0.46	p=0.74	p=0.76	p=0.65
	'Nuts, soy and high-fiber cereals' dietary pattern					
	HDL cholesterol n=1,825		**Triglycerides** n=1,820		**Insulin** n=1,810	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.12 (-0.25; 0.00)	-0.14 (-0.28; -0.01)	0.13 (-0.01; 0.26)	0.15 (0.01; 0.29)	0.17 (0.04; 0.30)	0.15 (0.01; 0.28)
Q3	-0.01 (-0.14; 0.11)	-0.04 (-0.18; 0.09)	0.08 (-0.05; 0.21)	0.10 (-0.04; 0.24)	0.10 (-0.03; 0.23)	0.08 (-0.05; 0.22)
Q4 high	-0.00 (-0.13; 0.12)	-0.01 (-0.15; 0.13)	0.08 (-0.05; 0.21)	0.12 (-0.03; 0.26)	0.08 (-0.05; 0.21)	0.07 (-0.05; 0.22)
Per SD	p=0.31	p=0.37	p=0.27	p=0.13	p=0.72	p=0.62
	'Margarine, snacks and sugar' dietary pattern					
	HDL cholesterol n=1,825		**Triglycerides** n=1,820		**Insulin** n=1,810	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.07 (-0.06; 0.19)	0.07 (-0.07; 0.21)	-0.12 (-0.25; 0.01)	-0.10 (-0.25; 0.04)	-0.02 (-0.15; 0.11)	-0.01 (-0.15; 0.13)
Q3	0.03 (-0.10; 0.15)	0.04 (-0.12; 0.21)	-0.10 (-0.24; 0.03)	-0.08 (-0.25; 0.09)	-0.05 (-0.18; 0.07)	-0.05 (-0.22; 0.12)
Q4 high	-0.11 (-0.23; 0.02)	-0.08 (-0.30; 0.14)	-0.12 (-0.25; 0.02)	-0.07 (-0.29; 0.16)	0.05 (-0.08; 0.17)	0.06 (-0.16; 0.29)
Per SD	p=0.14	p=0.91	p=0.13	p=0.77	p=0.56	p=0.64
	'Dutch Healthy Diet ndex' dietary pattern					
	HDL cholesterol n=1,825		**Triglycerides** n=1,820		**Insulin** n=1,810	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.05 (-0.08; 0.17)	0.04 (-0.08; 0.17)	0.07 (-0.06; 0.20)	0.08 (-0.06; 0.21)	0.04 (-0.09; 0.18)	0.03 (-0.10; 0.16)
Q3	-0.01 (-0.10; 0.14)	-0.02 (-0.15; 0.11)	0.10 (-0.03; 0.23)	0.11 (-0.03; 0.24)	0.09 (-0.04; 0.22)	0.09 (-0.04; 0.23)
Q4 high	-0.01 (-0.14; 0.11)	-0.02 (-0.15; 0.11)	0.05 (-0.08; 0.18)	0.05 (-0.08; 0.19)	0.09 (-0.04; 0.22)	0.08 (-0.05; 0.21)
Per SD	p=0.70	p=0.84	p=0.62	p=0.70	p=0.18	p=0.17

Values (regression coefficients with 95%CI) reflect differences in outcomes (age- and sex-specific SD scores) for quartiles 2 to 4, as compared to the lowest quartile. Trend tests were performed by using adherence score (SD scores) as a continuous variable in the model.
Multivariable model: adjusted for maternal age, gestational age at dietary assessment, smoking, alcohol and folic acid supplement use during pregnancy, educational level, family income, parity, pre-pregnancy BMI, stress during pregnancy, and child sex, breast feeding, TV watching at 2y, participation in sports at 6y and height at 6y.

Table 3.2.5 Associations of maternal dietary patterns with offspring cardiovascular outcomes at the age of 6 years

	'Vegetable, fish and oil' dietary pattern					
	Pulse wave velocity n=2,104		**Systolic BP** n=2,592		**Diastolic BP** n=2,592	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.09 (-0.21; 0.04)	-0.09 (-0.22; 0.04)	-0.06 (-0.17; 0.05)	-0.03 (-0.13; 0.08)	-0.07 (-0.18; 0.04)	-0.03 (-0.14; 0.08)
Q3	-0.10 (-0.23; 0.02)	-0.11 (-0.24; 0.02)	-0.16 (-0.27; -0.06)*	-0.09 (-0.20; 0.03)	-0.15 (-0.25; -0.04)*	-0.08 (-0.19; 0.03)
Q4 high	-0.19 (-0.31 ;-0.07)*	-0.20 (-0.33; -0.06)*	-0.09 (-0.20; 0.02)	-0.01 (-0.13; 0.10)	-0.14 (-0.24; -0.03)	-0.06 (-0.17; 0.06)
Per SD	p=0.02	p=0.03	p=0.20	p=0.79	p=0.11	p=0.98
	'Nuts, soy and high-fiber cereals' dietary pattern					
	Pulse wave velocity n=2,104		**Systolic BP** n=2,592		**Diastolic BP** n=2,592	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.03 (-0.16; 0.10)	-0.02 (-0.15; 0.12)	-0.03 (-0.14; 0.08)	-0.01 (-0.12; 0.11)	0.05 (-0.06; 0.16)	0.08 (-0.03; 0.19)
Q3	0.08 (-0.20; 0.05)	-0.06 (-0.19; 0.08)	-0.04 (-0.15; 0.07)	0.01 (-0.11; 0.12)	-0.01 (-0.12; 0.10)	0.05 (-0.06; 0.17)
Q4 high	-0.02 (-0.15; 0.11)	-0.01 (-0.15; 0.13)	-0.15 (-0.26; -0.04)*	-0.09 (-0.21; 0.02)	-0.06 (-0.16; 0.05)	0.00 (-0.12; 0.12)
Per SD	p=0.34	p=0.39	p<0.01	p=0.10	p=0.25	p=0.96
	'Margarine, snacks and sugar' dietary pattern					
	Pulse wave velocity n=2,104		**Systolic BP** n=2,592		**Diastolic BP** n=2,592	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.08 (-0.21; 0.04)	-0.10 (-0.24; 0.04)	-0.05 (-0.15; 0.06)	-0.03 (-0.15; 0.09)	-0.01 (-0.12; 0.10)	0.00 (-0.12; 0.12)
Q3	0.07 (-0.05; 0.19)	0.03 (-0.14; 0.19)	0.03 (-0.08; 0.13)	0.03 (-0.11; 0.17)	0.01 (-0.10; 0.12)	0.02 (-0.13; 0.16)
Q4 high	-0.01 (-0.13; 0.12)	-0.08 (-0.30; 0.15)	-0.00 (-0.11; 0.11)	0.01 (-0.18; 0.20)	-0.02 (-0.13; 0.09)	-0.02 (-0.21; 0.17)
Per SD	p=0.31	p=0.67	p=0.69	p=0.69	p=0.85	p=0.89
	'Dutch Healthy Diet index' dietary pattern					
	Pulse wave velocity n=2,104		**Systolic BP** n=2,592		**Diastolic BP** n=2,592	
	Crude	Multivariable	Crude	Multivariable	Crude	Multivariable
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.01 (-0.13; 0.12)	-0.01 (-0.14; 0.11)	-0.11 (-0.22; -0.01)	-0.09 (-0.20; 0.02)	-0.04 (-0.15; 0.07)	-0.02 (-0.13; 0.09)
Q3	-0.17 (-0.29; -0.05)*	-0.18 (-0.30; -0.05)*	-0.11 (-0.22; -0.00)	-0.07 (-0.18; 0.04)	-0.04 (-0.15; 0.07)	0.01 (-0.11; 0.11)
Q4 high	-0.09 (-0.21; 0.04)	-0.10 (-0.22; 0.03)	-0.05 (-0.15; 0.06)	-0.01 (-0.12; 0.10)	0.03 (-0.08; 0.13)	0.06 (-0.05; 0.17)
Per SD	p=0.02	p=0.03	p=0.40	p=0.99	p=0.74	p=0.27

Values (regression coefficients with 95%CI) reflect differences in outcomes (age- and sex-specific SD scores) for quartiles 2 to 4, as compared to the lowest quartile. Trend tests were performed by using adherence score (SD scores) as a continuous variable in the model.
Multivariable model: adjusted for maternal age, gestational age at dietary assessment, smoking, alcohol and folic acid use during pregnancy, educational level, family income, parity, pre-pregnancy BMI, stress during pregnancy, and child sex, breast feeding, TV watching at 2y , participation in sports at 6y and height at 6y. *p<0.0125

Metabolic outcomes

Table 3.2.4 shows the associations between maternal dietary patterns and offspring metabolic outcomes at the age of 6 years. There were no significant associations between any of the *a posteriori* dietary patterns or the *a priori* dietary pattern during pregnancy and child HDL-cholesterol, triglyceride levels or insulin levels at the age of 6 years.

Cardiovascular outcomes

Table 3.2.5 shows the associations of maternal dietary patterns with offspring cardiovascular outcomes at the age of 6 years. There were no significant associations between any of the *a posteriori* dietary patterns or the *a priori* dietary pattern during pregnancy and systolic or diastolic blood pressure of the child at the age of 6 years, after adjustment for confounders. Also, the 'Nuts, soy and high-fiber cereals' dietary pattern and 'Margarine, snacks and sugar' dietary pattern were not significantly associated with pulse wave velocity of the child. However, a higher adherence score on the *a posteriori* 'Vegetable, fish and oil' dietary pattern was associated with a lower pulse wave velocity of the child at the age of 6 years (SD change -0.20 (95%CI -0.33; -0.06), for the highest as compared to the lowest quartile). Also, a higher score for the modified version of the *a priori* 'Dutch Healthy Diet index' was associated with a lower pulse wave velocity of the child, but this was significant only in the third quartile.

Additional analyses

Additional analyses for the *a priori* dietary pattern including the alcohol component did not change the results (data not shown). Also, the results did not change after the exclusion of mothers with pre-pregnancy comorbidities or mothers who vomited daily or a few days per week (data not shown). Furthermore, additional adjustment for maternal pregnancy complications, total energy intake, child diet quality at age 1 year or child weight at age 6 years had no effect on the results (data not shown).

We observed no significant interactions between any of the dietary patterns and maternal pre-pregnancy BMI, child birth weight for gestational age, or child sex (p for interaction all NS).

Discussion

In a population-based prospective cohort from fetal life onwards, we observed no consistent associations of dietary patterns (*a posteriori* and *a priori*) with cardiometabolic risk factors individually or combined as a score after adjusting for potential confounders including sociodemographic and lifestyle factors. We observed a significant association between a higher adherence score on the *a posteriori* 'Vegetable, fish and oil' dietary pattern with a lower pulse wave velocity of the child at the age of 6 years, and a similar effect for a higher score on the *a priori* 'Dutch Healthy Diet index', but not with the 'Nuts, soy and high-fiber cereals' or the 'Margarine, snacks and sugar' dietary patterns.

Based on the developmental origins of health and disease hypothesis,[253] it could be expected that diet during pregnancy can have a long term effect on the offspring's cardiometabolic

profile, such as blood pressure, lipid profile and insulin sensitivity. Our results do not demonstrate this potential effect at an early age (6 years). Perhaps the effect could occur at a later age, however the age of our population does not permit us to evaluate these effects in subsequent age periods.

Previous research on the effects of maternal nutrition on offspring cardiometabolic health focused mostly on total energy intake, macronutrients, or micronutrients, and literature on overall diet is lacking. Studies on maternal diet often use birth weight as a marker of infant health.[246] However, observations from the Dutch famine have shown that malnutrition during pregnancy could affect offspring cardiometabolic health at middle-age, without influencing birth weight.[254] Furthermore, many studies used indirect measures of nutritional status, such as anthropometric measurements.[255] However, nutritional status is much more complex and much remains unknown about optimal diet during pregnancy for child health.[245] Thus, studies on the effects of overall maternal diet on cardiometabolic health in offspring are necessary.

The lack of association between maternal dietary patterns and cardiometabolic health in our study can be explained in several ways. First, the original hypothesis on the effects of maternal nutrition on child health is primarily based on studies that observed extreme malnourishment,[241,254] and many of the studies that found associations between maternal diet and infant outcomes were done in nutritionally at-risk populations.[244] Studying extremes might make it easier to detect associations, but it could also be that the associations only exist in extreme undernutrition or overnutrition. Our population has a selection towards a healthy population of pregnant women and perhaps the effect of maternal dietary patterns on offspring cardiometabolic health is just not relevant in our population. Also, the suggested relation between maternal diet and offspring health came to the attention because increased incidence of cardiometabolic diseases. Cardiometabolic diseases occur late in life, and it might be that the variation in outcome in children is too small and all within a range of healthy. While currently we found no effect, it could be that effects become visible when children are older and outcomes are more deviated towards cardiometabolic risk.

Second, if epigenetic mechanisms may influence cardiometabolic health, it may be the case that dietary effects are mainly caused by specific dietary components influencing gene expression or DNA methylation . For example, epigenetic effects have been described for methyl-donor nutrients, iron, zinc and flavonoids.[243] Although dietary pattern analyses can be useful for creating food-based dietary guidelines,[247] the downside of studying dietary patterns may be that effects of specific nutrients influencing epigenetic mechanisms may be diluted.[222] If associations between maternal diet and child cardiometabolic health are fully driven by certain nutrients, analyses using dietary patterns might not detect these effects, which might explain why we did not find clear associations.

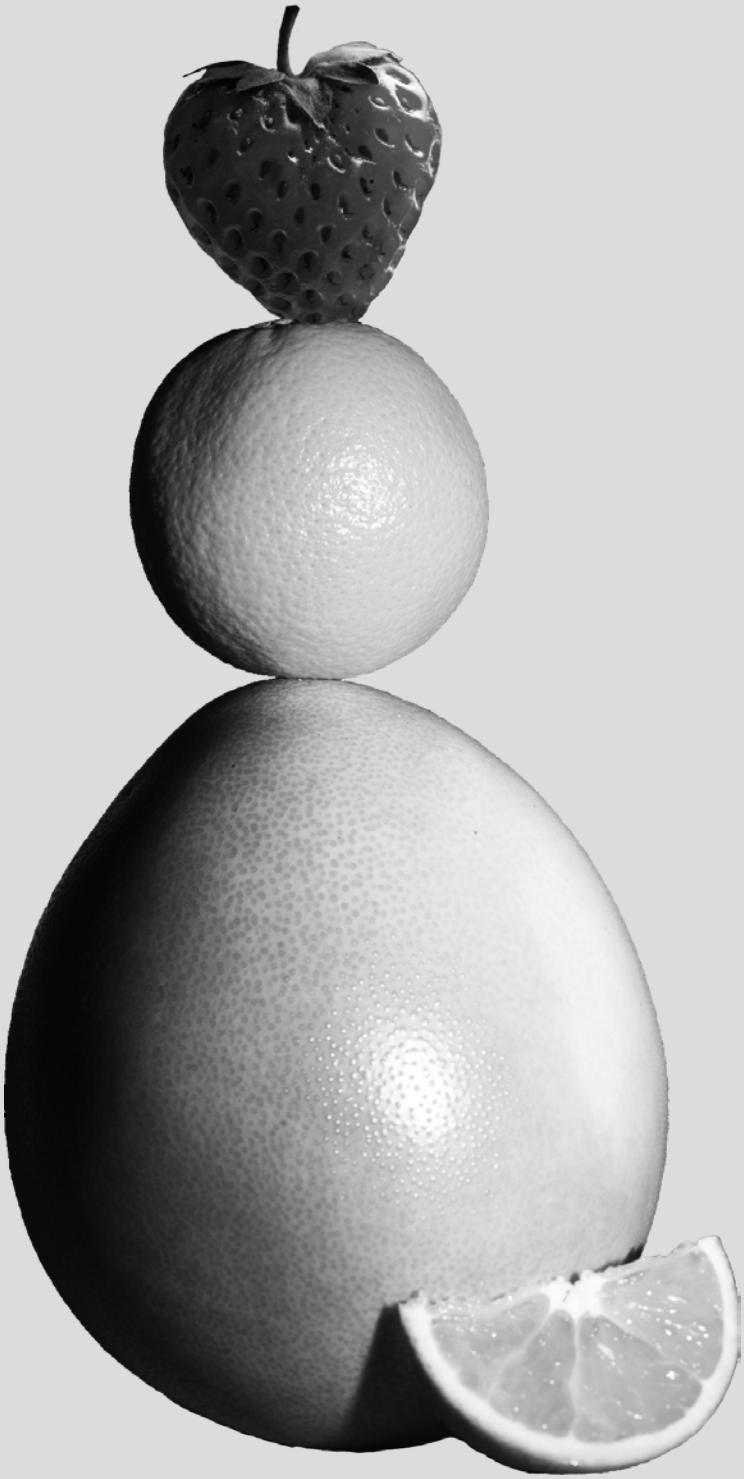
We studied both *a priori* and *a posteriori* dietary patterns during pregnancy. *A posteriori* patterns are not hypothesis-driven but data-

driven, but the hypothesis is important when defining food groups.[222] If food groups are too heterogeneous or contain food products with opposite effects on health, they may not be useful in predicting disease risk. Therefore, we created the food groups based on a possible relation with the outcome, for example we separated high-fat dairy products from low-fat dairy products, and separated cereals based on their fiber content. However, we were restricted by the design of the FFQ and were therefore for example not able to separate vegetables based on their folate content, which would have been interesting in light of the previously mentioned epigenetic programming effects.[243] *A priori* dietary patterns are hypothesis-oriented and the food groups should be correctly chosen for that purpose.[256] The Dutch Healthy Diet index was developed based on Dutch guidelines,[248] which we slightly modified based on our available data and for the use during pregnancy. Although we removed the alcohol component from the score because of known adverse effects of alcohol on the fetus, the *a priori* dietary pattern was mostly developed based on hypothesized effects for health of the mother herself, and not for fetal health. Nevertheless, to our knowledge, there are currently no dietary indexes for the use during pregnancy that are designed based on offspring health outcomes.

Dietary patterns can capture the totality of diet but also overall lifestyle, since dietary patterns cluster with other lifestyle behaviors as well.[257] Nevertheless, it is important to take into account sociodemographic and lifestyle factors that may confound the relation between maternal diet and child cardiometabolic health, including also vitamin supplementation.[256] We had detailed information on periconceptional folic acid supplement use and many other possible confounders, and we observed that adjustment for these factors had large effects on our results for blood pressure, but only a little effect on the association with pulse wave velocity. Nevertheless, a limitation may be that residual confounding might still be present due to lack of information on potential confounding factors, such as physical activity and sedentary behavior during pregnancy.

We used a self-administered FFQ to measure diet during pregnancy, which is considered an appropriate method to assess average dietary intake over an extended period of time in epidemiological studies.[258] However, as a consequence of self-reported dietary intake, measurement error might exist, which could have led to an underestimation of the true relation.[259]

In conclusion, in our population-based prospective cohort study, we examined the associations of different *a priori* and *a posteriori* dietary patterns during pregnancy with offspring cardiometabolic health. We found no independent associations between different maternal dietary patterns during pregnancy with blood lipids, blood pressure or insulin in offspring, but higher adherence to an *a posteriori* 'Vegetable, fish and oils' dietary pattern was associated with lower pulse wave velocity in the offspring at the age of 6 years. Further studies are needed to enable recommendations for maternal dietary patterns that are optimal for child cardiometabolic health.



Chapter 4

Child Nutrition and Cardiometabolic Health

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Abstract

Background Lutein is a carotenoid with strong antioxidant properties. Previous studies in adults suggest a beneficial role of lutein on cardiometabolic health. However, it is unknown whether this relation also exists in children.

Objective We examined the association between lutein intake at 1 year of age, and cardiometabolic outcomes at 6 years of age.

Methods We included 2,044 Dutch children from a population-based prospective cohort study. Diet was measured at 1 year of age with a food-frequency questionnaire. Lutein intake was standardized for total energy intake and beta-carotene intake. Blood pressure, anthropometrics, serum lipids and insulin were measured at age 6 years. Dual-energy X-ray-absorptiometry was performed to measure total and regional fat and lean mass. A continuous cardiometabolic risk factor score was created including the components body fat percentage, blood pressure, insulin, HDL-cholesterol and triglycerides.

Results Median (energy-standardized) lutein intake was 1317 mcg per day (95% range 87 – 6069 mcg/day). There were no consistent associations between lutein intake at 1 year of age and anthropometrics and body composition measures at 6 years of age. Also, lutein intake was not associated with a continuous cardiometabolic risk factor score, nor was it associated with any of the individual components of the cardiometabolic risk factor score.

Conclusion Results from this large population-based prospective cohort studie do not support the hypothesis that lutein early in life has a beneficial role for later cardiometabolic health.

Introduction

Lutein is a non-provitamin A carotenoid which is synthesized in many commonly eaten fruits and vegetable.s[260] Due to ingestion of these foods by animals, lutein is also present in animal products such as eggs and dairy. As an anti-oxidant, lutein has the potential to protect against oxidative stress by quenching lipid peroxide radicals, and could therefore protect from cardiovascular and metabolic diseases.[260]

Previous literature showed associations between higher lutein intake or blood levels and a decreased risk of metabolic syndrome.[76-80] In particular, lutein has been shown to be inversely related to waist circumference[80,108] and body mass index.[79,108] Nevertheless, reverse causation could apply since lutein is fat-soluble and adiposity thus might lead to lower levels of lutein due to lutein absorption in fat tissue. However, lutein has also been linked to adipocyte differentiation and this mechanism could suggest a causal relation in which lutein reduces abdominal adiposity.[261] Bioavailability of lutein depends on many other dietary factors, such as the presence of dietary fiber (which inhibits lutein uptake)[262] and the presence of dietary fat (which increases lutein uptake).[263]

We recently reviewed the literature about the relation between lutein and cardiometabolic health and observed that, despite these suggested positive effects of lutein in adults, studies in children are scarce (**chapter 2.1**).

To our knowledge, only one article has been published that related lutein to cardiometabolic health in children.[48] In this cross-sectional study among 1,339 U.S. adolescents, lutein levels were not significantly associated with risk of being diagnosed with metabolic syndrome, but higher lutein was significantly inversely associated with a continuous score of number of metabolic syndrome components.

It is important to study the effects of nutrition already early in life since dietary behaviors track throughout the life course,[264] and therefore early interventions can have benefits to improve health later in life. We aimed to assess the relation between lutein intake at the age of 1 year, with cardiometabolic outcomes at the age of 6 years, among Dutch children participating in a prospective population-based cohort study. A second objective was to assess whether these associations could be modified by dietary fat and fiber intake.

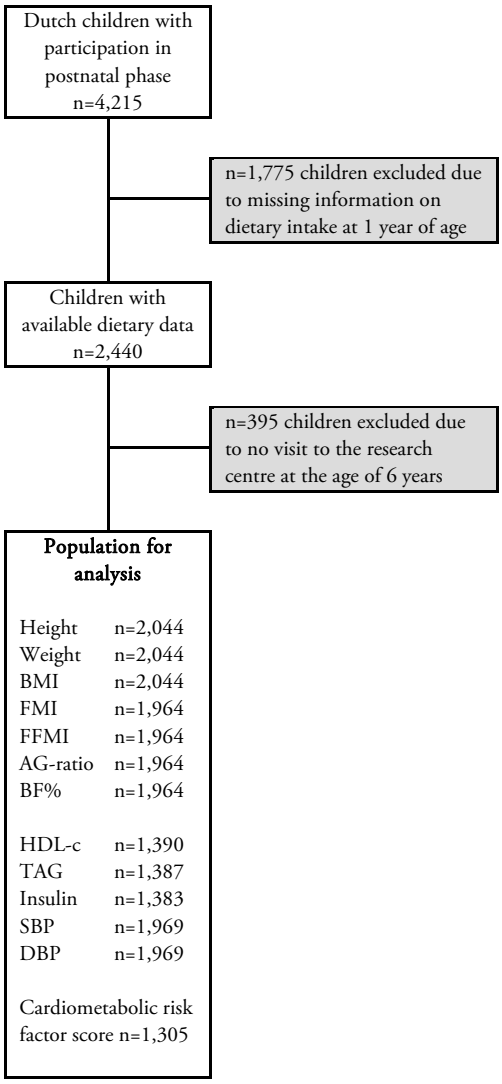
Methods

Study population

This study was embedded in The Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands, which has previously been described in detail elsewhere.[34] This study was approved by the Medical Ethical Committee at Erasmus MC, University Medical Centre, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all participating mothers. We restricted our analyses to Dutch children because the food-frequency questionnaire (FFQ) was designed and validated for dietary assessment of a Dutch population. The FFQ was implemented in a later stage of the study and was therefore available in 71% of the total population. Children without information on dietary intake (n=1,775) were excluded. Since

not all children had blood drawn, the population for analysis ranged from 1,305 to 2,044 children, depending on the outcome of interest (**Figure 4.1.1**).

Figure 4.1.1 Flow chart of study population



Dietary assessment

At the child's age of 13 months (median 12.9, 95% range 12.2 to 19.2), the primary caregiver (mother 86.2%, father 3.8%, both 9.8%, other 0.2%) completed a 211-item semi-quantitative FFQ[265]. This FFQ was validated against three 24-hour recalls in a representative sample of Dutch children (n=32), which showed the following intraclass correlation coefficients for macronutrients: total energy, 0.4; total protein, 0.7; total fat, 0.4; carbohydrates, 0.4; dietary fibre, 0.7.[252,265]

Since our objective was to assess the effects of lutein intake, we assessed lutein independent of beta-carotene. The correlation between intake of lutein and intake of beta-carotene was 0.94, thus adjusting for beta-carotene in the regression model was not possible due to multicollinearity. Therefore, we standardized lutein intake for beta-carotene using the residual method,[266] together with total energy to account for measurement error.[259] Standard deviation scores (SDS) for (beta-carotene- and energy-standardized) lutein intake were created and SDS of lutein intake were categorized into quartiles. The median absolute intake of lutein within each quartile was 264, 766, 1470 and 2274 mcg/day respectively.

The supplementary material shows the results for lutein unstandardized for beta-carotene (**Supplementary Material S4.1.1b** and **S4.1.2b**) and results for beta-carotene individually (**Supplementary Material S4.1.1C** and **S4.1.2c**).

Cardiometabolic outcomes

At a median age of 5.9 years (95% range 5.7 to 6.5), children visited our dedicated research facility at the Erasmus Medical Centre, Sophia's Children Hospital. Weight and height were measured (without shoes and heavy clothing) using an electronic scale (SECA) and stadiometer (Holtain Limited). Total and

regional fat mass was measured by Dual-energy X-ray absorptiometry (DXA) scans (iDXA; General Electric, 2008, Madison, WI, USA).[229] Percentage body fat was calculated as 100%*[total body fat mass(g)]/[total body mass (fat mass + lean mass + bone mass)(g)], android/gynoid fat mass ratio was calculated as (abdominal fat mass (g)/fat mass around hips, thighs and buttocks(g)). The fat free mass index was calculated as [lean mass (kg) + bone mass (kg)]/ [height^2 (m)]. The fat mass index was calculated as [fat mass (kg)]/ [height^2 (m)].

Non-fasting blood samples were drawn by antecubital venipuncture. Insulin, C-peptide, cholesterol (total, HDL and LDL), and triglyceride concentrations were measured with enzymatic methods (using a Cobas 8000 analyser, Roche, Almere, The Netherlands). Quality control samples demonstrated intra-assay and inter-assay coefficients of variation ranging from 0.69 to 1.57%.

Systolic and diastolic blood pressure (SBP and DBP) were measured at the right brachial artery with the child in supine position, using the validated automatic sphygmomanometer Datascope Accutor Plus TM (Paramus, NJ, USA).[267] Blood pressure was measured four times with one-minute intervals, and the first measurement was excluded for the calculation of mean SBP and DBP. Mean arterial pressure (MAP) was calculated as MAP = (mean SBP + 2*mean DBP)/3.

In addition to the individual cardiometabolic outcomes, we calculated a continuous score following examples of previously defined metabolic syndrome scores for children,[251] including body fat percentage, blood pressure (DBP and SBP), and serum levels of HDL-c, triglycerides, and insulin. The cardiometabolic risk factor score was calculated as the sum of age- and sex-specific SD scores of these five components, as proposed previously for pediatric populations.[251] Summarized, the cardiometabolic risk factor score was calculated as: SDS BF% + 0.5*SDS SBP + 0.5*SDS DBP + SDS triglycerides + (-1*SDS HDL-C) + SDS insulin, and was standardized to facilitate interpretation of effect estimates.

Covariates

Information on maternal and paternal age, household income, educational level, maternal parity, smoking, alcohol use and folic acid supplement use during pregnancy, was obtained from questionnaires during pregnancy. Maternal and paternal weight and height were measured at enrollment and BMI was calculated (kg/m^2). Information on sex, gestational age at birth and birth weight of the children was obtained from midwife and hospital registries. Information about breastfeeding, introduction of solid foods, history of cow's milk allergy and hospitalization in first year of life was derived from questionnaires at 6 and 12 months of age. TV watching was used as a proxy for sedentary lifestyle and was derived from the questionnaire at the 2 years. Participation in sports was used as a proxy for physical activity and was derived from the questionnaire at 6 years. Total energy intake, food intake and nutrient intake were derived from the FFQ at 13 months. We used a modified version of the diet quality score[252] including components for cereals, potatoes, fish, fats, sugar-containing beverages and snacks and candy.

Statistical analysis

Age- and sex specific SD scores were created for all outcomes based on the total population. Non-normally distributed variables were transformed before standardizing. Lutein was square root transformed. We used linear regression to estimate the SD change in cardiometabolic outcomes for each quartile of lutein intake, using the lowest quartile as reference category. Trend tests were performed using lutein intake (SDS) continuously.

Crude models were adjusted for child's age at the FFQ. Selection of confounders was based on literature or significant associations with exposure and/or outcome ($p<0.05$). Possible confounders were included in our final model if they changed the effect estimates of the univariate model with 5% or more on at least one outcome.[268] Analyses with android/gynoid fat ratio, body fat percentage or blood pressure as outcome were adjusted for child height (SDS) at 6 years.

We tested for interactions between lutein intake, dietary fat and dietary fiber intake since these factors may modify the relationship between lutein and cardiometabolic health. Additionally, we tested for sex interactions since there may be sex differences in factors associated with childhood obesity.[269] These interactions were tested in the multivariable model with lutein intake (SDS) continuously.

Missing data on the covariates were imputed using multiple imputation.[230] Analyses were performed in each of the 10 imputed data sets separately, and final results were pooled. A p-value below 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 21.0 (Chicago, IL, USA).

Table 4.1.1 Characteristics of the participants

	n	Median or percentage*
Children		
Sex (% girls)	2,044	50.7% (1,036)
Gestational age at birth (wk)	2,042	40.3 (35.9; 42.3)
Birth weight (gr)	2,043	3498 (566)
Introduction of solid foods	2,037	
0-3 mo		4.2% (86)
3-6 mo		55.8% (1,136)
After 6 mo		40.0% (815)
Breastfed	1,846	
Never		9.8% (181)
Partially in the first 4 mo		60.2% (1,112)
Exclusively for >4 mo		30.0% (553)
Age at FFQ (mo)	2,044	12.9 (12.2; 19.2)
Total energy intake (kcal/d)	2,044	1267 (736; 2077)
Total lutein intake (mcg/d)	2,044	1317 (87; 6069)
Age at centre visit (mo)	2,044	71 (68; 78)
BMI (kg/m^2)	2,044	15.7 (13.6; 19.0)
FMI (kg/m^2)	1,964	3.5 (2.4; 6.3)
FFMI (kg/m^2)	1,964	11.9 (10.4; 13.6)
Android/gynoid fat ratio	1,964	0.23 (0.15; 0.37)
Total body fat percentage	1,964	0.23 (0.16; 0.34)
Insulin (pmol/l)	1,383	114 (18; 392)
Total cholesterol (mmol/l)	1,386	4.2 (3.0; 5.6)
HDL-cholesterol (mmol/l)	1,389	1.3 (0.8; 2.1)
LDL-cholesterol (mmol/l)	1,386	2.3 (1.4; 3.6)
Triglycerides (mmol/l)	1,386	1.0 (0.4; 2.4)
SBP (mmHg)	1,968	101 (88; 119)
DBP (mmHg)	1,968	60 (47; 73)
Mothers		
Maternal age (y)	2,044	32.0 (4.1)
Maternal BMI (kg/m^2)	1,866	24.2 (4.0)
Educational level	2,022	
Primary or secondary		33.8% (683)
Higher		66.2% (1,339)
Net household income	1,787	
<2200 €/mo		20.3% (419)
>2200 €/mo		79.7% (1,424)
Folic acid supplement use	1,539	
Never		7.2% (111)
Started periconceptionally		63.4% (975)
Started in first 10 wks		29.4% (453)
Smoking	1,856	
Never during pregnancy		79.3% (1,472)
Until pregnancy was known		10.3% (191)
Continued during pregnancy		10.4% (193)

* Values are valid percentages or medians (95% range)

Results

Subject characteristics

Median energy intake of the children at 1 year of age was 1267 kcal/day, with a median lutein intake of 1317 mcg/day (95% range 87 to 6069 mcg/day)(**Table 4.1.1**). Main contributors to lutein intake were spinach, broccoli, Brussels sprouts, green beans, curly kale, legumes and fruits, which explained in total 67% of the variation in lutein intake.

All these products, except for curly kale, were also significantly contributing to beta-carotene intake, in addition to carrots and dairy products, and explained in total 79% of the variation in beta-carotene intake.

Body composition

There were no significant associations between lutein intake at the age of 1 year, and height, FMI, FFMI, android/gynoid fat ratio, and body fat percentage at the age of 6 years. The second quartile of lutein intake was associated with higher weight at 6 years, and the highest quartile of lutein intake was associated with higher BMI at 6 years, but for both outcomes there was no significant linear association (**Table 4.1.2**). The crude associations are shown in **Supplementary Material S.4.1.1**, and suggest that the increase in BMI was mainly driven by an increase in FFMI.

Cardiometabolic outcomes

There was no association between lutein intake at 1 year of age and the cardiometabolic risk factor score at 6 years of age (**Table 4.1.3**). The third quartile of lutein intake was associated with higher triglycerides at the age of 6 years, but there was no significant linear association. Also, there were no significant associations with any of the other components of the cardiometabolic risk factor score. In addition to the components of the risk factor score, we examined total cholesterol, LDL cholesterol, C-peptide, mean arterial pressure and pulse wave velocity, and found also no significant associations (data not shown). Crude associations of the cardiometabolic outcomes are shown in **Supplementary Material S4.1.2**.

Table 4.1.2 Associations between lutein intake at 1 year of age and body composition at 6 years of age

	Height n=2,044	Weight n=2,044	BMI n=2,044	FMI n=1,964	FFMI n=1,964	AG ratio n=1,964	Body fat% n=1,964
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.09 (-0.02; 0.20)	0.10 (0.01; 0.19)*	0.08 (-0.01; 0.17)	0.06 (-0.03; 0.14)	0.06 (-0.05; 0.17)	0.05 (-0.06; 0.16)	0.04 (-0.06; 0.14)
Q3	0.09 (-0.02; 0.20)	0.08 (-0.01; 0.17)	0.06 (-0.03; 0.15)	0.03 (-0.06; 0.11)	0.07 (-0.04; 0.17)	-0.04 (-0.14; 0.06)	0.00 (-0.09; 0.10)
Q4 high	0.04 (-0.07; 0.15)	0.09 (-0.00; 0.17)	0.10 (0.01; 0.19)*	0.03 (-0.05; 0.12)	0.09 (-0.01; 0.20)	-0.01 (-0.12; 0.09)	0.01 (-0.09; 0.10)
Per SD	0.01 (-0.03; 0.05)	0.03 (-0.01; 0.06)	0.03 (-0.00; 0.06)	0.01 (-0.02; 0.04)	0.02 (-0.02; 0.06)	-0.01 (-0.04; 0.03)	0.01 (-0.03; 0.04)

Values (regression coefficients with 95%CI) reflect the difference in outcomes (age-and sex-specific SD scores) per SD of exposure, or for quartiles 2 to 4, as compared to the lowest quartile.

Models are adjusted for maternal and paternal age and BMI, household income, maternal education, parity, smoking, alcohol and folic acid supplement use during pregnancy, and child sex ,birth weight (SDS), gestational age at birth, breastfeeding, timing of introduction of solids, hospitalizations in the first year of life, allergy to cow's milk, age at dietary assessment, total energy intake, diet quality score, TV watching at 2y and playing sports at 6y. Models for android/gynoid fat ratio and body fat percentage are additionally adjusted for child height at 6y.

*p<0.05

Table 4.1.3 Associations between lutein intake at 1 year of age and cardiometabolic outcomes at 6 years of age

	SBP n=1,969	DBP n=1,969	HDL-c n=1,390	TAG n=1,387	Insulin n=1,383	Cardiometabolic risk factor score n=1,305
Q1 low	Reference	Reference	Reference	Reference	Reference	Reference
Q2	-0.02 (-0.14; 0.11)	-0.06 (-0.18; 0.06)	-0.06 (-0.22; 0.09)	-0.07 (-0.23; 0.08)	-0.05 (-0.20; 0.10)	0.01 (-0.13; 0.16)
Q3	0.07 (-0.05; 0.20)	0.01 (-0.10; 0.13)	0.08 (-0.07; 0.23)	-0.23 (-0.37; -0.08)**	-0.10 (-0.24; 0.05)	-0.12 (-0.26; 0.02)
Q4 high	-0.01 (-0.14; 0.11)	-0.04 (-0.16; 0.08)	0.02 (-0.13; 0.18)	0.01 (-0.15; 0.16)	-0.02 (-0.17; 0.13)	-0.07 (-0.21; 0.08)
Per SD	-0.01 (-0.05; 0.03)	-0.01 (-0.05; 0.03)	0.02 (-0.04; 0.07)	-0.03 (-0.09; 0.02)	-0.01 (-0.07; 0.04)	-0.04 (-0.09; 0.01)

Values (regression coefficients with 95%CI) reflect the difference in outcomes (age- and sex-specific SD scores) per SD of exposure, or for quartiles 2 to 4, as compared to the lowest quartile.
Models are adjusted for maternal and paternal age and BMI, household income, maternal education, parity, smoking, alcohol and folic acid supplement use during pregnancy, and child sex ,birth weight (SDS), gestational age at birth, breastfeeding, timing of introduction of solids, hospitalizations in the first year of life, allergy to cow's milk, age at dietary assessment, total energy intake, diet quality score, TV watching at 2y and playing sports at 6y. Models for SBP and DBP are additionally adjusted for child height at 6y.
**p<0.01

Interactions

We found a significant interaction between lutein intake and fat intake on HDL-cholesterol, but stratification for below and above the median of total fat intake showed no significant associations.

We found a significant interaction between lutein intake and fiber intake on weight. After stratification for above and below the median of fiber intake, we observed that there was a significant positive association between lutein and weight, in the group with fiber intake below the median (increase in weight SD score 0.06 (0.02; 0.11)).

We found significant sex interactions for weight, body fat percentage and diastolic blood pressure. Stratification by sex showed that there was a significant positive association of lutein with weight in girls (0.06 SD (0.02; 0.11)), but not in boys. Stratification for sex showed no significant associations of lutein with body fat percentage or diastolic blood pressure in either boys or girls. There were no significant interactions on any of the other outcomes.

Discussion

In a population-based prospective cohort study, we assessed whether lutein intake at 1 year of age was associated with cardiometabolic outcomes at 6 years of age, and whether these associations could be modified by sex, dietary fat or dietary fiber intake. We observed that there were no consistent associations between lutein intake in toddlers and a cardiometabolic risk factor score at the age of 6 years, nor with any of the individual cardiometabolic risk factors. Also, these associations were not clearly modified by factors that may influence cardiometabolic risk or bioavailability of lutein such as sex, dietary fat and dietary fiber intake.

Our finding that lutein was not associated with cardiometabolic health is different from previous literature, though all studies were conducted in older populations. Previous studies in adults have shown that higher lutein was associated with lower risk of metabolic syndrome.[76-80] Only one of these studies assessed dietary intake[78], all other studies used

blood levels of lutein. Also, the only study in adolescents that assessed lutein in relation to cardiometabolic health used blood levels of lutein, and observed that higher lutein levels were associated with a lower metabolic syndrome score.[48]

As compared to the use of dietary intake, the use of blood levels of lutein might provide a more direct measurement of how much lutein is available. Absorption of lutein in the body depends on multiple factors, including other dietary factors such as fiber[262] and fat.[263] In our study, we observed significant interactions of lutein intake with fiber and fat intake with some outcomes, which could indeed be due to the fact that the bioavailability of lutein depends on dietary fiber and fat intake, and these factors are thus important to take into account in studies assessing dietary lutein. However, studies that assess blood levels of lutein already have a direct measure of the available lutein. Furthermore, when lutein is measured in the blood, also other factors that affect bioavailability are taken into account. This is of particular benefit because there are large inter-individual differences in the bioavailability of lutein,[131] which are only partly explained by known factors that we can adjust for (e.g. age and sex), but unmeasured factors (e.g. genetic variance) might play an equally important role.[131,270] All these factors contribute to the fact that the correlation between lutein intake and lutein levels is low,[131] which might explain why we did not observe beneficial effects of dietary intake of lutein.

However, the use of blood levels can also have challenges. As blood levels of antioxidants are being used to counteract oxidative stress, disease processes with high oxidative stress may result in depletion of lutein levels, and lower levels of lutein in metabolic disorders might thus be a result of these processes.[119] This reverse causation is of particular concern in cross-sectional studies, when there could already be an active disease process. However, also in pre-clinical stages of diseases there might already be increased oxidative stress, and thus lower levels of anti-oxidants. For example, it has been shown that subjects with obesity or metabolic syndrome have higher levels of oxidized LDL, a marker of oxidative stress, that might deplete antioxidant levels.[121] Also, it is important to address this topic in studies with sufficiently long follow-up periods. The studies assessing lutein in relation to metabolic syndrome were all cross-sectional, and thus the inverse relations that were observed could potentially be explained by reverse causation. Prospective studies in adults that investigated lutein (levels or intake) in relation to risk of type 2 diabetes found inconsistent results,[72-75] and these studies all had a follow-up of more than 10 years, and are thus less prone to risk of reverse causation.

The large sample size and the prospective longitudinal design are important strengths of our study. Also, we were able to adjust for a wide range of potential confounders such as sociodemographic and other dietary and lifestyle factors. It is important to note that all studies on lutein in relation metabolic syndrome and diabetes in adults were observational. Besides issues with reverse causation, observational studies are subject to confounding. Since nutritional and lifestyle factors are clustered,[257] and intakes of nutrients

are highly correlated, it is difficult to conclude from observational studies that any beneficial effect that is found is attributable to one specific nutrient. In the case of lutein, it could also be that not lutein is responsible for the observed effect, but another carotenoid, a group of carotenoids as a whole, or other nutrients, foods or nutritional factors that are correlated with lutein intake. Indeed we found in our study that beta-carotene and lutein were highly correlated. In addition to dietary factors, sociodemographic and lifestyle variables such as sedentary behavior and physical activity are highly related to diet, and could often not be fully adjusted for in these studies.

Another possible explanation for our negative findings, is that we were unable to detect a relevant difference in our study. It may be argued that associations between lutein and health are easier to detect in studies where extremely low lutein intake is present among participants, and that our study had negative findings due to a relatively high lutein intake in our population. Unfortunately, there are no dietary recommendations regarding lutein intake to establish whether children have sufficient intake. Also, there are no other studies on lutein intake in young children in relation to cardiometabolic health, but lutein intake in our study was comparable to a study in 6-year old Canadian children, which studied lutein in relation to cognitive function.[271]

We assessed lutein intake with the use of an FFQ, and as a result, measurement error in dietary intake may be present.[259] As recommended, we standardized lutein intake for total energy using the residual method,[266] which reduces the magnitude of systematic measurement error. However, random error may still be present, which generally leads to bias towards the null and could thus have led to an underestimation of the true effect.[272]

At last, for this analysis, we included only the subset of Dutch children who had complete nutritional data and who visited the research centre around the age of 6 years. Although this approach reduces heterogeneity of the study population and related measurement of diet, it may decrease the generalizability of our results, and studies in other populations should also evaluate this association.

In conclusion, although earlier studies in adults did show a beneficial effect of lutein on cardiometabolic health, we found no association between lutein intake in early life and cardiometabolic outcomes in children at 6 years of age. However, this was the first study in this age-group and further studies are needed to elucidate the role of lutein in young children.

Supplementary Material can be found online: http://hdl.handle.net/1765/77768

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Abstract

Background Intake of sugar-containing beverages (SCBs) has been associated with higher body mass index (BMI) in childhood. The potential effect of SCB intake during infancy is unclear

Objective We examined the association between SCB intake at 1 year of age and BMI development up to age 6 years, and body composition at the age of 6 years.

Methods This study included 2,371 Dutch children from a population-based prospective cohort. SCB intake at 1 year was assessed with a food-frequency questionnaire with validation and was standardized for total energy. BMI was calculated from repeated weight and height measurements and age-and-sex-specific standard deviation scores were calculated. Adiposity was measured using Dual-energy X-ray absorptiometry (DXA).

Results In girls, higher SCB intake at 1 year was significantly associated with higher BMI at ages 2, 3, 4 and 6 (at age 6 BMI(SDS) increase 0.11 (95%CI+0.00;0.23), high versus low intake). We observed a tendency towards higher android/gynoid fat ratio in girls with high intake (SD increase 0.14 (95%CI-0.02;0.29), versus low intake), but not with body fat percentage. In boys, there was no association with BMI or body composition, but boys with high SCB intake at 1 year were taller at age 6 years (SD increase 0.14 (95%CI+0.00;0.27), versus low intake).

Conclusions Higher SCB intake at 1 year of age was associated with higher BMI up to age 6 years in girls, but not in boys. Our results imply that the unfavorable effects of SCB intake start early in life and that dietary advice regarding limiting SCB intake should already be given early in life.

Introduction

The prevalence of childhood obesity has increased dramatically over the past decades. In 2010, it was estimated to be 11.7% in the developed countries.[12] Similarly, intake of sugar-containing beverages (SCBs) has increased and is the largest single source of energy and sugars, also in preschool children.[273, 274] A recent systematic review and meta-analysis by Malik et al.,[19] including 30 publications (observational and intervention studies), concluded that higher intake of sugar-containing beverages was associated with more weight gain, both in children and in adults.

In line with these findings, a double-blind randomized controlled trial among school children showed that intake of sugar-sweetened beverages (as compared to artificially sweetened beverages) increased body mass index (BMI), skinfold thickness, waist-to-height ratio and fat mass.[275] This study also supported the hypothesis that ingestion of calories from sugar-containing beverages is accompanied by a lower and shorter feeling of satiety as compared to calories from solid foods, leading to an increased total energy intake.[276,277]

Despite this compelling evidence in school children, adolescents and adults, the effects of sugar-containing beverages in preschool children are largely unknown. Fruit concentrates are frequently consumed in this age group, and although more nutritious than soft drinks, they contain a high amount of sugar. However, as compared with school children or adolescents, food intake of toddlers may depend more on what is offered to them by their parents. Therefore, the lower satiety of sugar-containing beverages might not directly lead to a higher total energy intake in these young children. The meta-analysis of Malik et al.[19] included only one study where SCB intake was assessed in preschool children (1,345 children aged 2 to 5 years), which did not find a significant association between sugar-containing beverage intake and weight gain after 6 to 12 months.[278] Thus the effect of sugar-containing beverages in early life might not be the same as the effect at a later age and there are no studies on sugar-containing beverage intake immediately after the weaning and lactation period.

We examined the association of sugar-containing beverage intake at 1 year of age (including fruit juices, fruit concentrates, lemonades, softdrinks and sportsdrinks) with repeatedly measured BMI from 13 months of age up to 6 years of age. Additionally, we examined the associations of early sugar-containing beverage intake with DXA-derived measures of adiposity at age 6 years (total fat percentage and android/gynoid fat ratio).

Subjects and methods

Study population

This study was embedded in The Generation R Study, a population-based birth cohort in Rotterdam, the Netherlands, which has previously been described in detail.[34] Briefly, mothers living in Rotterdam, the second largest city in the Netherlands, with a delivery date between April 2002 and January 2006 were eligible. Midwives and obstetricians informed eligible mothers about the study at their first prenatal visit in routine care. This study was

approved by the Medical Ethical Committee at Erasmus MC, University Medical Centre, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all participating mothers. We restricted our analysis to Dutch children because the food-frequency questionnaire (FFQ) was designed for dietary assessment of a Dutch population and was validated in Dutch children. Dutch is defined as two parents and four grandparents born in the Netherlands. The FFQ was implemented in a later stage and was therefore available in 71% of the total population. Children without information on SCB intake at 1 year of age (n=1,775) or BMI at any time point (n=69) were excluded. The population for analysis consisted of 2,371 children, of which DXA scans were available in 84% (n=2,001) (**Supplementary Material S4.2.1**).

Dietary assessment

At the child's age of 13 months (median 12.9, interquartile range (IQR) 1.2 months), the primary caregiver (mother 86.2%, father 3.8%, both 9.8%, other 0.2%) completed a 211-item semi-quantitative FFQ.[265] The FFQ was developed in cooperation with the division of Human Nutrition of Wageningen University, the Netherlands, and was based on an existing validated FFQ developed and described in detail previously.[279] This existing FFQ was modified by only comprising foods frequently consumed during the second year of life, according to a National Dutch food consumption survey in 941 Dutch children aged 9 to 18 months.[280] The final FFQ was validated against three days 24-hour recalls carried out by trained nutritionists in 32 Dutch children living in Rotterdam, the Netherlands, who were representative of our study population.[265] This validation study showed the following intraclass correlation coefficients for macronutrients: total energy: 0.4, total protein: 0.7, total fat: 0.4, carbohydrates: 0.4, and dietary fiber: 0.7.[265] For the current study, additional analyses were performed on this validation study to calculate an intraclass correlation coefficient for sugar-containing beverages (SCBs), which showed an intraclass correlation coefficient of 0.76 for SCB intake. Total SCB intake included intake of fruit juices, fruit concentrates, lemonades, softdrinks and sportsdrinks. As recommended by Willet et al,[266] SCB intake was standardized for energy using the residual method, which reduces the magnitude of systematic measurement error and removes extraneous variation in SCB intake.[266] However, as energy can also mediate the association between SCB intake and body composition, analyses were also performed without energy adjustment. Total energy intake included energy intake from all food items that were consumed, including formula feeding and breastfeeding. Only 6.8% of the population received any breastfeeding at the time of the FFQ. SCB intake was categorized as low, medium or high based on tertiles of the total population, and cutoffs were thus the same in boys and girls. Median (energy standardized) intake was 3.1, 8.0 and 14.9 servings/week for low, medium and high intake, respectively. One serving was considered to be 150ml.

Body composition

Well-trained staff obtained the child's height and weight during periodic visits to the

community health centres, based on the routine health-care program. We included measurements from 13 months up to 48 months of age, with a median number of 4 measurements (IQR 2) available per child. At age 6 years, all children were invited to our dedicated research facility where weight and height were measured (without shoes and heavy clothing) using an electronic scale (SECA, Almere, the Netherlands) and stadiometer (Holtain Limited, Dyfed, UK). Body fat was measured by DXA scans (iDXA; General Electric, 2008, Madison, WI, USA).[229] Percentage body fat was calculated as 100%*[total body fat mass(g)]/[total body mass (fat mass + lean mass + bone mass)(g)], android/gynoid fat mass ratio was calculated from predefined regions (enCORE2010) as [abdominal fat mass(g)/fat mass around hips, thighs and buttocks(g)] for which age- and sex-specific SD scores were calculated. For BMI, age- and sex-specific SD scores were obtained using Dutch reference growth curves (Growth Analyzer 3.0, Dutch Growth Research Foundation). Children were classified as overweight according age- and sex-specific cutoff points from the International Obesity Task Force.[228]

Covariates

Information on maternal and paternal age, maternal educational level (only primary/secondary school or higher), net household income (<2200 or >2200 euro/month), maternal smoking (never, until pregnancy was known, continued during pregnancy) and folic acid use (start periconceptional, start in the first 10 weeks of pregnancy, never) were obtained from questionnaires during pregnancy. Maternal body mass index (kg/m^2) was calculated from weight and height measured at enrollment. Maternal comorbidities included pregnancy complications (gestational diabetes, pregnancy-induced hypertension and preeclampsia) and pre-pregnancy comorbidities (hypertension, diabetes mellitus and hypercholesterolemia). Information on pregnancy complications was obtained from birth records after delivery and pre-pregnancy comorbidities were assessed by questionnaires at enrollment. Gestational age at birth, sex and birth weight of the children were obtained from midwife and hospital registries. Information about breastfeeding (never, partially in the first 4 months, exclusive for at least 4 months), timing of introduction of complementary feeding (0-3 months, 3-6 months, later than 6 months), history of cow's milk allergy (yes, no) and hospitalization in first year of life (yes, no) were derived from questionnaires at 6 and 12 months of age. TV watching (less or more than 2 hours per day) was used as a proxy for sedentary lifestyle and was derived from the questionnaire at the age of 2 years. Total energy intake and intake of sugar, confections, cakes and pastry were derived from the FFQ at 1 year. Participation in sports (yes, no) was derived from the questionnaire at 6 years of age.

Statistical methods

We used linear regression to assess the association of categories of SCB intake at 1 year (independent variable) with age- and sex-specific SD scores of BMI at ages 2, 3, 4 and 6 years, percentage fat mass and android/gynoid

fat ratio (outcome variables). Trend tests were performed using the categories as a continuous variable.

As there may be sex-related differences in factors associated with obesity,[269] we tested for a possible interaction between SCB intake and sex. Interaction was significant (p-value<0.05) in our crude model for BMI, therefore all analyses were stratified by sex.

Three multivariable models were analyzed: Model A only contained age at the measurements, model B was additionally adjusted for sociodemographic confounders and model C extends model B by also including the child's dietary and lifestyle factors. We considered model C as our main model. Selection of potential confounders was based on literature or if they changed the effect estimates of our univariate model with SCB intake and BMI with 5% or more.[268] Analyses of android/gynoid fat ratio or body fat percentage were adjusted for child's height (SDS) at age 6 years to account for child growth.

To estimate BMI (SDS) development over time, and taking into account that BMI measurements within subjects are clustered over time,[281] a linear mixed model (LMM)[282] was fitted, including all covariates (sociodemographic and lifestyle confounders) from Model C and interaction of SCB intake categories with age, with random effects for the intercept, age, age^2, age^3, and compound-symmetry correlation assumption and with all BMI measurements from 13 months onwards included in one model. The Bayesian Information Criterion and likelihood ratio tests were used to find the best model.

To reduce potential bias, missing data were multiple imputed[230] (m=5, for details and results: **Supplementary Material S4.2.1 and S4.2.2**). Analyses were performed in each of the 5 imputed data sets separately, and final results were pooled. In the LMM only observed BMI measurements were used.

As there is inconsistency throughout the literature regarding the definition of SCBs, additional analyses included investigation of different definitions of SCBs (excluding fruit juices, and including tea with added sugar). New (energy standardized) tertiles of intake were constructed for these definitions. Besides analyzing BMI, we investigated height and weight separately.

Logistic regression was used to estimate the risk of being overweight, and the risk of being in the highest 10^{th} percentile of body fat percentage and android/gynoid ratio.

Energy standardization was performed to reduce the magnitude of measurement error and to control for confounding. However, as energy is also an intermediate in the association between SCBs and body composition, additional analyses were performed without energy standardization and without adjustment for energy as confounder .

A p-value below 0.05 was considered statistically significant. P-values were rounded to two decimals. P-values below 0.01 were reported as <0.01 instead of the exact p-value. Statistical analyses were performed using the Statistical Package of Social Sciences for Windows (SPSS Inc, Chicago, IL, USA), version 21.0 and The R foundation for statistical computing, version 3.0.1. Vienna, Austria (2013-05-16).

Results

Table 4.2.1 shows the characteristics of the 2,371 children and their mothers. Mean total energy intake (kcal/day) was 1,343 in boys and 1,268 in girls. SCB intake explained 11% of the variation in total energy intake. Distribution of children across categories of intake was similar between boys and girls (boys/girls in low intake 392/394, in medium intake 393/399 and 398/395 in high intake).

Table 4.2.1 Characteristics of the children

	Boys n=1,183	Girls n=1,188
Gestational age at birth (wk)	40 (36; 42)	40 (36; 42.)
Birth weight (gr)	3557 ± 577	3427 ± 564
Energy intake (kcal/d)	1343 ± 350	1268 ± 340
Cakes & confections (gr/d)	23 (3; 83)	20 (1; 78)
SCB (servings/wk)	7 (0; 27)	7 (0; 26)
Introduction of solid foods		
0-3 mo	5.0% (59)	4.0% (47)
3-6 mo	55.1% (648)	56.3% (668)
After 6 mo	39.9% (470)	39.7% (471)
Hospitalization in the first year		
No	76.8% (739)	81.9% (775)
Yes	23.2% (223)	18.1% (171)
Breastfed		
Never	10.5% (112)	10.7% (115)
Partially in the first 4 mo	60.9% (648)	60.3% (648)
Exclusively for >4 mo	28.6% (304)	29.0% (312)
TV watching at 2y		
< 1 h/d	49.1% (541)	52.6% (587)
>1 h/d	50.9% (560)	47.4% (528)
BMI at 2y (kg/m^2)	16.7 ± 1.4	16.5 ± 1.4
BMI at 3y (kg/m^2)	16.1 ± 1.3	15.9 ± 1.3
BMI at 4y (kg/m^2)	15.8 ± 1.2	15.8 ± 1.3
BMI at 6y (kg/m^2)	15.8 ± 1.3	15.8 ± 1.4
Fat mass percentage at 6y	21.4 ± 3.8	25.8 ± 4.2
Android/gynoid fat ratio at 6y	0.24 ± 0.05	0.24 ± 0.06

Table 4.2.1 (continued) Characteristics of the mothers

	Boys n=1,183	Girls n=1,188
Maternal age (y)	32.0 ± 4.2	31.8 ± 4.1
Age of partner (y)	34.1 ± 5.1	34.1 ± 5.3
Maternal BMI (kg/m^2)	24.1 ± 4.0	24.2 ± 3.9
Folic acid supplement use		
Never	8.7% (77)	6.7% (60)
Started periconceptional	61.7% (545)	63.4% (571)
Started in first 10 wks	29.6% (262)	30.0% (270)
Educational level		
Primary or secondary	34.6% (404)	34.3% (404)
Higher	65.4% (762)	65.7% (774)
Net household income		
<2200 €/mo	21.6% (220)	19.1% (199)
>2200 €/mo	78.4% (797)	80.9% (843)
Maternal comorbidities		
Pre-pregnancy	2.4% (20)	3.3% (29)
Pregnancy-related	6.4% (67)	7.9% (83)
Smoking		
Never during pregnancy	79.4% (852)	78.8% (858)
Until pregancy was known	8.2% (88)	10.8% (128)
Continued during pregnancy	12.4% (133)	9.5% (103)

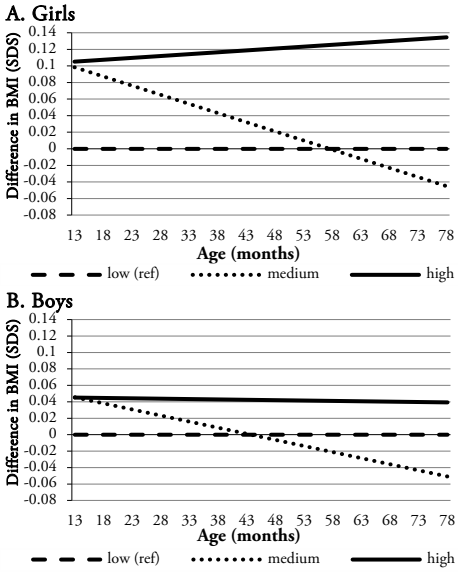
Values are valid percentages (absolute numbers), means ± SD, or medians (95% range).
Fat mass percentage and android/gynoid fat ratio were available in a subset of 2,001 children.
Number of missings for continuous variables were 3 (gestational age at birth), 3 (birth weight), 311 (BMI at age 2), 533 (BMI at 3y), 938 (BMI at 4y), 398 (BMI at 6y), 127 (age partner), 213 (BMI mother).

SCB intake and BMI

Table 4.2.2 shows that in girls, higher intake of SCBs was significantly associated with higher BMI SD scores at all ages. High intake was significantly associated with higher BMI as compared to low intake, and there was a significant linear trend over categories at all ages. In contrast, there was no association at any of the time points in boys. **Supplementary Material S4.2.3** shows absolute BMI (kg/m^2) and various adjustment levels.

Figure 4.2.1 shows the longitudinal relation between SCB intake at 1 year of age and BMI from 13 months up to the age of 6 years, as the difference in BMI (SD scores) for medium and high intake compared with low intake. Overall, there was no significant difference between trajectories of BMI in the three intake categories (p for interaction of SCB intake with age all non-significant), but the moderate intake group became closer to the reference group of low intake. Girls with high intake had a higher BMI, which remained higher up to 6 years. However, after adjustment for within-person correlation, BMI was not significantly different across intake categories (high versus low p=0.11).

Figure 4.2.1 Association between sugar-containing beverage intake at 1 year and BMI SD scores up to 6 years



The figures show the average trends of BMI (age- and sex-specific SD scores) over time as estimated by the linear mixed model. Trends are plotted for medium and high intake, as compared to low intake Adjusted for age at measurements, gestational age at birth, birth weight (SDS), age of parents, net household income, maternal BMI, education, smoking, folic acid use, pre-pregnancy and pregnancy related comorbidities, child history of hospitalization and allergy to cow's milk and child energy intake and intake of sugar, confections, cakes and pastry, breastfeeding, age at introduction of solid foods and TV watching at 2y.

SCB intake and body composition

Table 4.2.3 shows that there were no statistically significant associations of SCB intake with body fat percentage and android/gynoid fat ratio. However, we observed a borderline significant association between high SCB intake and higher android/gynoid fat ratio in girls (SD 0.14 (95%CI -0.02; 0.29) p=0.08, versus low intake). Different multivariable models are shown in **Supplementary Material S4.2.4**.

Additional analyses

Our analyses for weight and height separately showed that in girls, SCB intake tended to be associated with an increase in both weight and height (**Supplementary Material S4.2.5**). In boys, there was no association with weight (adjusted for height), but a positive association with height (p for trend <0.05). Boys with high SCB intake at 1 year of age had 0.14 SD increased height at 6 years of age (95%CI +0.00; 0.27), versus low intake.

We observed no significant associations between SCB intake at 1 year and risk of being overweight or obese at the ages 2, 3, 4 and 6 years (**Supplementary Material S2.2.6**). Also, we observed no significant associations between SCB intake at 1 year of age and risk of being in the highest 10th percentile of android/gynoid ratio or body fat percentage (data not shown).

After excluding fruit juices from total SCBs and construction of new tertiles, median (energy standardized) intake per tertile was 1.3, 6.5 and 13 servings/week. After the exclusion of fruit juices, the result for BMI at age 6 in girls was 0.11 SD (95%CI -0.01; 0.22), for highest versus lowest intake.

Table 4.2.2 Associations between sugar-containing beverage intake at 1 year and BMI at different ages (n=2,371)

	Age 2y	Age 3y	Age 4y	Age 6y
			Boys	
T1 low	Reference	Reference	Reference	Reference
T2	-0.02 (-0.15; 0.11)	-0.07 (-0.19; 0.06)	-0.04 (-0.15; 0.07)	-0.02 (-0.14; 0.10)
T3 high	-0.01 (-0.15; 0.12)	-0.01 (-0.15; 0.12)	-0.01 (-0.12; 0.09)	0.05 (-0.08; 0.18)
Trend	p=0.85	p=0.84	p=0.82	p=0.42
			Girls	
T1 low	Reference	Reference	Reference	Reference
T2	0.03 (-0.12; 0.16)	0.05 (-0.07; 0.17)	-0.01 (-0.12; 0.10)	0.01 (-0.11; 0.13)
T3 high	0.15 (0.01; 0.30)*	0.14 (0.01; 0.27)*	0.13 (0.01; 0.25)*	0.11 (0.00; 0.23)*
Trend	p=0.03	p=0.03	p=0.03	p=0.04

Values (regression coefficients with 95%CI) reflect the difference in outcome (age- and sex-specific SD scores) for tertiles 2 and 3, as compared to the lowest tertile. Trend tests were performed by using tertiles as continuous variable in the model.
Adjusted for age at FFQ, gestational age at birth, birth weight (SDS), age of parents, net household income, maternal BMI, education, smoking, folic acid use, pre-pregnancy and pregnancy related comorbidities, child history of hospitalization and allergy to cow's milk and child energy intake and intake of sugar, confections, cakes and pastry, breastfeeding, age at introduction of solid foods and TV watching at 2y. *p-value < 0.05

Only 70 boys and 63 girls consumed tea with added sugar. After including tea with added sugar and construction of new tertiles, median (energy standardized) intake per tertile was 3.2, 8.2 and 15.1 servings/week. After the inclusion of tea with added sugar, the result for BMI at age 6 years in girls was 0.11 SD (-0.00; 0.23), for highest versus lowest intake.

Additional adjustment for participation in sports at age 6 had no effect on our results (data not shown).

The results of the analyses of SCB intake without energy-adjustment are presented in **Supplementary Material S4.2.7** (for BMI) and **Supplementary Material S4.2.8** (body fat percentage and android/gynoid ratio). With absolute SCB intake and removal of energy as covariate, results for BMI at 2, 3 and 4 years were slightly attenuated, but the association with BMI at 6 years became stronger (0.14 SD (95%CI 0.02; 0.25), highest vs lowest intake in girls) (**Supplementary Material S4.2.7**).

The associations of absolute SCB intake with body fat percentage and android/gynoid ratio were also stronger, although still not significant (**Supplementary Material S4.2.8**).

Table 4.2.3 Associations between sugar-containing beverage intake at 1 year and body composition at 6 years

	Body fat percentage	Android/gynoid fat ratio
	Boys	
T1 low	Reference	Reference
T2	-0.07 (-0.22; 0.08)	0.00 (-0.16; 0.16)
T3 high	0.05 (-0.11; 0.20)	-0.02 (-0.14; 0.18)
Trend	p=0.53	p=0.77
	Girls	
T1 low	Reference	Reference
T2	-0.02 (-0.17; 0.12)	0.08 (-0.08; 0.23)
T3 high	0.09 (-0.06; 0.23)	0.14 (-0.02; 0.29)
Trend	p=0.25	p=0.09

Values (regression coefficients with 95%CI) reflect the difference in outcome (age-and sex-specific SD scores) for tertiles 2 and 3, as compared to the lowest tertile. Trend tests were performed by using tertiles as continuous variable in the model.
Adjusted for age at FFQ, height (SDS) at 6y, gestational age at birth, birth weight (SDS), age of parents, net household income, maternal BMI, maternal education, smoking, folic acid use and comorbidities, child history of hospitalization and allergy to cow's milk, energy intake, intake of sugar, confections, cakes and pastry, breastfeeding, age at introduction of solid foods and TV watching at 2y.

Discussion

In our prospective cohort study among Dutch children, we observed that SCB intake at the age of 1 year was associated with higher BMI up to the age of 6 years in girls, but not in boys. Also, girls with high intake of SCBs had a tendency towards higher android/gynoid ratio at age 6, but this was not statistically significant. Our finding that intake of SCBs in children was associated with a higher BMI confirms results from previous studies in older age groups, as shown in the systematic review by Malik et al.[19] However, our findings extend current research by demonstrating that these associations already occur during infancy. Furthermore, we observed effect modification by child sex, suggesting that the unfavorable effects of SCB intake on BMI are larger for girls than for boys. This might explain why a previous study in preschool children, in which boys and girls were combined, failed to show an association between SCB intake and BMI.[278] The effect modification by sex may be explained in several ways.

First, the satiety effect of SCBs might be different in girls and boys, which could make girls more susceptible to overeating when consuming SCBs. In an experimental study among young adults, it was observed that males had a good caloric compensation when eating a meal after drinking a SCB, whereas females had no such compensatory mechanism and therefore consumed significantly more calories in total after a SCB.[283] Another experimental study also observed poor caloric compensation after SCB intake in females, as compared to males.[284] To our knowledge, this has not yet been studied in children, but a recent study in school children has found that serum ghrelin (an appetite-stimulating hormone) was associated with adiposity in girls, but not in boys.[285] These studies highlight that it may be of importance to study sex differences in satiety responses and the relation of dietary factors with body composition.

As a second explanation, we speculate that boys better compensated their energy intake by having a higher energy expenditure, based on higher resting metabolic rate or higher activity-related energy expenditure. We partly adjusted for sedentary behavior (TV watching) and performed sensitivity analysis with additional adjustment for playing sports at 6 years, but this had no effect on the results. Although we observed no sex differences in TV watching or playing sports, energy expenditure might differ between boys and girls in our study.

Another explanation might be that girls have different growth patterns and differ in body composition already at a young age, with girls having a higher percentage body fat.[286] It could be hypothesized that there is an interaction between SCBs and body fat, and that the effects of SCBs are stronger in children who already have a higher body fat percentage. Indeed, we found that at age 6 years girls had a higher body fat percentage than boys, but further studies using detailed measurements of body fatness (for example by DXA) at an earlier age are needed to address potential interactions between SCBs and body fat and sex differences. In addition, the effect modification by sex might be a chance finding in our study, hence further replication of sex-specific effects may be warranted.

Trajectories of BMI were not different between the intake groups. Girls with high intake at age 1 year had a higher BMI, which remained higher up to 6 years, but this relationship was diluted after correction for within-person correlation, suggesting that the effect is partly explained by earlier differences in BMI. As a recent paper showed that parents may also change feeding practices in response to their child's BMI,[287] it is important to assess longitudinal relations between dietary factors and body composition measures in children, taking into account earlier BMI measures.

Although we did not observe an association between SCBs and BMI in boys, we did observe that high SCB intake was associated with greater height in boys. As taller children are more likely to become overweight adults,[288] increased height in childhood could also be considered unfavorable. Interestingly, a recent study that studied this association, showed that the height of boys aged 2 to 5 indeed predicted risk of obesity in adulthood, but the height of girls at that age did not.[289] In addition to the risk of obesity in later life, it has been observed that taller children have higher cardiovascular risk markers, independent of adiposity.[290] Therefore, more studies and longer follow-up is necessary before it can be concluded that SCB intake in early life does not have an adverse effect in boys.

The importance of calories from SCBs in relation to obesity has been well recognized,[291] and there seems to be enough evidence to conclude that reducing intake of SCBs will decrease obesity.[292] Health policies trying to reduce the burden of childhood obesity have therefore focused on limiting SCB intake, but these interventions have mostly targeted school nutrition,[293] and thus affect only school children. Currently, dietary guidelines do not contain recommendations regarding limiting SCB intake in preschool children.[294,295] Our results provide further indications that toddlers should also be targeted in prevention programs. Although parents generally consider lemonades and soft drinks unhealthy for their children, 100% fruit juices and fruit concentrates still have a healthy image and are even being used as a substitute for fresh fruit.[296] The FFQ that we used only specified fruit juices, so we could not separate fruit concentrates from non-nutritive beverages. We therefore did not know which exact types of SCBs were consumed in our population. For example, we did not know how much sugar was added to tea exactly, and we were not able to separate 100% fruit juices from fruit juices with added sugar. However, we additionally examined data from the validation study in Dutch toddlers,[265] and we observed that fruit concentrates and fruit juices were responsible for over 96% of the total SCB intake, so these could largely contribute to the observed effects of SCBs in our study.

The large sample size, prospective data collection and assessment of a wide range of sociodemographic and lifestyle factors are important strengths of our study. Also, the use of a population-based sample of young children facilitates the generalizability of our results. Furthermore, the longitudinal measurements of height and weight by professional staff and measurements of body composition with DXA scan, are strengths of our study as compared to studies using (single) parental reports of child weight status. Although loss to follow-up

usually is a potential source of bias in cohort studies, we had very limited loss to follow-up, with anthropometric measures being available for over 97% of the children.

We used an FFQ to assess dietary intake. As a result, measurement error in dietary intake may be present.[259] Nevertheless, in a validation study of the FFQ against 24-hour recalls, our FFQ showed an intraclass correlation coefficient of 0.76 for SCB intake, which is in line with previously reported results from validation studies on SCB intake in young children.[297-299] Also, SCB intake was energy-standardized which reduces the magnitude of systematic measurement errors and extraneous variation in SCB intake.[259] Nevertheless, it can be argued that total energy intake may be an intermediate in the analyses between SCB intake and body composition, since it has been shown that total energy intake mediates the effect of SCBs on body weight.[19] For that reason we performed additional analyses without energy standardization and without additional adjustment for total energy. We indeed observed in these analyses that results were slightly changed in both positive and negative directions, suggesting that both measurement error and mediation by energy intake may have existed. With the use of the residual method, systematic measurement error due to total energy has partly been removed, but random error may still be present, which might have led to an underestimation of the true effect.[272]

Because of the observational design of our study, we cannot conclude that SCB intake is causally related to higher BMI. However, we took many sociodemographic, lifestyle and nutrition factors into account. In addition, the causality of the relation between SCB intake and body weight has already been established by randomized double-blind intervention studies in school children, adolescents and adults.[19]

Given this previously reported evidence of a causal relationship between SCB intake and body weight in school children, our study suggests that the same relationship is also present in preschool children, even though they are depending more on their parents regarding their food and beverage consumption. Considering the evidence that decreasing SCB intake will reduce obesity, our study indicates that public health policies trying to limit SCB intake should also focus on children below the age of 2 years, a group which is currently not included in dietary guidelines.[294,295]

To our knowledge, this is the first study to assess the association between SCB intake before 2 years of age and body composition during childhood, with repeatedly measured BMI. We conclude that, in girls, SCB intake at 1 year of age is associated with higher BMI up to 6 years of age. A sex interaction in the association of SCBs and BMI has not been reported previously and should be replicated before final conclusions can be drawn. Nevertheless, our results highlight the importance of diet early in life and suggest that recommendations regarding limiting intake of SCBs should be made already at an early age, before consumption of SCBs starts (e.g. before weaning).

Supplementary Material can be found online: http://hdl.handle.net/1765/77768

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Abstract

Background Consumption of sugar-containing beverages (SCBs) in adults has been associated with an increased risk of metabolic syndrome. Although the effect of SCB on body weight in children is well established, little is known about the cardiometabolic effects in young children.

Objective We studied the associations between SCB intake at the age of 1 year and cardiometabolic health at the age of 6 years.

Methods This study was performed among 2,045 Dutch children from a population based prospective cohort. SCB intake was assessed with a food-frequency questionnaire at 1 year of age. Children visited the research center at the age of 6 years, and a continuous cardiometabolic risk factor score was created including: body fat percentage, blood pressure, insulin, HDL-cholesterol and triglycerides. We created age-and sex-specific standard deviation scores for all outcomes. Multivariable linear regression was performed with adjustment for sociodemographic and lifestyle variables.

Results In the total population, we observed an association between higher SCB intake at 1 year of age and a higher cardiometabolic risk factor score at the age of 6 years (0.13 SD (95%CI 0.01; 0.25), highest vs. lowest tertile). After stratification by sex, we found that boys in the highest tertile of SCB intake had a higher cardiometabolic risk factor score (0.18 SD (95%CI 0.01; 0.34)), as compared to the lowest tertile. There was no significant association in girls. We did not find associations of SCB intake with the individual cardiometabolic risk factors in the total population, or stratified by sex.

Conclusions Higher SCB intake at 1 year of age was associated with a higher cardiometabolic risk factor score at the age of 6 years in boys, but not in girls. Further research on sex-specific effects of SCBs is needed.

Introduction

Both experimental and observational studies have shown evidence of a relation between consumption of sugar-containing beverages (SCBs) and an increase in body weight in both children and adults.[19] Several studies in adults also have shown an association between consumption of SCBs and an increased risk of metabolic syndrome and type 2 diabetes mellitus.[21] Part of this association is explained by an increased risk of obesity.[19] It has been suggested that SCBs may also affect cardiometabolic health independent of weight gain.[300]

Also in school-age children, harmful effects of SCBs on cardiometabolic risk factors have been observed, such as on blood pressure, blood lipids and glucose intolerance.[95,301,302] In **chapter 4.2**, we reported that SCB intake in infancy was associated with higher BMI up to the age of 6 years in girls.[303] However, associations between SCB intake in infancy and cardiometabolic outcomes at school age have not been reported thus far. While consumption of SCBs such as carbonated soft drinks and sports drinks is low during infancy, fruit juices and fruit concentrates are frequently consumed.[280] These beverages might have a more healthy image because they are fruit-derived and contain valuable nutrients, but they also contain high amounts of sugar.[226] Although fructose and glucose are isocaloric, they have large differences in metabolism.[304] It has been hypothesized that fructose-sweetened beverages may have more detrimental effects on metabolic outcomes as compared to glucose-sweetened beverages.[305]

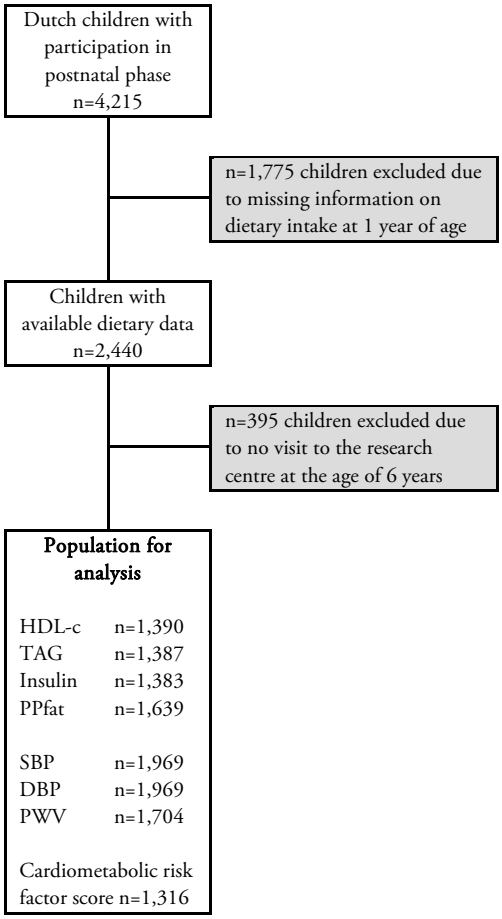
Despite these suggested adverse effects of SCBs on cardiometabolic health, there are no studies that report on associations between SCBs consumption in very young children and cardiometabolic health. Therefore, we aimed to examine the relation between SCB intake at the age of 1 year, with cardiometabolic outcomes at the age of 6 years, among Dutch children participating in a population-based prospective cohort study.

Subjects and Methods

Study population

This study was performed in children from the Generation R Study, a population-based prospective cohort from fetal life onwards, which has previously been described in detail[34]. This study was approved by the Medical Ethical Committee at Erasmus MC, University Medical Centre, Rotterdam Written informed consent was obtained from all participating mothers. We only included Dutch children for these analyses because the food-frequency questionnaire (FFQ) was designed for dietary assessment of a Dutch population and was validated in Dutch children.[265] Dutch ancestry was defined as having two parents and all grandparents born in the Netherlands. The FFQ was implemented in a later stage of the study and was therefore available in 71% of the total population. Children without information on SCB intake at 1 year of age (n=1,775) or without a visit to the research centre at age 6 years (n=395) were excluded. Because not all children had blood drawn, the population for analysis ranged from 1,305 to 1,964 children (**Figure 4.3.1**).

Figure 4.3.1 Flow chart of study population



Dietary assessment

At the child's age of 13 months (median 12.9, IQR 1.2 months), the primary caregiver (mother 86.2%, father 3.8%, both 9.8%, other 0.2%) completed a 211-item semi-quantitative FFQ.[265] This FFQ was validated against three 24-hour recalls in a representative sample of Dutch children (n=32), which showed an intraclass correlation coefficient of 0.76 for SCB intake.[10,265] Total SCBs included fruit juices, fruit concentrates, lemonades, softdrinks and sportsdrinks. One serving was considered to be 150ml. Energy adjustment was performed using the residual method to account for measurement error[266] and was standardized to the mean energy intake. We created sex-specific tertiles of SCB intake in the 2,045 children with data on SCB intake and a visit to the research centre, and the same tertiles were used for all outcomes.

Cardiometabolic outcomes

At a median age of 5.9 years (95% range 5.7 to 6.5 years) children visited our dedicated research facility at the Erasmus Medical Centre, Sophia's Children Hospital. Non-fasting blood samples were drawn by antecubital venipuncture and insulin, C-peptide, total, HDL, and LDL cholesterol (HDL-c and LDL-c), and triglyceride (TAG) concentrations were measured with enzymatic methods (using a Cobas 8000 analyser, Roche, Almere, The Netherlands). Systolic and diastolic blood pressure (SBP and DBP) were measured at the right brachial artery four times with one-minute intervals with the child lying, using the validated automatic phygmomanometer Datascope Accutor Plus TM (Paramus, NJ, USA).[267] Mean SBP and DBP were calculated using the last three measurement for all children who had a maximum of one out of these three missing. Mean arterial pressure (MAP) was calculated as MAP=(mean SBP + 2*mean DBP)/3. Total body fat was measured by Dual-energy X-ray absorptiometry (DXA) scans (iDXA; General Electric, 2008, Madison, WI, USA).[229] Percentage body fat (BF%) was calculated as 100%*[total body fat mass(g)]/[fat mass + lean mass + bone mass(g)]. Results of body fat percentage are presented in **chapter 4.2**.

In addition to the individual cardiometabolic outcomes, we calculated a continuous score following examples of previously defined metabolic syndrome scores for children,[251] including the components: BF%, blood pressure (including DBP and SBP), HDL-c, TAG, and insulin. The cardiometabolic risk factor score was calculated as the sum of age- and sex-specific SD scores of these five variables, as proposed previously for pediatric populations.[251] The SD scores for HDL-c were multiplied by -1 since a higher HDL-c represents a better cardiometabolic profile. The SD scores for SBP and DBP were multiplied by 0.5 so they each contribute half to the blood pressure component. The cardiometabolic risk factor score was thus calculated as: SDS BF% + 0.5*SDS SBP + 0.5*SDS DBP + SDS TG + (-1*SDS HDL-c) + SDS insulin.

Covariates

Information on maternal age (continuous), educational level (secondary school or lower vs. higher education), smoking during pregnancy (never smoked or quitted when pregnancy was known vs. continued during pregnancy) and folic acid supplement use (never used, started periconceptionally or started in first ten weeks) was obtained from questionnaires during pregnancy. Maternal weight and height was measured at enrollment and BMI (kg/m^2) was calculated. Information about breastfeeding duration was derived from a combination of delivery reports and questionnaires at 6 and 12 months of age. TV watching (hours/day) was used as a proxy for sedentary lifestyle and was derived from the questionnaire at age 2 years. Total energy intake (kcal/day) was derived from the FFQ. With the information of the FFQ a diet quality score (theoretical range from 0 to 10) was previously created[252] which was slightly modified by excluding the SCB component, thus the modified score theoretically ranged from 0 to 9.

Statistical methods

Age-and sex-specific SD scores were created for all outcomes based on the total Generation R population with available measurements. Insulin was not normally distributed and was therefore roottransformed before standardizing. We used linear regression models to assess the association of SCB intake and cardiometabolic outcomes. We estimated the SD difference in outcomes for the middle and highest tertile of SCB intake, as compared to the lowest tertile. Trend tests were performed using tertiles as a continuous variable.

Three multivariable models were analyzed: Model A was a crude model that only contained age at dietary assessment and total energy intake (and sex, when analyses were not stratified by sex), model B was a larger multivariable model which additionally included sociodemographic and lifestyle factors and model C was additionally adjusted for child height and weight at age 6 years, since these may be mediators in the association between SCB and cardiometabolic health.[289,303] Weight was not included in models that included a measure of adiposity as outcome. Selection of potential confounders was based on literature. Also, we included potential confounders if they changed the effect estimates of our univariate model of SCB intake with the cardiometabolic risk factor

score with 5% or more,[268] and were included in for all outcomes. Hence, the same multivariablemodels were used for all outcomes. Child Diet Quality Score did not induce a 5% change but was nonetheless included as covariate to limit residual confounding by overall diet.

We checked for an interaction with SCB intake and sex, by adding an interaction term to model B. There were no significant interaction, but since we previously found sex-differences in the associations of SCB intake and body composition,[303] all analyses were performed in the total population as well as stratified by sex.

Since there is inconsistency throughout the literature regarding SCB definition, we performed sensitivity analyses using different definitions of SCBs (excluding fruit juices, and including tea with added sugar). New energy-standardized and sex-specific tertiles of intake were constructed for these definitions. Sensitivity analyses were performed in model B stratified for sex, with cardiometabolic risk factor score as outcome. To check if certain cardiometabolic components were driving the results for the cardiometabolic risk factor score, analyses were repeated by excluding the components one by one.

To reduce potential bias, missing data on covariates were imputed using the Fully Conditional Specification method (predictive mean matching) assuming no monotone missing pattern.[230] Analyses were performed in each of the 10 imputed data sets separately, and final results were pooled. A p-value below 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 21.0.

Results

Population

Table 4.3.1 shows the characteristics of the 2,045 children and their mothers. Total energy intake (kcal/day) was 1314 in boys, and 1224 in girls. Mean (absolute) SCB intake was 7.1 servings per week in boys, and 7.0 servings per week in girls. Mothers were on average 32 years old at enrollment in the study, most of them had higher education, and most mothers used folic acid supplements periconceptionally.

Table 4.3.1 Characteristics of the participants

	Boys n=1,009	**Girls** n=1,036
Children		
Age at FFQ (mo)	12.8 (12.2; 19.4)	12.9 (12.2; 19.1)
SCB (servings/wk)	7.1 (0; 26.9)	7.0 (0; 26.0)
Energy intake (kcal/d)	1314 (755; 2098)	1224 (720; 2037)
Diet quality score	4.0 (1.9; 6.4)	3.8 (1.6; 6.4)
Breastfed (mo)	3.5 (0; 12)	3.5 (0; 12)
TV watching at 2y		
<1 h/d	50.1% (505)	52.9% (548)
>1 h/d	49.9% (504)	47.1% (488)
Age at centre visit (y)	5.9 (5.6; 6.5)	5.9 (5.6; 6.6)
Mothers		
Maternal age (y)	32 (22.8; 40.0)	32 (22.7; 39.7)
Maternal BMI (kg/m^2)	23.2 (18.3; 34.6)	23.4 (19.1; 35.2)
Educational level		
Primary or secondary	33.2% (331)	34.4% (353)
Higher	66.8% (665)	65.6% (674)
Folic acid supplement use		
Never	7.4% (56)	7.0% (55)
Started periconceptional	63.1% (475)	63.1% (231)
Started in first 10 wks	29.5% (222)	29.4% (501)
Smoking during pregnancy		
Never or quit	88.3% (802)	90.8% (862)
Continued	11.7% (106)	9.2% (87)

Values are valid percentages (absolute numbers) or medians (95% range)
Data on TV watching was missing in 55 boys and 66 girls.
Data on maternal BMI was missing in 90 boys and 88 girls.

Associations of SCB intake with the cardiometabolic risk factor score

Table 4.3.2 shows the associations of SCB intake at the age of 1 year, with the cardiometabolic risk factor score at the age of 6 years, in the total population and stratified by sex..

In the total population, higher SCB intake at 1 year of age was associated with a higher cardiometabolic risk factor score at 6 years of age.

Table 4.3.2 Associations between sugar-containing beverage intake at 1 year of age and the cardiometabolic risk factor score at 6 years of age

	Cardiometabolic risk factor score	
	Total population n=1,316	
	Model A	Model B
T1 low	Reference	Reference
T2	-0.00 (-0.13; 0.12)	-0.02 (-0.14; 0.11)
T3 high	0.16 (0.04; 0.28)*	0.13 (0.01; 0.25)*
Trend	p=0.01	p=0.04
	Boys n=681	
T1 low	Reference	Reference
T2	0.02 (-0.15; 0.19)	0.03 (-0.13; 0.20)
T3 high	0.18 (0.02; 0.35)*	0.18 (0.01; 0.34)*
Trend	p=0.03	p=0.04
	Girls n=635	
T1 low	Reference	Reference
T2	-0.03 (-0.21; 0.15)	-0.09 (-0.27; 0.10)
T3 high	0.11 (-0.06; 0.30)	0.06 (-0.12; 0.24)
Trend	p=0.20	p=0.47

Values (regression coefficients with 95%CI) reflect the difference in outcome (age- and sex-specific SD scores) for tertiles 2 and 3, as compared to the lowest tertile. Trend tests were performed by using tertiles as continuous variable in the model.
Model A is adjusted for age at FFQ and total energy intake (and child sex in analysis of the total population).
Model B additionally includes maternal age, BMI, education level, smoking during pregnancy, folic acid supplement use during pregnancy, breastfeeding, diet quality score, and TV watching at 2y.

After adjustment for sociodemographic and lifestyle factors, the highest tertile of SCB intake was associated with a 0.13 SD higher cardiometabolic risk factor score at the age of 6 years (95%CI 0.01; 0.25), as compared to the lowest quartile.

After stratification for child sex, we observed that boys in the highest tertile of SCB intake had a higher cardiometabolic risk factor score (0.18 SD (95%CI 0.01; 0.34)), as compared to boys in the lowest tertile of SCB intake, after adjustment for sociodemographic and lifestyle factors.

In girls, there was no significant association between SCB at 1 year of age and the cardiometabolic risk factor score at 6 years of age (0.06 SD (95%CI -0.12; 0.24), highest vs. lowest tertile of intake).

Additional adjustment for child height did not materially change the results (data not shown).

Associations of SCB intake with cardiovascular risk factors

Table 4.3.3 shows the associations between SCB intake at 1 year and systolic and diastolic blood pressure and pulse wave velocity at the age of 6 years, in the total population and stratified by child sex.

After adjustment, we found no significant association with any of the individual cardiovascular risk factors in the total population, or stratified by sex. However, for boys, the associations for systolic and diastolic blood pressure were in the direction of a higher cardiometabolic risk factor score.

There were no significant associations of SCB intake at 1 year with mean arterial pressure at 6 years (data not shown).

Table 4.3.3 Associations between sugar-containing beverage intake at 1 year of age and cardiovascular outcomes at 6 years of age, in the total population and stratified by sex

	Systolic blood pressure n=1,950		Diastolic blood pressure n=1,950		Pulse wave velocity n=1,704	
	Model A	Model B	Model A	Model B	Model A	Model B
Total population						
T1 low	Reference	Reference	Reference	Reference	Reference	Reference
T2	0.03 (-0.08; 0.14)	0.02 (-0.09; 0.12)	0.06 (-0.04; 0.17)	0.05 (-0.05; 0.16)	0.00 (-0.11; 0.12)	0.01 (-0.11; 0.12)
T3 high	0.05 (-0.06; 0.16)	0.02 (-0.08; 0.13)	0.11 (0.01; 0.22)*	0.09 (-0.02; 0.19)	-0.01 (-0.13; 0.10)	-0.01 (-0.13; 0.11)
Trend	p=0.34	p=0.67	p=0.03	p=0.10	p = 0.81	p=0.86
Boys						
T1 low	Reference	Reference	Reference	Reference	Reference	Reference
T2	-0.01 (-0.15; 0.14)	-0.01 (-0.16; 0.13)	0.01 (-0.14; 0.16)	0.01 (-0.14; 0.16)	0.10 (-0.08; 0.28)	0.10 (-0.08; 0.28)
T3 high	0.10 (-0.05; 0.24)	0.08 (-0.06; 0.23)	0.14 (-0.00; 0.29)	0.13 (-0.02; 0.28)	0.03 (-0.15; 0.20)	0.03 (-0.15; 0.21)
Trend	p=0.40	p=0.28	p=0.05	p=0.08	p = 0.77	p=0.76
Girls						
T1 low	Reference	Reference	Reference	Reference	Reference	Reference
T2	0.07 (-0.09; 0.23)	0.04 (-0.12; 0.20)	0.10 (-0.04; 0.25)	0.08 (-0.06; 0.23)	-0.06 (-0.21; 0.09)	-0.07 (-0.22; 0.08)
T3 high	0.00 (-0.16; 0.16)	-0.03 (-0.19; 0.13)	0.08 (-0.07; 0.22)	0.04 (-0.10; 0.18)	-0.05 (-0.20; 0.10)	-0.04 (-0.19; 0.11)
Trend	p=0.99	p=0.66	p=0.31	p=0.59	p = 0.54	p=0.60

Values (regression coefficients with 95%CI) reflect the difference in outcome (age- and sex-specific SD scores) for tertiles 2 and 3, as compared to the lowest tertile. Trend tests were performed by using tertiles as continuous variable in the model.
Model A is adjusted for age at FFQ and total energy intake (and child sex in analysis of the total population).
Model B additionally includes maternal age, BMI, education level, smoking during pregnancy, folic acid supplement use during pregnancy, breastfeeding, diet quality score and hours of TV watching at 2y.

Associations of SCB intake with metabolic risk factors

Table 4.3.4 shows the association of SCB intake at 1 year of age with metabolic outcomes at 6 years of age, in the total population and stratified by child's sex. There were no significant associations, but SD scores with children with higher SCB intake were towards higher cardiometabolic risk, in particular for blood lipids (0.12 SD higher triglycerides (95%CI -0.01; 0.34) and 0.12 SD lower HDL cholesterol (95%CI -0.25; 0.01) for highest vs lowest tertile of SCB intake).

Stratified by sex, we observed similar associations, which were strongest in boys.

There were no significant associations of SCB intake with total cholesterol, LDL cholesterol and C-peptide levels at the age of 6 years (data not shown).

Sensitivity analyses

We additionally performed sensitivity analysis with the cardiometabolic risk factor score as outcome by excluding each component of the socre one by one.

The association of highest tertile of SCB intake with higher cardiometabolic risk factor score at age 6 years in boys (0.18SD (95%CI 0.01; 0.34)), was not clearly driven by a singular component.

Table 4.3.4 Associations of sugar-containing beverage intake at 1 year of age with metabolic outcomes at 6 years of age, in the total population and stratified by sex

	HDL cholesterol n=1,390		Triglycerides n=1,387		Insulin n=1,383		Preperitoneal fat n=1,639	
	Model A	Model B	Model A	Model B	Model A	Model B	Model A	Model B
Total population								
T1 low	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
T2	-0.06 (-0.19; 0.07)	-0.07 (-0.20; 0.06)	-0.04 (-0.17; 0.09)	-0.04 (-0.17; 0.10)	0.02 (-0.11; 0.14)	0.02 (-0.11; 0.15)	-0.04 (-0.13; 0.06)	-0.05 (-0.15; 0.05)
T3 high	-0.11 (-0.24; 0.02)	-0.12 (-0.25; 0.01)	0.12 (-0.01; 0.25)	0.12 (-0.01; 0.25)	0.02 (-0.11; 0.15)	0.03 (-0.10; 0.16)	0.05 (-0.05; 0.15)	0.02 (-0.08; 0.12)
Trend	p=0.08	p=0.06	p=0.06	p=0.06	p=0.76	p=0.66	p=0.30	p=0.63
Boys								
T1 low	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
T2	-0.12 (-0.30; 0.06)	-0.13 (-0.31; 0.05)	-0.03 (-0.21; 0.15)	-0.03 (-0.21; 0.16)	0.05 (-0.12; 0.22)	0.07 (-0.10; 0.24)	-0.05 (-0.18; 0.08)	-0.06 (-0.19; 0.07)
T3 high	-0.13 (-0.31; 0.05)	-0.14 (-0.32; 0.04)	0.16 (-0.03; 0.34)	0.15 (-0.03; 0.34)	0.06 (-0.11; 0.22)	0.08 (-0.09; 0.25)	-0.04 (-0.16; 0.09)	-0.06 (-0.19; 0.08)
Trend	p=0.15	p=0.13	p=0.09	p=0.11	p=0.52	p=0.33	p=0.61	p=0.41
Girls								
T1 low	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
T2	0.03 (-0.16; 0.21)	0.02 (-0.17; 0.21)	-0.06 (-0.24; 0.12)	-0.05 (-0.24; 0.13)	-0.02 (-0.21; 0.18)	-0.04 (-0.24; 0.16)	-0.02 (-0.18; 0.09)	-0.05 (-0.18; 0.09)
T3 high	-0.08 (-0.26; 0.10)	-0.09 (-0.28; 0.10)	0.08 (-0.11; 0.26)	0.09 (-0.10; 0.27)	-0.02 (-0.21; 0.17)	-0.03 (-0.23; 0.16)	0.10 (-0.04; 0.24)	0.10 (-0.04; 0.24)
Trend	p=0.39	p=0.33	p=0.40	p=0.34	p=0.85	p=0.75	p=0.06	p=0.15

Values (regression coefficients with 95%CI) reflect the difference in outcome (age- and sex-specific SD scores) for tertiles 2 and 3, as compared to the lowest tertile. Trend tests were performed by using tertiles as continuous variable in the model.
Insulin was root-transformed before standardization.
Model A is adjusted for age at FFQ and total energy intake (and child sex in analysis of the total population).
Model B additionally includes maternal age, BMI, education level, smoking during pregnancy, folic acid supplement use during pregnancy, breastfeeding, diet quality score and TV watching at 2y.

The association attenuated mostly after excluding HDL-c (0.14 SD (95%CI -0.02; 0.29)) or triglycerides (0.14 SD (95%CI -0.02; 0.29), and hardly after excluding blood pressure (0.16 SD (95%CI -0.05; 0.32)) or insulin (0.17 SD (95%CI 0.00; 0.33)). After excluding BF%, the association became slightly stronger (0.21 SD (95%CI 0.03; 0.39)).

Sensitivity analyses with different definitions of SCBs showed that the association remained similar after including tea with added sugar (0.18 SD (95%CI -0.02; 0.35)), but largely attenuated after excluding fruit juices (0.06 SD (95%CI -0.10; 0.22)).

Discussion

In this population-based prospective cohort study, we observed that higher SCB intake at 1 year of age was associated with a higher cardiometabolic risk factor score at 6 years of age. We only observed this association in boys, and not in girls, although there was no significant statistical interaction. There were no clear associations with any of the individual cardiovascular or metabolic risk factors.

Our finding that higher SCB intake was associated with a higher cardiometabolic risk factor score is in line with studies in adults,

which reported a higher risk of metabolic syndrome and type 2 diabetes mellitus in relation to SCB consumption.[21] However, the observation that additional adjustment for child weight did not affect the associations for metabolic and cardiovascular risk factors is not in line with a study in adults, which suggested that approximately half of the effect of SCBs on type 2 diabetes was mediated through obesity.[306] This study was performed in women only, and in our population, we found no association of SCB intake with cardiometabolic health in girls, despite the fact that they had an increased BMI (**chapter 4.2**).[303] In contrast, we previously found that boys with high intake did not have a higher BMI,[303] but we observed in the current study a significant associations towards a higher cardiometabolic risk factor score.

Despite the large amount of research interest on the effects of SCBs on cardiometabolic health,[20,300] there are no studies that assessed the effects of SCB intake below the age of 2 years. One study examined the relation between SCBs and cardiometabolic health in preschool children,[302] and this small cross-sectional study observed among 467 children 3 to 5 years of age, that that higher SCB intake was associated with higher LDL cholesterol. Interestingly, the association of SCB intake with BMI and waist circumference in this previous study was not present in these children aged 3 to 5 years, but was found only in children aged 9 to 11 years.[302] This gives additional indications that SCB intake may affect metabolic outcomes, without affecting body weight. Unfortunately, this study did not report results stratified by sex in the age-group 3 to 5 years, and to our knowledge, there are no other studies performed on the relation between between SCBs and cardiometabolic outcomes in preschool children.

Previous studies have found sex differences in the associations of obesity with blood lipids and insulin resistance,[269,307] which may be related to difference in health behaviors.[269] We previously speculated that the observed differences between boys and girls in the relation of SCBs with BMI might be caused by a lower level of satiety in girls as compared to boys, which was observed in experimental studies in adults.[283,284] Previous studies also have shown sex differences in development of components of metaboli syndrome and age at onset,[308-310] and there might be biological differences, already in children.[307] However, it has been proposed that the vast majority of sex differences in health outcomes are due to social and cultural differences[311]. For example, a review on different aspects of parenting in relation to child overweight showed differences in parenting depending on child sex.[312]

If a sex interaction truly exists, either biological or social, this could be of importance for future public health programs but further studies are needed to elucidate any sex-specific effects. A limitation of our study is that we have only measured cardiometabolic health at one time point. Future studies with repeated measurements of cardiometabolic health are important to clarify any sex differences in the long run.

In sensitivity analyses, we showed that the results attenuated after the exclusion of fruit juices, which suggest that fruit juices contribute to a large extent to the observed associations.

Indeed, experimental research in adults showed that fructose-sweetened beverages increased visceral adiposity, insulin resistance, and hyperlipidemia, while glucose-sweetened beverages did not.[305] In addition, a large observational study with 71,346 participants showed that higher consumption of fruit juices was associated with a higher risk of type 2 diabetes mellitus (HR 1.18 (95%CI 1.10; 1.26), per daily serving).[313] However, it has also been suggested that only fruit juices with added sugar increase the risk of type 2 diabetes mellitus, and 100% fruit juices do not.[314] Our FFQ did not separate 100% fruit juices from fruit juices with added sugar. Hence, we could not study the role of these different types of beverages.

To reduce the magnitude of potential systematic measurement error, we adjusted SCB intake for total energy using the residual method,[266] and our FFQ showed good validation for SCB intake against 24-hour recalls (intraclass correlation coefficient of 0.76).[303] Nevertheless, random error might still be present which may have led to an underestimation of the true effect.[272]

We performed detailed measures of cardiometabolic health, and combined the individual risk factors into a continuous cardiometabolic risk factor score. The benefits of a continuous score as compared to dichotomous metabolic syndrome definitions are that the continuous score is less prone to to erros and more sensitive to pick up differences, since more information is being used.[251]

Although causality remains unclear, the available evidence from both observational and experimental studies suggest that SCBs can affect health outcomes of both children and adults unfavorably, and initiatives targeting SCB intake reduction should be explored.[292]

In conclusion, we observed that higher intake of SCBs at 1 year of age is associated with a higher cardiometabolic risk factor score in school-aged boys. We extend the current literature by showing for the first time that an association between SCBs and cardiometabolic health already exists for consumption of SCBs before the age of 2 years. Future public health policies should target a reduction in SCB intake also in these very young children.

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Abstract

Background Dietary patterns are linked to obesity in adults, however, the relation between overall diet and body composition in childhood is unclear.

Objective We examined associations between different dietary patterns at the age of 1 year and body composition at the age of 6 years.

Methods This study was peformed in 2,026 Dutch children from a population-based cohort. Food intake at 1 year was assessed with a food-frequency questionnaire. At 6 years, we measured anthropometrics and body fat mass (with dual-energy X-ray absorptiometry) and calculated body mass index (BMI), fat mass index (FMI), and fat-free mass index (FFMI). Three dietary pattern approaches were used: 1)A priori-defined diet quality based on a dietary-guideline driven diet score. 2)'Health-conscious' and 'Western' patterns derived from principal component analysis (PCA) based on variation in food intake. 3)Two patterns derived with reduced-rank regression (RRR) based on variations in FMI and FFMI.

Results Children in the highest quartile of the a priori-defined diet score or the 'Health-conscious' pattern at 1 year had a higher FFMI at 6 years (0.19 SD (95%CI 0.08; 0.30) per SD increase in diet score, versus lowest quartile), but no difference in FMI. The PCA-derived 'Western' pattern was not associated with body composition. The first RRR-derived pattern was characterized by high intake of meat, fish, sauces, and sugar-containing beverages and remained positively associated with both FMI and FFMI after adjustment for confounders. The second RRR-pattern was characterized by a high intake of whole grains, pasta and rice, and vegetable oils and remained positively associated with FFMI, but was no longer significantly associated with FMI.

Conclusions Our results suggest that patterns characterized by high intakes of vegetables, grains, and vegetable oils in early childhood are beneficial for later body composition, while patterns charachterized by high intake of refined grains, meat and sugar-containing beverages may be unfavorable.

Introduction

Childhood adiposity is of great concern because of its adverse consequences for both short and long term health.[214] Diet in early childhood may be an important target for prevention of childhood obesity, but nevertheless, there are not many studies that examined overall diet of preschool children in relation to later body composition.[315,316] Analysis of dietary patterns can be used to characterize overall food intake and has emerged as a complementary approach for studying diet in addition to examining intakes of individual nutrients or foods.[222,246] Three main approaches have been used to identify dietary patterns. Dietary patterns can be defined *a priori*, for instance on the basis of existing dietary guidelines; they can be defined *a posteriori*, using information on dietary intake of the population, or they can be derived *a posteriori* based on the variation in specific markers related to health.[317]

Previous studies in adults have shown that high adherence to *a posteriori*-derived 'Western' or or 'Empty calorie' dietary patterns is associated with higher risk of obesity, while high adherence to so-called 'Prudent' or 'Heart Healthy' dietary patterns.[318-320] Likewise, higher scores on *a priori*-defined diet quality indices, such as the Mediterranean diet score, the Healthy Eating Index (HEI), or the Diet Quality Index (DQI), have been associated with a lower prevalence of obesity and less weight gain.[321-324] Studies in children also reported associations between higher scores on an *a posteriori* 'Snacking' dietary pattern, or on a pattern characterized by high fat and low fiber intake and a higher risk for obesity,[325 326] and similarly, between higher *a priori*-defined diet quality scores and a lower risk for obesity.[327,328] However, studies on overall diet in young children shortly beyond the weaning period in relation to later obesity are scarce[315].

Therefore, we explored associations between dietary patterns in children at the age of 1 year and body mass index (BMI), fat mass index (FMI), and fat-free mass index (FFMI) at the age of 6 years.

We applied three different approaches for dietary patterns: 1) an *a priori*-defined diet quality score[252] based on dietary guidelines for preschool children; 2) *a posteriori*-derived dietary patterns based on variations in food intake,[265] extracted using principal component analysis; and 3) *a posteriori*-derived patterns based on variations in body composition outcomes, identified using reduced-rank regression.

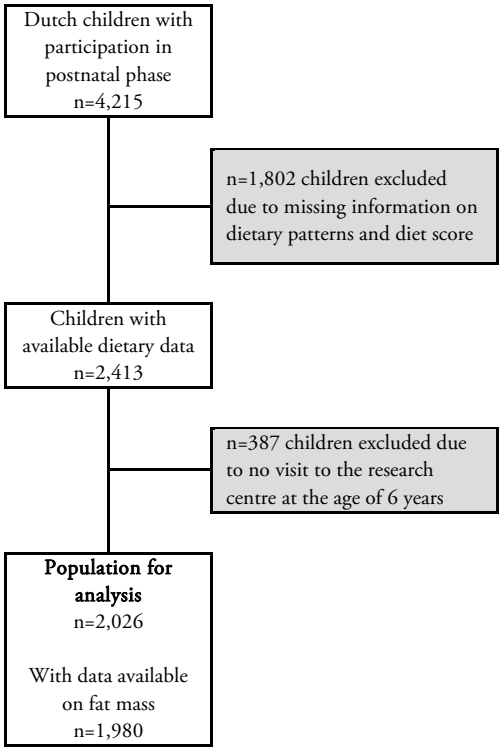
Methods

Subjects

This study was embedded in the Generation R Study, a population-based cohort study from fetal life onward in Rotterdam, the Netherlands.[34] The study was conducted according to the guidelines of the Helsinki Declaration and approved by the Medical Ethics Committee of Erasmus Medical Centre, Rotterdam. All parents provided written informed consent. To avoid the influence of cultural differences in dietary patterns, our analyses were restricted to children with a Dutch ethnicity.[329] A total of 4,215 Dutch children participated in the preschool follow-

up.[34] Data on dietary patterns were available in 2,413 of these children. At the age of 6 years, 2,026 (84%) of these children visited the research centre and had anthropometrics measured and body fat measures were available in 1,980 of them (**Figure 4.4.1**).

Figure 4.4.1 Flow chart of study population



Dietary patterns

Dietary intake was assessed at a median age of 12.9 months (95% range 12.2 to 19.2) using a 211-item semi-quantitative food-frequency questionnaire (FFQ), which included foods that are frequently consumed by Dutch 9 to 18 months old children.[265] The FFQ was evaluated against three 24-hour recalls in a representative sample of 32 Dutch children.[265] Interclass correlation coefficients for nutrient intakes ranged from 0.4 to 0.7.[252, 265] An *a priori* dietary pattern was defined using a previously developed diet quality score for preschool children.[252] This score was developed using international dietary guidelines as a basis and includes intake of the following ten food groups: high intake of vegetables; fruit; bread and cereals; rice, pasta, potatoes, and legumes; dairy; meat, poultry, eggs and meat substitutes; fish; and fats and oils; and low intake of candy and snacks; and sugar-sweetened beverages (**Table 4.4.1 and Supplementary Material S4.4.1**). The score ranges from 0 to 10 on a continuous scale, with a higher score representing a healthier diet. The diet score was standardized to a recommended energy intake for 1 to 3-year old children of 1200 kcal/d.[252]

A posteriori-derived dietary patterns on the basis of variation in food group intake were extracted using principal component analysis (PCA), that explained the maximum variation in the intake of 21 food groups.[265] Two major dietary patterns were previously extracted in our population: a 'Health-conscious' dietary pattern, characterized by high intakes of fruits, vegetables, oils, legumes, pasta, and fish; and a 'Western-like' dietary pattern characterized by high intakes of snacks, animal fats, refined grains, confections and sugar-containing beverages. Together, these two PCA-derived patterns explained 24.5% of the total variance in food intake (**Table 4.4.1**).

A posteriori dietary patterns on the basis of variation in FMI and FFMI were identified using reduced-rank regression (RRR), in order to identify patterns that best predict child body composition.[330] For this method, we used age- and sex-adjusted FMI and FFMI as response variables and the 21 food groups as predictor

variables. Two dietary patterns were extracted that explained the maximal variance in FMI and FFMI: the first RRR pattern was positively correlated with both FMI and FFMI, whereas the second was positively correlated with FFMI, but inversely with FMI. The two RRR-derived patterns together explained 2.6 % of the variation in FMI and FFMI (**Table 4.4.1**).

Body composition

Children's body composition was measured by well-trained staff at a median age of 5.9 years (95% range 5.7 to 6.5) in our research centre in the Sophia Children's Hospital, Rotterdam. Height was determined in standing position to the nearest millimeter without shoes with a Harpenden stadiometer (Holtain Limited, Dyfed, U.K.). Weight was measured using a mechanical personal scale (SECA, Almere, the Netherlands) and body mass index (BMI) was calculated (body weight (kg)/height (m)^2).

Total body, android, and gynoid fat mass were measured using a Dual-energy X-ray absorptiometry (DXA) scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA), which analyzed fat, lean and bone mass of the total body and specific regions using enCORE software v.13.6.[331]

Table 4.4.1 Food groups included in the dietary patterns

	Positive (+) or negative (-) scoring	Factor loadings from PCA[2,3] (after varimax rotation)		Factor loadings from RRR[3]	
	Diet Quality Score	'Health-conscious' pattern	'Western-like' pattern	RRR pattern 1	RRR pattern 2
Refined cereals	Not included		0.57	0.22	
Whole cereals	+				0.41
Pasta and rice	+	0.62			0.46
Dairy	+				0.27
Fruit	+	0.32			0.28
Soya substitutes	Not included				
Vegetables	+	0.74		0.40	0.38
Potatoes	+	0.61		0.34	
Soups and sauces	Not included		0.23	0.44	
Savory snacks	-		0.59		
Confections	-		0.72		
Vegetable oils	+	0.50			0.37
Other fats	+		0.58		0.20
Fish	+	0.22		0.42	
Shellfish	+				
Meat	+	0.21	0.27	0.30	
Eggs	+				
Legumes	+	0.59			
Sugar-containing beverages	-		0.59	0.30	
Non-sugary beverages	Not included				0.28
Composite dishes	Not included				
Explained variation (%) in food group intake		16.3	8.2	5.7	12.5
Explained variation (%) in FMI and FFMI		0.4	0.1	1.8	0.8

[1]Further details in Supplementary Material, reprinted from Voortman et al., 2015
[2]Reprinted from Kiefte-de Jong et al., 2013
[3]Only factor loadings ≥|0.2| are reported

We calculated fat mass index (FMI) (fat mass (kg)/height $(m)^2$) and fat-free mass index (FFMI) (fat-free mass (kg)/height $(m)^2$).[332] Secondary outcome measures included android/gynoid fat ratio (android fat mass divided by gynoid fat mass); and body fat percentage (BF%), (fat mass as percentage of total body weight). We calculated age- and sex-specific SD scores for all outcomes based on the total Generation R study population.

Covariates

We selected covariates as potential confounders based on a previously described association with one of the dietary patterns.[252,265] Information on maternal age, parity, folic acid supplement use, paternal education, paternal smoking and household income were obtained using questionnaires at enrollment in the study.[34] Education level and household income were categorized into three groups according to Dutch standard classifications.[333,334] Maternal smoking and alcohol use during pregnancy were assessed using questionnaires in each trimester and was categorized into never; until pregnancy was known; or continued during pregnancy.[335] Maternal anthropometrics were measured at enrollment at the research centre, without shoes and heavy clothing.[34]

Information on breastfeeding was obtained from delivery reports and postnatal questionnaires and was categorized as never breastfeeding; any partial breastfeeding in the first 4 months of life and; full breastfeeding in the first 4 months of life.[265] Timing of introduction of solid foods in the first year of life was assessed using the FFQ at 1 year and categorized into three groups: <3 months, 3-6 months, or ≥6 months.[336] Child's height and weight around 1 year of age were measured at the Community Child Health Centres and BMI SD scores were calculated using Dutch reference curves.[337] Information about TV watching at the age of 2 years (h/d), was used as a proxy for sedentary behavior; and about participation in sports (yes, no) at the age of 6 years, as an indicator of physical activity, were assessed using questionnaires.

Statistical analyses

The dietary pattern scores were analyzed as continuous variables and categorized into quartiles with the first quartile as reference. All dietary patterns were expressed in SD scores to facilitate comparability of the results. A higher SD score represents a higher adherence to the dietary pattern. All body composition outcomes were expressed as age- and sex-specific SD scores and analyzed continuously.

We used multivariable linear regression to assess the associations of the dietary patterns with body composition measures. Crude models were adjusted for child sex, age at dietary assessment, and total energy intake. The multivariable models were further adjusted for maternal age, BMI at enrollment, parity, folic acid supplement use, smoking and alcohol use during pregnancy; paternal smoking and education; household income; and child breastfeeding in the first four months of life, timing of introduction of solid foods, and TV watching at the age of 2 years.

To assess whether the associations were different by sex, BMI (SDS) at 1 year, sedentary behavior at 2 years, or participation in sports at 6 years we evaluated the statistical

interaction by adding the product term of the covariate and the dietary patterns to the multivariable models. To reduce potential bias associated with missing data, missing values of covariates were multiple imputed (n=10 imputations) using the Fully Conditional Specification method (predictive mean matching), assuming no monotone missing pattern.[230, 240] Analyses were performed in each of 10 imputed datasets separately and results were pooled. We performed sensitivity analyses in which we excluded children who still received breast milk or a substantial amount of infant formula (i.e., more than 500 kcal/d, n=386) at the time of the FFQ. Also, we additionally adjusted for playing sports at 6 years. Finally, we adjusted the multivariable models for BMI (SDS) at 1 year to assess whether dietary patterns at 1 year of age were associated with body composition at 6 years of age independent of baseline BMI. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Subject characteristics

Characteristics of the children and their parents are presented in **Table 4.4.2.** Most of the mothers were nulliparous (63.0%) and did not smoke during pregnancy (79.5%); and most of the children received exclusive (30.2%) or partial (60.1%) breastfeeding in their first four months of life. Mean (±SD) diet quality score at the age of 1 year was 4.2 (± 1.3). At the age of 6 years, median body fat percentage was 23.1% and median BMI was 15.7 kg/m^2.

Table 4.4.2 Characteristics of the participants

	n	Median or percentage*
Parents		
Maternal age (y)	2,026	32.3 (22.9; 39.9)
Maternal BMI (kg/m^2)	1,849	23.3 (18.9; 34.8)
Parity		
0	1,242	63.0%
1	557	28.2%
≥ 2	173	8.8%
Folic acid supplement use		
Started periconceptional	970	63.5%
Started in first 10 wks	448	29.3%
Never	110	7.2%
Alcohol		
Never during pregnancy	517	30.8%
Until pregnancy was known	278	16.6%
Continued during pregnancy	881	52.6%
Smoking		
Never during pregnancy	1,466	79.5%
Until pregnancy was known	186	10.1%
Continued during pregnancy	193	10.5%
Paternal smoking		
No	947	61.7%
Yes	603	38.3%
Paternal education		
Primary or secondary	542	29.0%
Higher	1,328	71.0%
Net household income		
<2200 €/mo	359	20.3%
≥2200 €/mo	1,413	79.7%
Children		
Sex		
Boys	1,002	49.5%
Girls	1,024	50.5%
Breastfeeding		
Exclusively for ≥ 4 mo	552	30.2%
Partially in the first 4 mo	1,101	60.2%
Never	176	9.6%
Introduction solid foods		
After 6 mo	800	39.6%
3-6 mo	1,136	56.3%
0-3 mo	83	4.1%
TV watching at 2y (h/d)	1915	0.9 (0.0; 2.0)
Child age at FFQ (mo)	2,026	12.9 (12.2; 19.2)
Total energy intake (kcal/d)	2,026	1267 (737; 2080)
Child age at centre visit (y)	2,026	5.9 (5.7; 6.5)

* Values are valid percentages or medians (95% range).

Dietary patterns and body composition

In multivariable adjusted models, a higher adherence to the PCA-derived 'Health-conscious' dietary pattern or a higher diet quality score at the age of 1 year was associated with a higher BMI and FFMI at the age of 6 years (**Table 4.2.3**). These patterns were not associated with FMI (**Table 4.4.3**), with BF%, or with android/gynoid ratio (**Supplementary Material S4.4.2**). Adherence to the PCA-derived 'Western' dietary pattern at the age of 1 year was not consistently associated with body composition at the age of 6 years.

The first RRR-derived pattern, which was positively correlated with FMI and FFMI, was characterized by high intakes of refined grains, meat, potatoes, fish, soups and sauces, and sugar-containing beverages (**Table 4.4.1**). After adjustment for confounders, a higher adherence to this patterns remained positively associated with both FMI and FFMI (**Table 4.4.3**) and was also associated with a higher BF% and a higher android/gynoid ratio (**Supplementary Material S4.4.2**). The second RRR-pattern, which was positively correlated with FFMI and inversely correlated with FMI, was characterized by high intakes of whole grains, pasta and rice, dairy, fruit, vegetable oils and fats, and non-sugar-containing beverages (**Table 4.4.1**). After adjustment for confounders, a higher score on this pattern remained positively associated with FFMI, but was no longer associated with FMI (**Table 4.4.3**). However, this second RRR-pattern remained associated with a lower BF% and a lower android/gynoid ratio in the multivariable model (**Supplementary Material S4.4.2**).

Table 4.4.3 Associations between dietary patterns at 1 year of age and body composition at 6 years of age

	BMI n=2,026	FMI n=1,980	FFMI n=1,980
Diet Quality Score			
Q1 low	Reference	Reference	Reference
Q2	0.07 (-0.02; 0.16)	0.03 (-0.06; 0.11)	0.09 (-0.02; 0.20)
Q3	0.07 (-0.02; 0.16)	-0.01 (-0.09; 0.08)	0.14 (0.02; 0.25)*
Q4 high	0.18 (0.08; 0.27)**	0.07 (-0.01; 0.16)	0.19 (0.08; 0.30)**
Per SD	0.06 (0.02; 0.09)**	0.02 (-0.01; 0.05)	0.06 (0.02; 0.10)**
PCA Health-conscious pattern			
Q1 low	Reference	Reference	Reference
Q2	0.03 (-0.07; 0.12)	0.02 (-0.07; 0.10)	0.02 (-0.09; 0.13)
Q3	0.10 (0.01; 0.19)*	0.03 (-0.05; 0.12)	0.13 (0.02; 0.24)*
Q4 high	0.14 (0.05; 0.24)**	0.04 (-0.05; 0.13)	0.17 (0.06; 0.29)**
Per SD	0.04 (0.01; 0.08)*	0.01 (-0.03; 0.04)	0.05 (0.01; 0.09)*
PCA Western pattern			
Q1 low	Reference	Reference	Reference
Q2	0.02 (-0.07; 0.11)	0.02 (-0.06; 0.11)	-0.01 (-0.12; 0.10)
Q3	0.12 (0.02; 0.21)*	0.06 (-0.03; 0.15)	0.15 (0.04; 0.27)**
Q4 high	0.04 (-0.07; 0.14)	-0.01 (-0.11; 0.09)	0.09 (-0.04; 0.22)
Per SD	0.00 (-0.04; 0.05)	-0.01 (-0.05; 0.03)	0.02 (-0.04; 0.07)
RRR pattern 1			
Q1 low	Reference	Reference	Reference
Q2	0.12 (0.03; 0.21)**	0.10 (0.01; 0.18)*	0.08 (-0.03; 0.19)
Q3	0.12 (0.03; 0.21)**	0.09 (0.01; 0.18)*	0.07 (-0.04; 0.18)
Q4 high	0.25 (0.16; 0.35)**	0.18 (0.10; 0.27)**	0.23 (0.11; 0.35)**
Per SD	0.11 (0.07; 0.15)**	0.10 (0.06; 0.13)**	0.09 (0.04; 0.14)**
RRR pattern 2			
Q1 low	Reference	Reference	Reference
Q2	-0.06 (-0.15; 0.03)	-0.06 (-0.15; 0.02)	-0.02 (-0.13; 0.10)
Q3	0.07 (-0.03; 0.16)	-0.01 (-0.10; 0.08)	0.18 (0.06; 0.29)**
Q4 high	0.06 (-0.04; 0.17)	-0.07 (-0.17; 0.03)	0.19 (0.06; 0.32)**
Per SD	0.02 (-0.02; 0.06)	-0.03 (-0.07; 0.00)	0.07 (0.02; 0.11)**

Values (regression coefficients with 95%CI) reflect the difference in outcome (SDS) per SD of exposure, or for quartiles 2 to 4, versus lowest quartile. Adjusted for maternal age, BMI, parity, smoking, alcohol and folic acid use during pregnancy,household income, paternal smoking and education, child sex, breastfeeding, age at FFQ, introduction of solid foods, energy intake and TV watching at 2y *p<0.05, **p<0.01

Additional analyses

Additional adjustment for BMI (SDS) at 1 year only slightly attenuated the effect estimates and all associations with FFMI remained significant (**Supplementary Material 4.4.3**). The inverse association between the second RRR-pattern and FMI became statiscally significant again. We observed no significant interactions of the dietary patterns with child sex, BMI (SDS) at 1 year, or participation in sports at 6 years.

We observed a significant interaction (p=0.03) of the second RRR-derived dietary pattern with TV watching at 2 years on FMI. Stratification using a cut-off for TV watching of 1 h/d[338] revealed that a higher score for the second RRR pattern was associated with a lower FMI in children who watched TV <1 h/d (-0.07 SD (95%CI -0.11; -0.03) per 1 SD increase in dietary pattern score), but not in those who watch ≥1 h/d (0.00 (95%CI -0.06; 0.05)).

We performed a sensitivity analysis in which we excluded children who still received breastfeeding or a substantial amount of infant formula at the time of dietary measurement (n=386). After excluding these children, the associations of the 'Health-conscious' dietary pattern with BMI and FFMI slightly attenuated and were no longer statistically significant, whereas the association between the diet score and FFMI remained significant (**Supplementary Material S4.4.3**).

Discussion

In a large population-based cohort study in young children, we observed that higher adherence to *a priori*-defined diet quality or to an *a posteriori*-defined 'Health-conscious' dietary pattern at the age of 1 year was associated with a higher FFMI, but not with FMI at the age of 6 years. Using reduced-rank regression, we additionally identified dietary patterns that predict child body composition. A pattern that was associated with a higher FFMI, but not with FMI, was characterized by high intakes of whole grains, pasta and rice, dairy, fruit, vegetable oils and fats, and non-sugar-containing beverages. Additionally, a pattern positively associated with both FMI and FFMI was identified, which was characterized by high intakes of refined grains, meat, potatoes, fish, soups and sauces, and sugar-containing beverages. These associations were all independent of total energy intake and parental and child sociodemographic and lifestyle factors.

Interpretation and comparison with previous studies

We observed small but statistically significant positive associations between better *a priori*-defined diet quality or higher scores on PCA- or RRR-derived 'Health-conscious' dietary patterns in early childhood and subsequent FFMI, but not with FMI. These three patterns were characterized by high intakes of foods generally considered to be healthy (vegetables, fruit, whole grains, and vegetable oils). The associations with a higher FFMI suggest that these dietary patterns can be beneficial for health in later life, as higher lean mass is associated with improved cardiovascular and metabolic health.[339-341]

Three previous prospective studies examined the association of overall diet in early

childhood with body composition later in life,[342-344] of which only one separately assessed fat and fat-free mass.[344] In line with our results, this latter study in 536 U.K. children observed that a higher adherence to a PCA-derived 'infant guidelines' dietary pattern at the age of 12 months was associated with higher lean mass index but not FMI at the age of 4 years.[344] This pattern was characterized by a high intake of fruit, vegetables, cooked meat and fish, and rice and pasta; and low intake of commercial baby foods.[345] Other dietary patterns were not examined. The other two studies both assessed predefined diet quality on the basis of dietary guidelines. Adherence to the 'Raine Eating Assessment in Toddlers' index at 1 to 3 years of age in 2,562 Australian children was not consistently associated with BMI during childhood and adolescence.[343] In a large cohort of U.K. children (n=4,798), a higher score on a 'Complementary Feeding Utility Index' at the age of 6 months was also not associated with BMI at the age of 7 years after adjustment for sociodemographic variables, but was associated with a lower waist circumference.[342]

We observd less consistent associations between dietary patterns and later body fat or fat distribution. Although in our population adherence to a 'Western' dietary pattern was associated with increased FMI in crude models, this association was explained by sociodemographic and lifestyle factors. The only pattern associated with later body fat after adjustment for confounders was an RRR-derived pattern constructed on the basis of variation in body composition. The first RRR-pattern, which was characterized by high intake of refined grains, meat, potatoes, fish, soups and sauces, and sugar-containing beverages, was associated with a higher FMI, a higher body fat percentage and a higher android/gynoid fat ratio. The second RRR-pattern, charactherized by intake of whole grains, pasta and rice, and vegetable oils, was associated with a lower body fat percentage, but was associated iwht a lower FMI only after additional adjustment for BMI (SDS) at the age of 1 year.

We used reduced rank regression as exploratory approach to identify which patterns in early childhood explain most variation in body composition. In contrast to several previous studies,[326] [346] but in line with one other study,[347] we chose to use body composition measures as response variables, rather than nutrient intakes or biomarkers. We used this approach because we were interested in exploring which patterns best predict body composition. Consequently, the main results are primarily the food groups characterizing the patterns, rather than the results of regression analysis on the association of the patterns with body composition. Additionally, patterns based on variation in FMI and FFMI can be used in future studies to evaluate the relation between diet and other health outcomes, as body composition is a possible intermediate factor in many diet-disease associations.

Strengths and limitations

An important strength of our study is that we had a prospective study design with detailed information available on a large number of potential confounders. Previously, several family sociodemographic and lifestyle characteristics have been related to child dietary

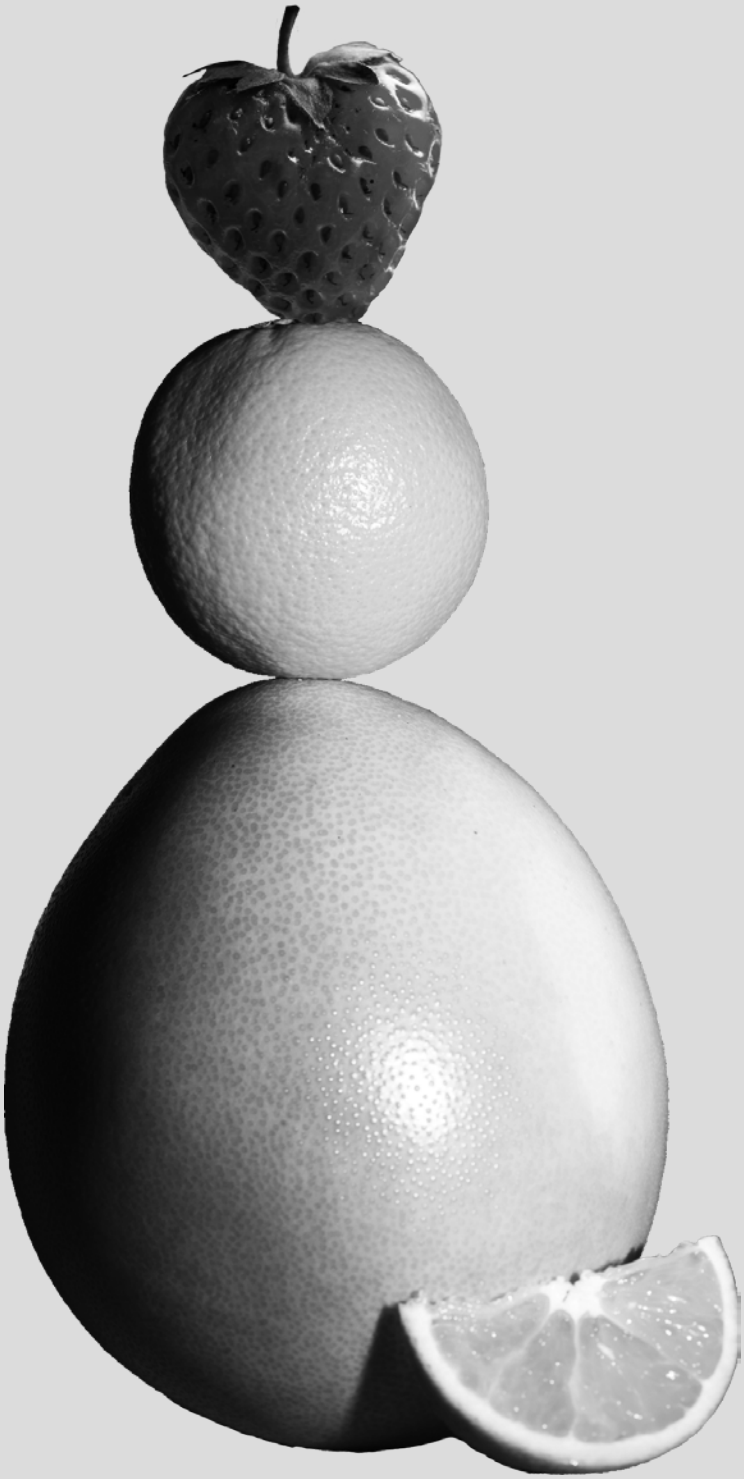
patterns,[265] and to child body composition.[348] These factors are thus important to take into account when studying the relation between diet and body composition. Previous studies were not always able to adjust for important factors such as parental BMI and lifestyle. Furthermore, while loss to follow up is usually an important limitation of prospective studies, more than 80% of all children in our study population with information on food intake participated in the body composition measurements at the age of 6 years.

A limitation of our study is that we measured food intake with an FFQ, which is known to be prone to substantial measurement error. However, an FFQ measures habitual diet rather than diet on just one or a few days, and is considered appropriate to use for dietary pattern analysis.[349] A major strength of our study is that we performed detailed measurements of child body composition using DXA. Many previous studies assessed body composition based on total body weight, while in our study we were able to distinguish between fat mass and fat-free mass index. We observed that higher adherence to healthier dietary patterns was associated with a higher BMI but that this reflected a higher fat-free mass rather than fat mass, supporting the notion that BMI alone is not a good proxy for childhood obesity.[350]

Conclusion

Dietary patterns characterized by high intake of fruit, vegetables, grains, and vegetable oils at the age of 1 year, were associated with a higher FFMI, but not with FMI, at the age of 6 years. Using reduced-rank regression we additionally identified a pattern that predicted a higher FMI and FFMI, which was characterized by high intakes of refined grains, meat, potatoes, fish, soups and sauces, and sugar-containing beverages. Future studies should explore whether these differences in body composition track into later life and whether these differences are independent of later dietary patterns.

Supplementary Material can be found online: http://hdl.handle.net/1765/77768



Chapter 5

General Discussion

5.1 Summary of background, results and main conclusions

Nutrients and health across the life course

Lutein

Carotenoids have a high antioxidant capacity and thus have the ability to counteract oxidative stress.[351] Therefore, they may have an important role in several disease processes and organ systems, which might already start early in life. Through consumption of carotenoid-rich foods by the mother, breast milk has a high level of carotenoids and newborns that are being breastfed have therefore much higher carotenoid levels relative to infants who receive infant formula.[352] Previous studies have shown breastfeeding to be beneficial for many different health outcomes, such as respiratory tract infections, allergies and obesity,[353] and since these outcomes have been related to oxidative stress as well,[354,355] carotenoids may play an important role in these associations. The carotenoid lutein, mostly known for its preventive effect on macular degeneration,[35] is of particular interest, given that the amount of lutein is extremely high in colostrum.[356] However, in our systematic reviews, we identified only three articles that assessed the effects of lutein during fetal life or in early childhood on cardiometabolic outcomes (**chapter 2.1**). These studies examined either maternal or cord blood levels of lutein, and an intervention study examined consumption of lutein-fortified infant formula. Based on these studies, there was no consistent association between lutein and anthropometrics or growth. Cardiometabolic health of children was only assessed in a study among 1,339 US adolescent, which found that higher lutein levels were associated with lower number of risk factors from the metabolic syndrome.[48] The effects of lutein in early childhood on cardiometabolic health have not yet been reported. Given this research gap, we studied the relation between lutein intake in early life with cardiometabolic health in childhood within the Generation R Study (**chapter 4.1**). We assessed the associations of lutein intake at the age of 1 year with multiple measures of body composition and cardiometabolic health at the age of 6 years. We observed no consistent relation between lutein intake and any of the outcomes. While this does not confirm the hypothesis of beneficial effects of lutein on cardiometabolic health, this is the first study of lutein intake and cardiometabolic health in children. In our systematic review, the vast majority of the literature was in adults, predominantly middle-aged and elderly (**chapter 2.1**). Studies were of average quality and from the 67 studies in adults, only three had an interventional design.

In our meta-analysis for cardiometabolic diseases, we found that evidence from observational studies showed higher lutein intake or levels to be associated with lower risk of coronary heart disease, stroke and metabolic syndrome. Studies on intermediate factors indicated that this lower risk might be driven by beneficial effects of lutein on inflammatory processes, such as lower levels of C-reactive protein, and by preventing atherosclerosis. The relation between lutein and inflammatory markers was also confirmed by some experimental studies. Given the high global burden of cardiometabolic diseases,[1] lutein might be of importance and its role deserves

further evaluation, as conclusions about a causal effect of lutein on cardiometabolic health across the life course cannot yet be drawn.

Choline

Choline can be important for health through various pathways. Choline serves as methyl donor in the methylation cycle, is a precursor for the neurotransmitter acetylcholine and is, in its form as phosphatidylcholine, a structural component of cell membranes[134]. Choline can be formed de-novo in the liver under influence of estrogen, but this is only in small amounts and choline is thus defined as an essential nutrient since 1998.[133] Foods rich in choline are animal-based products such as eggs, meat and dairy products. Choline is also present in high amounts in human breast milk. The presence of choline in breast milk has been one of the suggested explanations why breastfeeding is associated with better cognitive performance in children.[357]

The beneficial effects of choline on cognition may also start already during fetal life. Animal studies suggested a causal link between the availability of choline during pregnancy and offspring cognitive performance[33]. In our systematic review, we found several human studies addressing this topic, but there is not enough evidence to confirm the suggest effects (**chapter 2.3**). The demand of choline increases during pregnancy and lactation because of the higher need for methyl groups, as choline can serve as a methyl donor through its precursor effect of betaine.[134] Via this pathway, availability of choline during fetal life may be associated with birth defects.[140] Indeed, we showed that higher choline during pregnancy might decrease risk of neural tube defects, although evidence is scarce.

It is unknown if choline in early life may influence cardiometabolic health in later life, as we found no studies that addressed this relation. In adults, however, there are several mechanisms proposed in which choline might have an effect on cardiovascular health. Choline could potentially lower homocysteine through the methylation pathway[135] and hyper-homocysteinemia has been linked to cardiovascular disease.[207] Through this pathway, choline might thus be beneficial for cardiometabolic health. In contrast, choline-containing nutrients can be used as fuel by gut bacteria, after which trimethylamine-N-oxide (TMAO) is formed. Increased levels of TMAO have been linked to increased risk of cardiovascular diseases,[209] in which case choline would be harmful. The findings from our systematic review are mostly in line with this latter hypothesis, and a high choline intake might thus increase the risk of cardiometabolic diseases. However, choline is also needed to eliminate fat from the liver in the form of (V)LDL, and deficiency of choline may lead to non-alcoholic fatty liver disease (NAFLD).[137] Although we did not find evidence in our review for choline lowering risk of NAFLD in the general population, some studies suggest that choline might improve insulin sensitivity,[173-176] which may go through a lower risk of NAFLD. Studies on insulin sensitivity, fatty liver disease and cardiovascular diseases were of limited quality and there were no intervention studies that addressed these aspects.

Previously, choline has been suggested to have important effects on cognitive function because it serves as a precursor for acetylcholine.[134] There might be some benefits of choline use during recovery from stroke,[32] and it might enhance cognitive function in persons with cognitive deficits,[141] but results in our review were inconclusive and a beneficial effect of choline on cognition in the healthy population could thus not be concluded.

Foods and child cardiometabolic health

Sugar-containing beverages

The consumption of sugar-containing beverages (SCBs) has increased rapidly in the past decades, and is currently the most important single source of energy and sugar intake, in all age-groups.[273] The high consumption of SCBs is of great concern given that they only contain low amount of valuable nutrients, but contributes to high intake of sugars and energy. In adults, there is substantial evidence from both observational and experimental studies that high SCB intake can lead to a higher risk of obesity, metabolic syndrome and type 2 diabetes.[19,21] The effects of SCBs have also been studied in adolescents and in school children, which showed that SCB intake leads to higher body weight.[19,275] Given this evidence, public health initiatives aiming to prevent these diseases have put more emphasis on SCB intake, and multiple intervention strategies have focused on reducing the intake of SCBs.[293] Surprisingly however, the effects of SCB intake in preschool children were unaddressed, and to our knowledge, there were no studies looking at SCB intake in infancy. This might in part be because softdrinks, an important contributor to SCBs, are not frequently consumed in this age group,[280] but fruit juices and fruit concentrates are, and they also contain a high amount of sugar.[226] It could also be that this age group has not yet been studied, because the mechanisms by which SCBs are harmful in these older age groups, might not be applicable in these young children. For example, high SCB intake is proposed to lead to weight gain because the calories of SCBs hardly lead to a feeling of satiety and therefore a higher energy intake,[277] but it could be hypothesized that SCB intake does not necessarily lead to weight gain in toddlers, because their energy intake depends mostly on what is offered to them by their parents. We showed that SCB intake at 1 year of age was associated with a higher BMI up to the age of 6 years in girls, but not in boys (**chapter 4.2**). Nevertheless, we showed that high SCB intake in boys at 1 year of age was associated with greater height at the age of 6 years, which is also associated with a higher risk of obesity in later life.[289] In addition, we examined the relation of SCB intake with cardiometabolic outcomes at the age of 6 years, and observed that boys with high SCB intake at 1 year of age had a higher cardiometabolic risk factor score at 6 years of age (**chapter 4.3**). When studying individual components of the cardiometabolic risk factor score, the association did not seem to be driven by any component in particular. In girls, intake of SCBs at 1 year of age was not associated with the cardiometabolic risk factor score at age 6, nor was it associated with any of the individual components. Our results indicate that SCB intake in toddlerhood may have harmful effects

on health in later life, in which sex differences may be present. It is not known if these sex differences are caused by biological mechanisms or by social differences between boys and girls. The causality of the relation between SCB intake and cardiometabolic health was already established by other studies, and in this thesis we have extended the current literature by showing that these associations are already present in early life. Moreover, we observed sex differences in the relation between SCB intake and body composition and cardiometabolic health, which deserves further clarification.

Overall diet and child cardiometabolic health

Maternal diet

A few decades ago, Barker et al. observed a relation between low birth weight and cardiovascular disease mortality later in life.[215] Following this hypothesis, now well-known as the 'Barker hypothesis', the Hungerwinter study was designed, which evaluated the effects of exposure to famine in fetal life.[254] This study showed that severe undernutrition during pregnancy had adverse effects on the fetus, and that this increased the susceptibility for cardiometabolic diseases in later life.[254] These initial studies have led to many evaluations on the effects of exposures during fetal life on later health, of which nutrition is an important aspect. However, research has mostly focused on specific nutrients or foods,[27,358] or used maternal BMI[359] or birth weight[360] as measure of nutritional status. It remains unknown which dietary patterns during pregnancy are associated with cardiometabolic health of the child.[244,245]

We studied the relation between dietary patterns during first trimester of pregnancy, and body composition and cardiometabolic health in offspring at the age of 6 years (**chapter 3.1 and 3.2**). In our population, we identified three empirically driven dietary patterns using principal component analysis. Higher adherence to the 'Vegetable, fish, and oil' dietary pattern was associated with a more favorable body composition of the child. Also children of these mothers had a lower score on several cardiometabolic risk factors at the age of 6 years. However, all these associations were explained by other maternal sociodemographic and lifestyle factors, such as education level, body mass index and smoking and alcohol use during pregnancy. Similar findings were observed for the 'Fruits, soy and high-fiber' cereal dietary pattern. We found no association between the 'Margarine, snacks and sugar' dietary pattern and cardiometabolic health in offspring. We additionally examined whether maternal adherence to predefined dietary guidelines[248] was associated with child cardiometabolic health at the age of 6 years. Similarly, we observed that the relation between higher adherence to this guideline-based dietary pattern was associated with a lower score on several cardiometabolic risk factors, but that this was also explained by maternal sociodemographic and lifestyle factors. These results suggest that that there is no independent relation between indices of whole diet during pregnancy and offspring cardiometabolic health at the age of 6 years in our population of healthy young pregnant women. Further studies in other populations may be needed.

Infant diet

Diet during infancy is suggested to affect health in later life.[361] Infant diet starts with breastmilk or infant formula only, which changes throughout the first year of life to a whole diet consisting of many different food groups.[362] During this weaning period, preferences for foods and tastes may develop, which can track throughout later childhood and even into adulthood.[28,363,364] This partly explains why early diet may be associated with health in later life. However, diet in infancy may also have a direct effect to influence later health, for example through differences in growth trajectories, as can be seen between breastfed and bottle-fed children.[365] While there is a lot of research interest in effects of breastfeeding and formula feeding on later health outcomes, the relation between whole diet in early life and health outcomes in later life remains largely unstudied.

In **chapter 4.4**, we described the associations of different *a priori*-defined and *a posteriori*–derived dietary patterns of children at the age of 1 year, with body composition at the age of 6 years. We observed that higher adherence to an *a posteriori* 'Health conscious' dietary pattern[265] and higher adherence to an *a priori* 'Diet Quality Score'[252] was associated with a higher body mass index (BMI). Evaluation of other indices of child body composition revealed that this association was explained by a higher fat-free mass index (FFMI). This suggests that adherence to a healthy diet might increase fat-free mass, without affecting fat mass index (FMI). We found no associations between adherence to an *a posteriori* 'Western' dietary pattern[265] and child FMI or FFMI.

To explore which dietary patterns were associated with FMI and FFMI in our population, we used reduced rank regression to identify dietary patterns explaining the maximum variation in FMI and FFMI. We identified a pattern positively associated with FFMI and inversely associated with FMI, which was characterized by a high intake of whole grains, pasta and rice, dairy, fruit, vegetable oils and fats and non-sugar-containing beverages. Additionally, we identified a pattern positively associated with both FMI and FFMI, which was characterized by a high intake of refined grains, meat, potatoes, fish, soups and sauces, and sugar-containing beverages. Our results indicate that dietary patterns in infancy that are considered healthy are associated with FFMI, but not with FMI, in childhood. We identified no patterns in early life that were specifically associated with FMI in childhood.

5.2 Approach to nutritional epidemiology: methodological aspects and implications

Several approaches can be used to examine the relation between nutrition and health. In this thesis, we examined specific nutrients (lutein and choline), food products (sugar-containing beverages) and overall diet (*a priori* and *a posteriori* dietary patterns). Each approach has its own advantages and disadvantages and methodological challenges. Results from these different approaches will also have different implications for science and preventive medicine. This will be discussed in this chapter.

Nutrients

In the relation between diet and disease, the focus is often on nutrients, because nutrients can be the bioactive compounds of the diet influencing health outcomes. Studying nutrients can therefore also be very informative on the pathways by which diet affects disease risk, and give more insight in the functioning of the human body.[18] In addition, it can be useful for future research in other fields, as effects on organ systems or disease processes may affect multiple outcomes.

However, studying nutrients also has many methodological challenges. First of all, in observational studies it is difficult to measure how much of a certain nutrient is being consumed. Dietary assessments have measurement error, for example because of inaccurate self-report and incomplete food-composition tables.[239] In addition, even if dietary intake would be measured accurately, it is not known how much of the nutrient is actually absorbed into the body, as the bioavailability depends also on the intake of other nutrients, and many other factors such as age, sex and genetics.[18]

Furthermore, it is not always possible to find relationships between nutrients and disease in the general population, because nutrients have a large range that is considered optimal, and it is very likely that it only affects disease risk in extremes, for example due to deficiency. When assessing nutrient intake of a population that is mostly well-nourished, variability might thus be too low, which makes it difficult to detect associations.[18]

When a relation is observed between nutrient intake and risk of disease, it needs to be determined if the nutrient of interest really is responsible for the observed effects. A very important issue when studying nutrients is the risk of confounding, either within the diet or outside the diet. Within diet, a nutrient can be confounded by other nutrients in the food group that they are derived from. For example, vegetables are the major source of carotenoids, and the suggested effect of lutein might then as well be caused by another carotenoid.[69]

In addition, nutrients from totally different foods can be confounding the relation. For example, high carotenoid intake may be correlated to low intake of saturated fatty acids and this low intake of saturated fatty acids may lead to the beneficial relationship, rather than the high carotenoid intake.[366] But even if it is not confounding by other nutrients, it is important to note that food sources might be of importance as well. In the case of sugar-containing beverages (SCBs), the unfavorable effects of sugar may be mostly caused by the lack of satiety due to the liquid form. This effect may thus be different from sugar in its solid form, and analyzing total sugar without evaluating the source of sugar, may lead to inconsistent results or invalid conclusions.

Besides confounding within the diet, a relation between nutrient intake and disease risk can be confounded by other lifestyle factors that cluster with diet. Lutein is considered a useful biomarker for vegetable intake, which is in general related to a more healthy lifestyle. Other factors of a healthy lifestyle, such as frequent physical activity and not smoking, are thus potential confounders for the association of a higher lutein intake with better cardiometabolic health.[367]

While these disadvantages make it difficult to establish causal nutrient-disease relationships, they can be covered to some extent by adequate study design. Measurement error of reported nutrient intake can be partly removed during statistical analyses, for example by using energy adjustment[266] or regression calibration.[368] The bioavailability issues that remain, can be solved by measuring biomarkers of dietary intake, as for example blood levels of lutein. The issues with confounding highlight the need to adjust for other factors, both within and outside of diet. For example, we observed that the correlation between lutein intake an beta-carotene intake was 0.94, and analyzing lutein without adjusting for beta-carotene, might thus lead to invalid conclusions about lutein. Hence, in observational studies, it is important to measure other nutrients, other foods, and other lifestyle factors, and take them into account as confounders in the analyses.

The original studies in this thesis were of observational design, which limits any conclusions related to the causality of the observed association. Randomized controlled trials can be performed to determine if the particular nutrient really has a causal effect. If there is enough evidence from observational studies, intervention studies that are randomized and, if feasible, double-blind controlled may be performed to establish a causal relationship.

If benefits of certain nutrients have been causally established, an important advantage is that interventions can focus directly on improving nutrient status of the population at risk. This can be done in various ways such as supplementation, or food fortification.[369] Supplementation can be a good strategy if only certain groups of persons need to be targeted in this respect, for example the recommendation for women to take folic acid supplements before conception. If the proposed beneficial effects of choline supplements during pregnancy would be established, the same approach could be useful for choline. However, while this strategy theoretically seems simple and effective, this might in practice not always feasible. For example, only a small percentage of women follows the recommendation of starting with folic acid supplement one month before conception.[370] With that in mind, a different strategy to improve nutrient status of a population could be by mandated food fortification. This strategy is also recommended by the World Health Organization[371]. Although this has shown to be effective in increasing levels of folic acid of the population, it did not eradicate neural tube defects completely and too high intake might not be without hazards.[372]

Foods

Although nutrient status can be improved by supplementation or fortification,[369] this is only done in exceptional cases and in specific populations. In general, people do not obtain nutrients from supplements, but nutrients are derived from consumption of foods. It is thus important to translate recommendations regarding nutrient intake into recommended intake of foods. Even if effects of nutrients have been detected, translation to food-based dietary recommendations remains important to reach the general public and improve public health.[222]

When a nutrient has been suggested to be beneficial for health, it is thus necessary to examine if foods that are major sources of the nutrient, have the same health effects. For example, a high lutein and choline intake can be established by consuming eggs, but eggs also contain cholesterol, and it is still questioned whether eggs can be consumed unlimited.[373]

While nutrients may indeed be causing potential effects on health, the hypothesis relating the specific nutrient to disease outcomes may arise from observations of suggested effects of food groups.[18] For example, the preventive effect of lutein on macular degeneration was discovered due to the observation that spinach, but not other sources of beta-carotene, was associated with a lower risk of macular degeneration.[374] If only nutrients would be studied, certain relations between diet and disease might not be detected. For example, the relation of SCBs with obesity and cardiometabolic disease might have been missed if only total sugar was being examined. This again illustrates the importance of also having a food-based approach in nutritional epidemiology.

Nevertheless, there can be interactions between nutrients from different foods as well, and the risk of confounding within diet as mentioned for nutrient intake, is also present when examining foods. Caution should thus be taken when interpreting results from observational studies, since people do not eat single foods, but a combination of different foods. For example, a relation between white bread consumption and adverse health effects might not be caused by harmful compounds of white bread, but they might be caused by the low consumption of dark bread and therefore a lack of fiber and valuable nutrients that come from consumption of whole grain. Another example is that people who eat more fish tend to eat less meat, and a relation between higher fish consumption with better health might suggest beneficial effects of fish oils, while it could also be that the better health is caused by a lower consumption of unhealthy components of meat.

In addition, the limitations as discussed for the nutrient approach, such as measurement error and confounding by lifestyle factors, also apply to the food-based approach. The causality of any health effect of foods cannot always be assessed in randomized controlled trials easily, since dietary exposures are often not feasible for a randomized or blinded study design. Nevertheless, intervention studies that examined the effects of SCB consumption in a double blind randomized controlled trial have shown that indeed SCBs are causally related to weight gain.[19] The results from these studies can form a basis for evidence-based dietary guidelines on limiting SCBs consumption. A benefit of the food-based approach is then also that advice can be implemented in existing dietary patterns directly. For example, SCBs can be substituted with water without the need to change other aspects of the diet. Interventions aimed to reduce SCB intake by promoting water have indeed showed to effectively decrease intake of SCBs.[375-378] Some of these studies also showed that measures of cardiometabolic health were improved after the intervention,[377,378] which provides further evidence of unfavorable effects of SCBs on cardiometabolic health.

Overall diet

Within an overall dietary pattern, many correlations exist between foods and nutrients. For this reason, research has emerged on examining the whole of diet beyond nutrients or foods, taking into account these correlations and the interactive, synergistic and antagonistic effects that nutrients can have.[222] Although dietary patterns may not give information about biological mechanisms behind the relation between nutrition and health, a great benefit of assessing an overall dietary pattern is that it seems to be more powerful to detect associations of nutrition with health as compared to single nutrients or foods, especially for the complex diet-disease relations in cardiometabolic diseases.[222] An important advantage of the dietary pattern approach is also that results can be translated more easily into dietary guidelines. For the general population, dietary patterns are easy to interpret and this might facilitate the adherence to interventions.

Dietary pattern analyses can have different approaches, and in this thesis we used the empirically driven *a posteriori*-approach and the theoretically driven *a priori*-approach. The empirical dietary patterns, for example identified using principal component analysis, are based on the collected dietary data in our population, and thus reflect actual dietary patterns. The *a priori* dietary patterns are based on guidelines or expert opinions, and are designed to reflect an optimal diet. *A priori* dietary patterns are thus based on previous knowledge and the advantage is then that they can be used in any population and be easily replicated. A limitation is however that these patterns are based on existing knowledge, which might not always be sufficient. An optimal diet during pregnancy has for example not yet been established.[245] When assessing dietary patterns, both *a priori* and *a posteriori*, a lot of arbitrary choices need to be made, for example regarding the composition of food groups. These choices could influence the validity of the patterns and the ability to detect associations, and detected associations should therefore be replicated in other populations.

Dietary patterns, especially derived empirically, do not so much reflect clustering of diet based on biological factors, but more on a cultural clustering of foods.[18] Also other lifestyle factors are clustered with these dietary patterns,[367] and associations between dietary patterns and disease risk might thus not be caused by actual dietary factors. Indeed, in **chapter 3.1 and 3.2**, we showed that the relation between dietary patterns during pregnancy and offspring health was mainly explained by other maternal lifestyle factors, and these factors are thus important to take into account.

Even though observational evidence can be promising, additional evidence from intervention studies on dietary patterns can be more convincing in elucidating a true causal relationship. Testing causality with dietary patterns is however quite complicated. A double-blind intervention is almost impossible, and full adherence to a recommended diet is extremely difficult and requires a great amount of collaboration with the complete environment, in order to have the right foods, amounts, frequency and preparation. So far only few intervention studies on dietary patterns exist. The 'PREvención con Diet

MEDiterránea study' (PREDIMED) is one of the most well-known intervention studies on the Mediterranean diet.[379] The results of this study have already contributed to the evidence that the Mediterranean diet is beneficial for cardiometabolic health. Although limitations of the design still remain, it highlights the importance of examining whole diets in relation to health.

However, even if beneficial effects of a dietary pattern are well-established and the translation for the public is easy, it might not be easy to implement a whole new dietary pattern in existing lifestyle. The Mediterranean diet for example has many proposed health benefits and because it can also be easily preserved it is very attractive for the Mediterranean population,[380] but adherence is more difficult for Americans or Northern Europeans.[381] Adherence to a dietary pattern goes beyond food preferences, and a dietary pattern is difficult to maintain if foods are difficult to find[382] or if unhealthier foods are less expensive.[383] This stresses the importance of the environment in the relation between nutrition and cardiometabolic health.

5.3 Future perspectives: directions for research and preventive medicine

Nutrients and health across the life course

Lutein

Literature on lutein suggested that higher intake or blood levels were associated with lower risk of cardiometabolic diseases, possibly due to reduced atherosclerosis and lower inflammation (**chapter 2.1 and 2.2**). The results of lutein in adults are thus promising, but most of the evidence is derived from observational studies. Future experimental research is needed to assess the causality of the associations. Currently no recommendations for adequate dietary intake or optimal blood levels of lutein exist. Therefore, if beneficial effects have been causally established, the impact on dietary recommendations can be promising. Also, future studies may focus on identifying risk groups for lutein deficiency since lutein may also be depleted as a consequence of disease processes such as in metabolic syndrome.[119-121] The majority of the literature on lutein is in middle-aged and elderly, but the effects of lutein might be different in different life stages. Given the role of oxidative stress in child health, the role of lutein in early life needs to be further evaluated in younger age-groups.

Choline

Results from animal and *in vitro* studies suggested a causal effect of choline during fetal life on cognition in later life.[33] We showed in **chapter 2.3** that there is not yet sufficient evidence for possible beneficial effects of choline during pregnancy in humans. However, results from intervention studies that are currently on the way or are awaiting longer follow-up, will be important in establishing this potential relation.[145,384] Results of these trials will determine whether public health initiatives are needed to improve choline status of pregnant women. Also in adults, choline has been suggested to be beneficial for cognition, but we showed that there is not much evidence to support this in healthy general populations.

In addition, choline might not only affect cognitive function, but could also have an effect on other organ systems.[134] We showed some directions towards increased risk of cardiometabolic diseases, and the possible harmful effects of choline thus need to be carefully evaluated. Future studies should also examine the possible pathways underlying the suggested harmful effects on cardiometabolic health, for which the focus currently is on trimethylamine-N-oxide (TMAO).[210] Caution should be taken on public health initiatives such as supplementation or fortification of choline before these possible harmful effects are elucidated.

Foods and child cardiometabolic health

Sugar-containing beverages (SCBs) are known to be harmful for body composition and cardiometabolic health in school children, adolescents and adults,[19,21] and we have shown that these associations are also already present in toddlers (**chapter 4.2 and 4.3**). In contrast to SCBs in older age groups, SCB intake in toddlers consists of mostly fruit juices and fruit concentrates. Since the effect of SCB has also been causally established, it is important to create awareness about the unfavorable effects of SCBs especially for mothers of young children. Intervention programs already target primary and secondary schools,[293] but our results indicate that new initiatives should be undertaken to reduce the consumption of SCBs also in toddlers. Currently, children below the age of 2 years are not included in the Dietary Guidelines for Americans.[295] Public health professionals might specifically focus on limiting use of SCBs in daycare centres as well as educating health care professionals involved in preventive health care for young children.

There is sufficient observational evidence on the relation of SCBs with body weight.[292] However, research on SCBs should further explore sex differences in the associations of SCBs with body composition and cardiometabolic health. Satiety might play a role, since it is suggested that boys can compensate better for liquid calories.[283,284] However, evidence for this is scarce. Future studies, both observational as experimental, should thus examine if associations are different between boys and girls, rather than simply adjusting for sex. This would help in determining whether public health interventions aimed at reducing childhood overweight should be sex-focused.[385]

Overall diet and child health

We observed that associations between relatively healthy *a priori*- and *a posteriori*-derived dietary patterns during pregnancy with better cardiometabolic health in offspring were mainly explained by other maternal sociodemographic and lifestyle characteristics (**chapter 3.1 and 3.2**). Our study was performed in a fairly healthy population of pregnant women, while the largest effects of diet during pregnancy may be expected in nutritionally at risk populations.[358] Studies on overall diet during pregnancy and child health are lacking, and further studies are needed to address this aspect, especially in populations with low diet quality. It remains debated what the optimal diet is during pregnancy and more research on overall diet is needed to enable construction of future guidelines.[245]

Because pregnant women are attending medical care and may be more health conscious during pregnancy, it creates an important window of opportunity for gynaecologists and midwives to give lifestyle counseling to these families. This could help in improving the obesogenic environment of the future offspring and even if there would be no intrauterine effect of nutrition, a healthier parental lifestyle would be beneficial for the offspring, and preserve cardiometabolic health.

In addition to overall diet during pregnancy, we examined several dietary patterns at the child's age of 1 year, and observed that the previously constructed 'Diet Quality Score', 'Health-conscious' dietary pattern and 'Western' dietary pattern were not associated with child measures of fat mass (**chapter 4.4**). The pattern that we created based on fat mass index, was also associated with a higher fat-free mass index, and might therefore not be specific enough for guidelines aiming to reduce adiposity. Future studies should aim to identify a pattern strongly associated with lower or higher fat mass, in order to make adequate recommendations for public health interventions. We did observe that higher adherence to a 'Health-conscious' dietary pattern and a higher 'Diet Quality Score' was associated with a higher fat-free mass index. Future research and longer follow-up is needed in order to determine whether these dietary patterns are also associated with a better cardiometabolic profile and lower disease risk in later life. In addition, further replication of dietary patterns in relation to childhood body composition in other populations and at other ages is needed.

Cardiometabolic health

In many of the studies in this thesis, we evaluated cardiometabolic health at the child's age of 6 years. At 6 years of age, trajectories towards disease risk are still being developed and deviations from healthy will become more apparent at a later age.[26] Therefore, it is important to continue to follow these children in order to evaluate the associations with cardiometabolic health at a later age. This is especially important for the studies in which we did not demonstrate the expected effects, such as the lack of an effect of maternal dietary patterns or child lutein intake, since the children might have been too young to detect any difference in cardiometabolic outcomes.

While we assessed several well established risk factors for cardiometabolic diseases, such as adiposity, blood lipids and blood pressure, it is not yet established to what extent this affects cardiometabolic risk in adulthood. For example, android/gynoid fat ratio is related to cardiometabolic health in adults.[386,387] In children, android/gynoid fat ratio has been related to higher insulin resistance in children,[388] but these children were older and especially at a young age, the value of android/gynoid requires to be further studied in population-based cohort studies with long follow-up. Furthermore, changes in BMI over time may be used as risk factor for diseases.[389] In adults, this always reflects a change in body weight. In children however, changes in BMI also reflect changes in height. In addition, an increase in height may actually follow an increase in weight, and in childhood height itself has also been associated with obesity risk in adulthood,[289]

After the age of 1 year, there is a decline in BMI due to a more rapid growth in height as compared to weight, and reaches the lowest point between age 5 to 7 years, which is known as the adiposity rebound.[390] From this point onwards, BMI rises up to the end of growth.[390] The adiposity rebound differs largely between children and both the age of adiposity rebound as the absolute BMI at the lowest point have been associated with cardiometabolic health.[391,392] Also, the increase in BMI may be at an earlier age than the increase in fat mass index.[393] Hence, further follow-up of the cohort is needed to adequately assess the role of the adiposity rebound and different aspects of body composition. Interestingly, a study that examined the adiposity rebound, with the use of BMI, has suggested that there are sex differences in the timing of the adiposity rebound,[394] which again highlights the importance in studying differences between boys and girls when examining body composition and cardiometabolic health. The adiposity rebound may also play a role in the sex differences that were observed in this thesis, and therefore these associations need to be reevaluated at a later age.

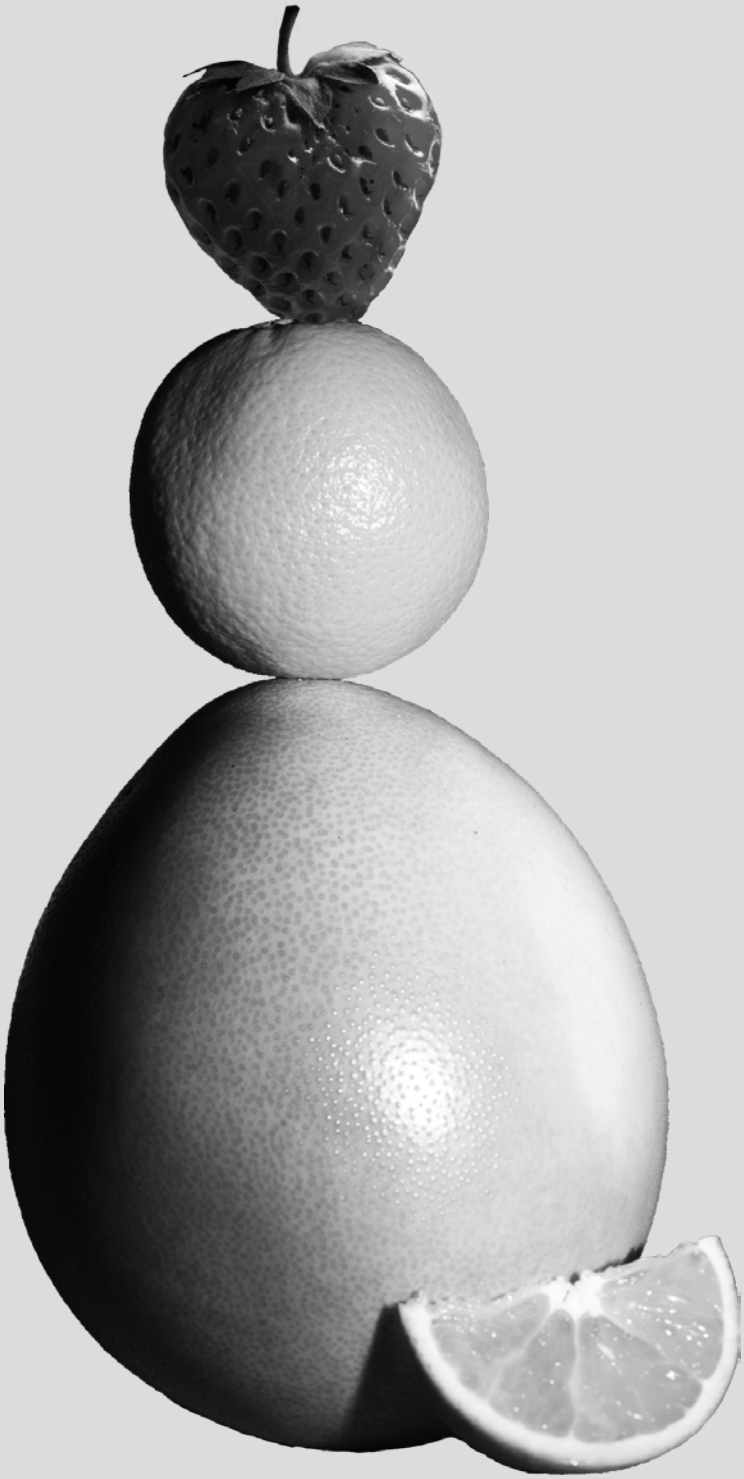
In this thesis, we have demonstrated in several chapters that studying child BMI can be misleading when no distinction is made between fat mass and fat-free mass. While a higher fat mass can increase the risk of cardiometabolic diseases,[10,11,14], a higher fat-free mass is associated with better cardiometabolic health,[339-341] and it is thus important to distinguish fat mass from fat-free mass. However, in practice, total body mass index is mostly used. This is to a large extent because it is a measurement that is easy to use for parents and health care professionals, especially as compared to for example DXA-scanning, which is timeconsuming and expensive. Other measurements of total or regional fat mass have been suggested which could be more suitable, such as bio electrical impedance analysis[395]. While this could be an alternative for estimating body fat percentage, this measure was only deemed valid when subjects were within a normal body fat range[396], and would thus be less useful for measuring adiposity. In adults, waist circumference is recommended to be used in addition to BMI in order to establish disease risk[397], but for children this is not yet been established. Future research should assess if measures of fatness or abdominal adiposity in addition to BMI could be useful in clinic for predicting disease risk in children.

Within the Generation R Study, currently the cardiometabolic health of the children around the age of 9 years is being evaluated,[34] and the results of these examinations can provide further clarification about the associations observed in this thesis. In addition to the measurements that were performed at the age of 6 years, Magnetic Resonance Imaging (MRI) is performed at the age of 9 years, which will provide valuable additional information on the cardiovascular health and body fat distribution of the children. Additionallty, diet of the children was assessed with a food-frequency questionnaire, which enables future studies to examine if the effects of nutrition in early life are independent of diet later in life, and to explore the relation between diet in early life and diet at school-age.

5.4 Conclusion

In this thesis, the role of nutrition in cardiometabolic health in several stages of life was examined, using different approaches to nutritional epidemiology, namely nutrients (lutein and choline), food groups (sugar-containing beverages), and overall diet (*a priori* and *a posteriori* dietary patterns). With any nutritional exposure, the most important question that arise from observational studies is whether observed associations are causal, and this should be evaluated in experimental research. Confounding within diet is important, but it is demonstrated that also confounding outside diet is of great importance, as this thesis showed that the relation between maternal dietary patterns during pregnancy and offspring body composition was explained by maternal sociodemographic and lifestyle factors other than diet. The causality of the adverse effects of sugar-containing beverages on cardiometabolic health however has already been established in children, and in this thesis it is shown that this association is also present in the very young consumers, i.e. below the age of 2 years.

Therefore, to improve cardiometabolic health of young children, health promotion programs should focus on reducing the intake of sugar-containing beverages also in these very young children. Additionally, it was observed that sex differences exist in the relation of nutrition with cardiometabolic health already at this early age, and whether they are biological or cultural, they deserve more attention in research and preventive medicine in order to improve mechanistic understanding and effectiveness of interventions. Also, it was demonstrated that body mass index as a measure of adiposity for young children can be misleading when no distinction is made between fat mass and fat-free mass. Future research should thus continue to assess different measures of body fat mass which may be used to improve clinical practice regarding adiposity. In addition, the implementation of evidence-based nutritional advice to improve and maintain health is difficult in an obesogenic environment, and collaboration between nutritional scientists, medical doctors and the government is thus of paramount importance to reduce the burden of cardiometabolic diseases.



Chapter 6

Summary & Samenvatting

Short summary

In **chapter 1**, we describe the background of the research presented in this thesis. Cardiometabolic diseases are a major public health problem. One of the most important risk factors for cardiometabolic diseases is obesity. A healthy lifestyle could prevent a large proportion of cardiovascular mortality, and nutrition could play a major role in maintaining cardiometabolic health. Early life can be of paramount importance in this, as it is hypothesized that nutrition in early life can influence disease susceptibility in later life. Therefore, we aimed to study the role of nutrition in several stages of life using different approaches to nutritional epidemiology, namely nutrients, foods and dietary patterns, on cardiometabolic health in later life.

In **chapter 2**, we performed systematic reviews of the literature on the health effects of lutein and choline.

In **chapter 2.1**, we present a systematic review and meta-analysis of the literature on the effects of lutein on cardiometabolic health. The pooled results showed that higher lutein intake or blood levels were associated with a lower risk of coronary heart disease, stroke and metabolic syndrome, but not with type 2 diabetes mellitus. However, this evidence was solely based on observational studies. Literature on risk factors showed associations of higher lutein with lower levels of atherosclerosis and inflammatory markers. We found no consistent associations with other aspects of cardiometabolic health, and literature on health effects of lutein during fetal life or childhood is scarce. Our review shows that the role of lutein in cardiometabolic health is promising, but that future studies are needed to evaluate the causality of the associations.

In **chapter 2.2**, we present a systematic review of the literature on the effects of choline on cardiometabolic, respiratory and neurological health. While evidence from *in vitro* and animal studies suggests a causal relation between choline availability during pregnancy and cognition in later life, there is no conclusive evidence from human studies. In healthy adults, results regarding effects on cognition are inconsistent. We observed a beneficial effect on insulin sensitivity in some studies, but the overall evidence is scarce. Results were inconsistent for body composition, lipid levels and cardiovascular outcomes, but there were some indications that high choline intake might increase risk of cardiovascular disease. Further studies are needed to elucidate the effects of choline, especially on cardiometabolic health.

In **chapter 3**, we assessed the associations of maternal dietary patterns during pregnancy, with offspring body composition and cardiometabolic health, within the Generation R Study.

In **chapter 3.1**, we describe the associations of three different *a posteriori*-derived dietary patterns with child body composition. With principal component analysis we extracted a 'Vegetable, fish and oil', a 'Fruits, soy and high-fiber cereals' and a 'Margarine, snacks and sugar' dietary pattern. In an unadjusted model, we observed that higher adherence to the 'Vegetable, fish and oil' or the 'Fruits, soy and high-fiber cereals' dietary pattern was association with a more favorable body

composition, such as a lower body fat percentage and a lower android/gynoid fat mass ratio. However, after adjustment for maternal sociodemographic and lifestyle factors, all the associations disappeared. The 'Margarine, snacks and sugar' dietary pattern was not associated with any of the measures of body composition. In conclusion, our results from a cohort of healthy pregnant women do not show an independent relation between dietary patterns during pregnancy and offspring body composition.

In **chapter 3.2**, we assessed the relation of dietary patterns during pregnancy with measures of cardiometabolic health in offspring. We observed that there were no consistent associations between the *a posteriori*-derived dietary patterns and the cardiometabolic risk factor score, or with any of the individual components. We additionally calculated adherence to an *a priori*-defined dietary pattern based on dietary guidelines. We observed that higher adherence to the *a priori* dietary pattern was not consistently associated with offspring cardiometabolic outcomes. Our results are not in line with the hypothesis that maternal nutrition during pregnancy affects offspring cardiometabolic health.

In **chapter 4**, we evaluate the associations of nutrition of the child in early life with cardiometabolic health later in childhood, within the Generation R Study.

In **chapter 4.1**, we aimed to assess the role of lutein intake in children of 1 year of age, with cardiometabolic health at the age of 6 years. We found no consistent associations between lutein intake and any of the measures of body composition. Lutein intake was also not associated with a cardiometabolic risk factor score at the age of 6 years, or with any of the individual components. This study was the first in this age group that assessed the relation between lutein intake and cardiometabolic health. However, given the benefical effects of lutein on cardiometabolic health shown in adults, further studies need to examine the role of lutein in young children.

In **chapter 4.2**, we describe the relation between sugar-containing beverages in children at the age of 1 year, with body composition measures up to the age of 6 years. We observed that higher sugar-containing beverage intake was associated with higher BMI up to 6 years of age in girls, but not in boys. However, in boys, sugar-containing beverage intake at age 1 was associated with greater height at age 6. Our results suggest that sugar-containing beverage intake already at a young age can affect body composition unfavorably, and that the effects might be different between boys and girls.

In **chapter 4.3**, we present the results for the association of sugar-containing beverage intake with cardiometabolic health at the age of 6 years. We observed that higher sugar-containing beverage intake at 1 year of age was associated with a higher cardiometabolic risk factor score at 6 years of age. Stratification by sex revealed that this association was present in boys, but not in girls. Our findings highlight the need for dietary advice regarding limiting sugar-containing beverages, and that this advice should be given already before the age of 2 years. Possible sex differences in the associations of sugar-containing beverage intake and cardiometabolic health should be further explored.

In **chapter 4.4**, we assessed overall diet of the children at 1 year of age, using different approaches to dietary pattern analysis. We observed that higher adherence to *a priori*-defined diet quality or to an *a posteriori*-derived 'Health-conscious' dietary pattern at the age of 1 year was associated with a higher fat-free mass index at the age of 6 years, but not with fat mass index. Using reduced-rank regression, we identified a pattern that was associated with a higher fat free mass index, but not with fat mass index, which was characterized by high intake of whole grains, fruit and vegetable oils. A second dietary pattern that was identified using reduced-rank regression was positively associated with both fat mass index and fat free mass index, and was characterized by a high intake of refined grains, meat and sugar-containing beverages. These associations were all independent of total energy intake and sociodemographic and lifestyle factors. Our results confirm associations previously found in adults, but further studies on overall diet in early childhood in relation to body composition in later life are needed.

In **chapter 5**, a general discussion regarding the results of this thesis has been described. First, we give a summary of the background behind the specific studies in this studies, and present the main findings and conclusions from the individual chapters. Second, we discuss the different approaches to nutritional epidemiology. Throughout this thesis, we examined nutrients (lutein and choline), food products (sugar-containing beverages) and overall diet (*a priori* and *a posteriori* dietary patterns). The advantages and disadvantages of the different approaches are discussed, focusing on methodological aspects of design and analysis, and the implications that the results may have for future research and public health policies. The future directions for science and preventive health care are subsequently described for the specific results from this thesis: for nutrients and health across the life course, foods and child cardiometabolic health, and overall diet and child cardiometabolic health. In addition, we discuss possibilities for future research on measures of cardiometabolic health in children.

Uitgebreide samenvatting voor niet-ingewijden

Cardiometabole ziekten, waaronder hart- en vaatziekten en suikerziekte, zijn wereldwijd een groot probleem. Deze ziekten vergroten het risico op vroegtijdig overlijden en zorgen daarnaast ook op de korte termijn voor veel gezondheidsproblemen. Overgewicht is één van de belangrijkste risicofactoren voor het ontstaan van cardiometabole ziekten. Een gezonde leefstijl zou voor een groot gedeelte het ontstaan van deze ziekten kunnen voorkómen, en gezonde voeding kan dus in belangrijke mate bijdragen aan het behouden van cardiometabole gezondheid. Voeding in het vroege leven kan hierin een bijzondere rol spelen, omdat gedacht wordt dat voeding in het vroege leven het ontstaan van ziekten op latere leeftijd kan beïnvloeden. In dit proefschrift is daarom de relatie bestudeerd tussen voeding tijdens verschillende fasen van het leven en cardiometabole gezondheid. Er is op verschillende manieren gekeken naar voeding, namelijk naar voedingsstoffen, voedingsmiddelen en voedingspatronen. De achtergrond van dit proefschrift is verder uiteengezet in **hoofdstuk 1**.

In **hoofdstuk 2** is gekeken naar de effecten van de voedingsstoffen luteïne en choline op cardiometabole gezondheid gedurende alle levensfasen. Dit is gedaan door systematisch literatuuronderzoek. Hierbij werd gekeken naar de resultaten van gepubliceerde studies over de effecten van luteïne en choline en werden alle studies uitgebreid samengevat. De resultaten van de verschillende studies werden gecombineerd om tot een conclusie te komen.

Hoofdstuk 2.1 gaat over de effecten van luteïne, een voedingsstof die te vinden is in groenten, eieren en zuivelproducten. Luteïne is een belangrijke antioxidant en zou een gunstige invloed kunnen hebben op cardiometabole gezondheid. De gecombineerde resultaten van de onderzoeken die gedaan zijn naar de invloed van luteïne laten zien dat mensen met een hoger luteïne (voedselinname of bloedwaarden) een lager risico hadden op het krijgen van een hartinfarct of een beroerte. Ook hadden zij een lager risico op het metabool syndroom. Dit is een benaming voor enkele gezondheids-problemen die vaak tegelijkertijd aanwezig zijn, namelijk hoge bloeddruk, verstoorde bloedsuikerspiegels, slechte cholesterolwaarden en overgewicht. Voor deze gezondheids-problemen afzonderlijk werd er echter geen duidelijke relatie gevonden, en ook was er geen lager risico op het ontstaan van suikerziekte. Wel waren er onderzoeken die luteïne relateerden aan minder ontstekingsfactoren in het bloed en minder aderverkalking. Dit zou wellicht kunnen verklaren op welke manier luteïne het risico op hart- en vaatziekten zou verlagen.

Omdat een hoge inname van luteïne voor een groot gedeelte komt door een hoge inname van groenten, weet je echter niet zeker of de gevonden gunstige effecten inderdaad toe te schrijven zijn aan luteïne. Het zou ook kunnen dat andere voedingsstoffen uit het voedingspatroon of andere factoren van een gezonde leefstijl een rol spelen. Experimentele studies waarbij al deze factoren gelijk worden gehouden en alleen luteïne wordt verhoogd kunnen uitsluitsel geven over de effecten van luteïne voor cardiometabole gezondheid.

In **hoofdstuk 2.2** zijn vervolgens de effecten beschreven van choline op gezondheid tijdens verschillende fases van het leven. Choline is een voedingsstof die met name te vinden is in dierlijke producten zoals vlees, eieren en zuivel. Moedermelk bevat veel choline, en wellicht kunnen de positieve effecten van borstvoeding voor een gedeelte toegeschreven worden aan deze voedingsstof. Choline is een B-vitamine, en kan op verschillende manieren belangrijk zijn voor het lichaam. Er zijn aanwijzingen dat choline tijdens de zwangerschap belangrijk is voor de ontwikkeling van het ongeboren kind, met name voor een goede hersenontwikkeling en voor lager risico op aangeboren afwijkingen, zoals een open ruggetje. Het literatuuronderzoek liet echter zien dat er maar weinig onderzoek is gedaan bij mensen, en dat er nog geen bewijs is vanuit experimentele studies. Eerder onderzoek heeft kleine gunstige effecten laten zien van choline op cognitieve functies, zoals geheugen en concentratie, bij patiënten met cognitieve problemen. Het onderzoek in dit proefschrift heeft deze relatie echter niet gevonden in de gezonde bevolking. Onderzoek dat over choline gepubliceerd is heeft zich met name gericht op gezondheid van de hersenen en er is weinig onderzoek gedaan naar de effecten op cardiometabole gezondheid. Er zijn kleine, maar mogelijk relevante, aanwijzingen dat te veel choline een ongunstige effect heeft op cardiometabole gezondheid. Een hoge inname van choline zou het risico op cardiometabolie ziekten kunnen verhogen. De mogelijke effecten van choline moeten daarom zorgvuldig onderzocht worden, met name wat betreft cardiometabole gezondheid.

In **hoofdstuk 3 en 4** van dit proefschrift is onderzoek beschreven dat verricht is als onderdeel van het Generation R onderzoek. Het Generation R onderzoek is een langlopend bevolkingsonderzoek in Rotterdam. In dit onderzoek worden de gezondheid, groei en ontwikkeling van kinderen onderzocht, vanaf het begin van de zwangerschap. Aan het Generation R onderzoek mochten alle zwangere vrouwen uit Rotterdam deelnemen, maar in dit proefschrift zijn alleen vrouwen met Nederlandse ouders bestudeerd, omdat voedingspatronen beïnvloed kunnen worden door culturele aspecten

Tijdens de zwangerschap zijn de aanstaande ouders onderzocht en is er met behulp van vragenlijsten informatie verzameld over leeftijd, opleiding en inkomen, en leefstijlfactoren zoals stress, roken, alcoholgebruik en het gebruik van foliumzuursupplementen. Ook zijn gegevens verkregen via de verloskundigen, gynaecologen en consultatiebureaus.

Aan het begin van de zwangerschap hebben de moeders een uitgebreide vragenlijst ingevuld over hun voedingspatroon tijdens het eerste trimester van de zwangerschap. Toen hun kind 1 jaar oud was, hebben de moeders opnieuw een uitgebreide vragenlijst ingevuld over het voedingspatroon van hun kind.

Op de leeftijd van 6 jaar werden de kinderen samen met hun ouders uitgenodigd voor een bezoek aan het onderzoekscentrum van Generation R, alwaar ze uitgebreid werden onderzocht. Lengte, gewicht, vetmassa en vetvrije massa, en bloeddruk werden gemeten. Ook werd er bloed geprikt voor de bepaling van cholesterolwaarden en insulinewaarden.

Hoofdstuk 3 beschrijft de relatie van het voedingspatroon van de moeder tijdens de zwangerschap en de lichaamssamenstelling en cardiometabole gezondheid van hun kind. Eerder onderzoek heeft een verband gelegd tussen een laag geboortegewicht en het risico op cardiometabole ziekten op latere leeftijd. Ondervoeding tijdens de zwangerschap kan leiden tot een laag geboortegewicht van het kind en zou dus de onderliggende factor kunnen zijn voor dit verband. Tijdens de zwangerschap past het ongeboren kind zich aan aan de omstandigheden in de baarmoeder, en deze aanpassingen vergroten de kans op overleven op de korte termijn, maar kunnen ongunstig zijn op de lange termijn. Eerder heeft het Hongerwinter onderzoek aangetoond dat kinderen van moeders die zwanger waren tijdens de hongerwinter inderdaad vaker hart- en vaatziekten en suikerziekte hadden op volwassen leeftijd. Daarnaast kan ook een teveel aan energie tijdens de zwangerschap leiden tot ongunstige aanpassingen van het ongeboren kind. Onderzoek heeft zich tot op heden met name gericht op voedingsstoffen en er is maar weinig bekend over de lange termijn effecten van voedingspatronen tijdens de zwangerschap.

In **hoofdstuk 3.1** is onderzocht wat de relatie is tussen verschillende voedingspatronen van de moeder tijdens de zwangerschap en de lichaamssamenstelling van het kind op de leeftijd van 6 jaar. De resultaten van dit onderzoek lieten zien dat kinderen van moeders die meer volgens een patroon aten gekenmerkt door een hoge inname van groenten, vis en plantaardige oliën of volgens een patroon met een hoge inname van fruit, soja en volkoren-producten, op 6-jarige leeftijd een gunstigere lichaamssamenstelling hadden. Deze kinderen hadden namelijk een lager vetpercentage en hadden minder vaak overgewicht, in vergelijking met kinderen van moeders die minder aten volgens deze patronen. Omdat voedingspatronen ook gerelateerd zijn aan andere leefstijl en sociaaldemografische factoren is ook onderzocht wat het effect is van deze factoren op de relatie tussen voedingspatronen en lichaamssamenstelling. Het bleek dat de relatie voornamelijk verklaard werd door deze andere factoren. Er was dus geen direct verband van het voedingspatroon van de moeder met de lichaamssamenstelling van hun kind. Een patroon gekenmerkt door een hoge inname van boter, zoetwaren en tussendoortjes was helemaal niet gerelateerd aan lichaams-samenstelling van het kind.

In **hoofdstuk 3.2** is vervolgens onderzocht wat de relatie tussen de eerder genoemde voedingspatronen en de cardiometabole gezondheid van de kinderen. De resultaten van dit onderzoek lieten geen verband zien tussen voedingspatronen van de moeder tijdens de zwangerschap met bloeddruk, insulinewaarden of cholesterolwaarden van de kinderen. Ook is onderzocht of de mate waarin moeders zich houden aan de richtlijnen voor goede voeding verband hield met cardiometabole gezondheid van de kinderen, maar ook daar werd geen duidelijk verband gezien.

De onderzoeksresultaten van **hoofdstuk 3** bevestigen de mogelijke effecten van voeding tijdens de zwangerschap op gezondheid van het kind niet. Vervolgonderzoek is nodig om te bestuderen of de relatie wellicht wel aanwezig is in andere bevolkingsgroepen.

In **hoofdstuk 4** van dit proefschrift is gekeken naar de voeding van het kind op de peuterleeftijd in relatie tot cardiometabole gezondheid op de kinderleeftijd. Er is veel onderzoek gedaan naar de effecten van voeding bij pasgeborenen, bijvoorbeeld borstvoeding of flesvoeding. Er is echter nog weinig bekend over de effecten van voeding op de peuterleeftijd. Voeding op de peuterleeftijd is erg belangrijk, omdat voorkeuren voor smaken en producten zich ontwikkelen in het vroege leven en aanhouden gedurende de kindertijd tot aan de volwassenheid. Daarnaast kan voeding in het vroege leven ook effect hebben op latere gezondheid omdat voeding de groei en ontwikkeling van kinderen rechtstreeks kan beïnvloeden.

Het literatuuronderzoek in **hoofdstuk 2.1** liet zien dat de antioxidant luteïne misschien gunstig kan zijn voor de cardiometabole gezondheid van volwassenen. Er kwam uit het onderzoek echter ook naar voren dat er nog geen onderzoek is gedaan naar luteïne in relatie tot cardiometabole gezondheid van jonge kinderen. De doelstelling van **hoofdstuk 4.1** was daarom te onderzoeken of er een relatie is tussen luteïne inname en lichaamssamenstelling en cardiometabole gezondheid binnen het Generation R onderzoek. De resultaten van dit onderzoek lieten zien dat er in geen relatie was tussen luteïne inname op 1-jarige leeftijd en lichaamssamenstelling en cardiometabole gezondheid op 6-jarige leeftijd. Omdat dit het eerste onderzoek bij kinderen was dat deze relatie bestudeerde, kunnen er nog geen duidelijke conclusies worden getrokken. Vervolgonderzoek in andere bevolkingsgroepen en op andere leeftijden is daarom nodig.

Hoofdstuk 4.2 en 4.3 hebben zich gericht op de inname van suikerrijke dranken bij kinderen op de peuterleeftijd. Suikerrijke dranken bevatten veel calorieën maar hebben weinig effect op het verzadingsgevoel. Inname van suikerrijke dranken kan dus ongemerkt leiden tot een hoge energie-inname en een hoger lichaamsgewicht tot gevolg hebben. Inname van suikerrijke dranken kan ook het risico op het metabool syndroom verhogen. Dit is niet alleen vanwege een hoger lichaamsgewicht, maar suikerrijke dranken kunnen ook de andere factoren van het metabool syndroom beïnvloeden, namelijk cholesterolwaarden, bloedsuikerspiegels en bloeddruk.

Er is bij basisschoolkinderen, adolescenten en volwassen al veel onderzoek gedaan naar deze mogelijke effecten van suikerrijke dranken, ook door middel van experimentele onderzoeken. Er is daarom voldoende bewijs dat suikerrijke dranken cardiometabole gezondheid ongunstig beïnvloeden. Gezondheidsprogramma's hebben zich daarom ook gericht op het verminderen van de inname van suikerrijke dranken. Deze gezondheidsprogramma's hebben zich echter voornamelijk gericht op kinderen op basisscholen en middelbare scholen. Dit is relevant omdat kinderen in de schoolleeftijd inderdaad een hoge inname van suikerrijke dranken zoals frisdranken en sportdranken kunnen hebben. Dat is in de peuterleeftijd nog nauwelijks het geval. Dit is wellicht de reden dat er weinig onderzoek is gedaan naar de gevolgen van suikerrijke dranken op de peuterleeftijd. Peuters drinken echter al wel vruchtensappen en vruchtenconcentraten. Daarvan zijn dezelfde ongunstige effecten op de cardiometabole gezondheid te verwachten.

Hoofdstuk 4.2 laat zien dat inname van suikerrijke dranken op de leeftijd van 1 jaar inderdaad gerelateerd is aan gewichtstoename. Deze relatie was echter alleen zichtbaar bij meisjes, en niet bij jongens. Meisjes met een hoge inname van suikerrijke dranken op de leeftijd van 1 jaar hadden een hoger BMI vergeleken met meisjes met een lage inname van suikerrijke dranken, een verschil dat aanhield tot de leeftijd van 6 jaar. Deze relatie werd niet verklaard door sociaaldemografische of leefstijlfactoren, en ook niet door een hogere inname van zoetwaren of tussendoortjes. Bij jongens was er geen relatie tussen inname van suikerrijke dranken op de peuterleeftijd en BMI in de eerste levensjaren. Wel waren jongens met een hoge inname van suikerrijke dranken op 1-jarige leeftijd langer op de leeftijd van 6 jaar dan jongens met een lage inname. Verder onderzoek is nodig om te beoordelen wat de gevolgen hiervan zijn op latere leeftijd.

In **hoofdstuk 4.3** is vervolgens de cardiometabole gezondheid van de kinderen onderzocht. Op basis van de factoren van het metabool syndroom is een totaalscore gemaakt van cardiometabole risicofactoren. Deze score bestond uit het vetpercentage, de bloeddruk, de cholesterolwaarden en de insulinewaarden van de kinderen op de leeftijd van 6 jaar. Jongens die een hoge inname van suikerrijke dranken hadden op de leeftijd van 1 jaar hadden een hogere cardiometabole risicofactor score op 6-jarige leeftijd dan jongens met een lage inname. Deze relatie werd gevonden met de totaalscore, en geen van de individuele factoren leek deze relatie te verklaren. Bij meisjes vonden we geen relatie tussen inname van suikerrijke dranken en de cardiometabole gezondheidsfactoren.

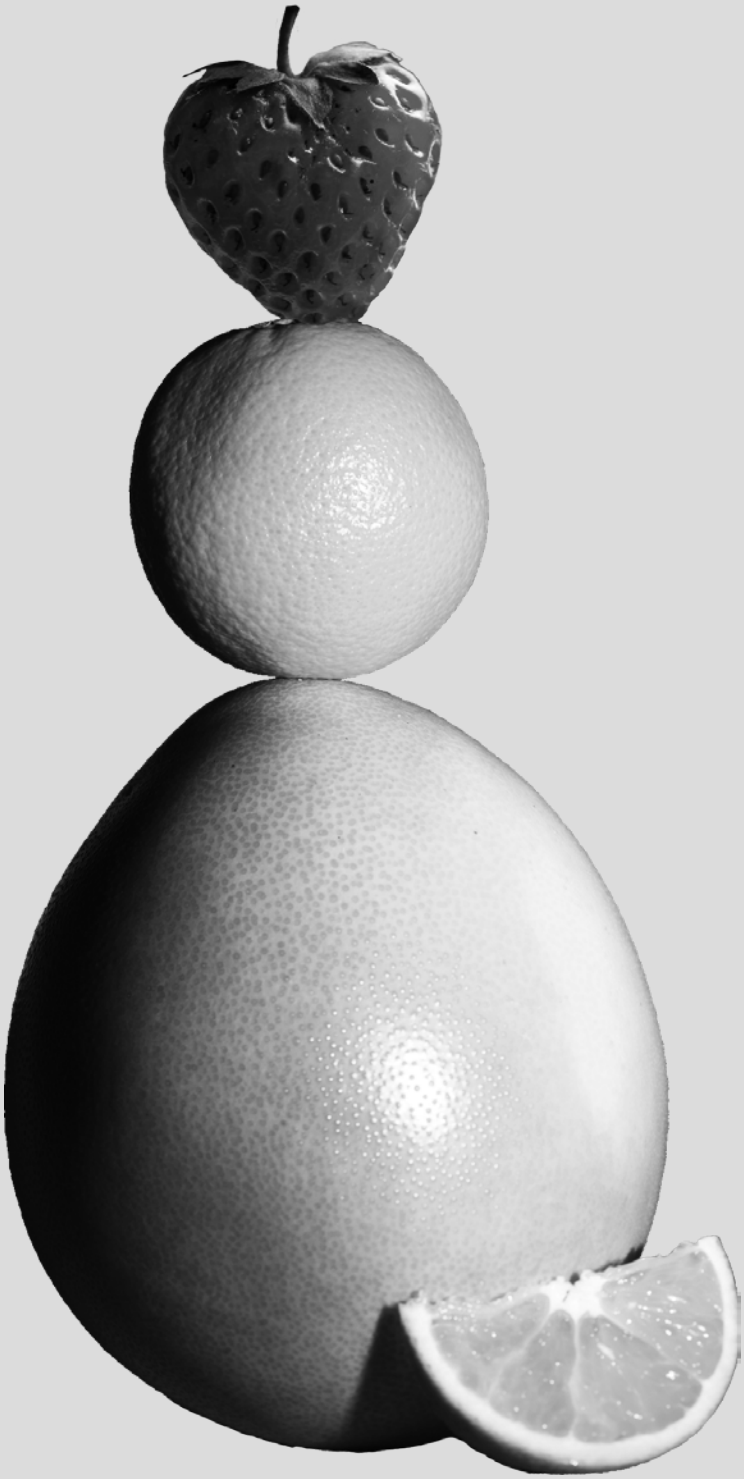
De bevindingen van **hoofdstuk 4.2 en 4.3** tonen aan dat suikerrijke dranken al op jonge leeftijd nadelige effecten kunnen hebben en benadrukken dat advies noodzakelijk is over het beperken van suikerrijke dranken voor kinderen in de peuterleeftijd.

De verschillen tussen jongens en meisjes waren nog niet eerder gerapporteerd. Andere onderzoeken hebben eventuele verschillen tussen jongens en meisje van de effecten van suikerrijke dranken niet bestudeerd. Het is dus onduidelijk of deze verschillen ook aanwezig zijn in andere bevolkingsgroepen. Het is ook niet duidelijk of de verschillen tussen jongens en meisjes veroorzaakt worden door biologische verschillen, zoals hormonale invloeden, of door culturele verschillen, zoals leefstijlfactoren. Verder onderzoek is nodig om te bevestigen of er inderdaad verschillen zijn tussen jongens en meisjes wat betreft de effecten van suikerrijke dranken op lichaamssamenstelling en cardiometabole gezondheid, en waar deze verschillen mogelijk door verklaard kunnen worden.

In **hoofdstuk 4.4** is gekeken naar het gehele voedingspatroon van de kinderen op de leeftijd van 1 jaar, in relatie tot lichaamssamenstelling op de leeftijd van 6 jaar. Het voedingspatroon van de kinderen is op verschillende manier bestudeerd. Kinderen die op 1-jarige leeftijd meer aten volgens een 'gezondheidsbewust' patroon hadden een hoger BMI op 6-jarige leeftijd. Specifieker gekeken naar vetmassa en vetvrije massa, bleek dat het hogere BMI bij deze kinderen verklaard werd door een hogere vetvrije massa. Dezelfde relatie werd gezien bij kinderen die in hoge mate voldoen aan de voedingsrichtlijnen.

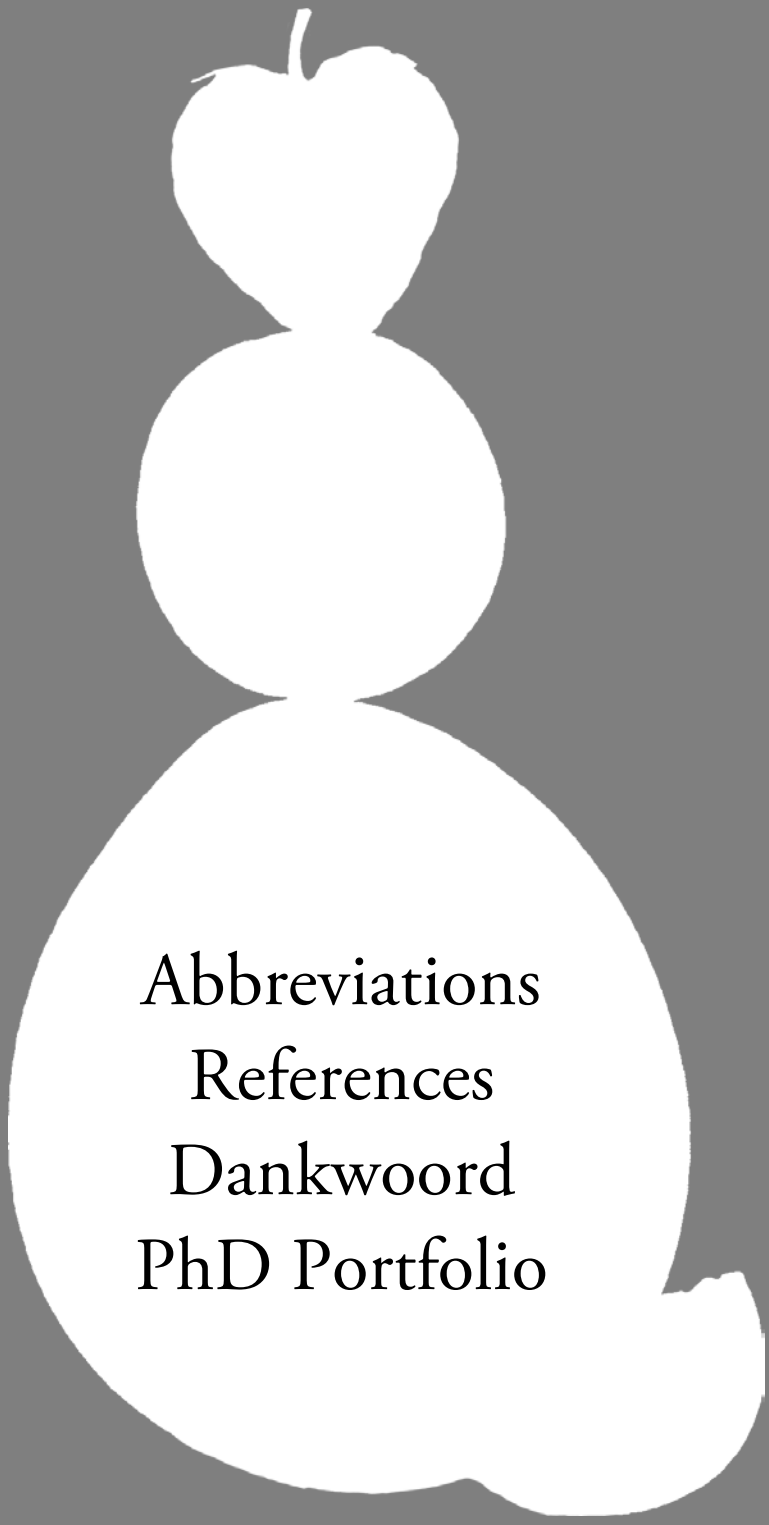
Ook werd gezocht naar voedingspatronen op basis van de lichaamssamenstelling van de kinderen. Een patroon dat gerelateerd was aan een hoger vetpercentage, werd gekenmerkt door een hoge inname van geraffineerde graanproducten, aardappels, vlees, vis, soepen en sauzen en suikerrijke dranken. Het patroon dat gerelateerd was aan een lager vetpercentage werd gekenmerkt door een hoge inname van volkorenproducten, rijst en pasta, zuivel, groenten en fruit, plantaardige oliën en suikervrije dranken. Deze resultaten laten zien dat voedingspatronen op de peuterleeftijd de lichaamssamenstelling kunnen beïnvloeden. Gezondheidsprogramma's gericht op de preventie van overgewicht moeten zich dus ook richten op het verbeteren van het voedingspatroon van jonge kinderen.

Tot slot geeft **hoofdstuk 5** een algemene discussie over het onderzoek beschreven in dit proefschrift. Er wordt een samenvatting gegeven van de achtergrond van de verschillende studies en de belangrijkste bevindingen en conclusies van elk hoofdstuk worden genoemd. Ook worden de voor- en nadelen van de verschillende benaderingen besproken: voedingsstoffen, voedingsmiddelen, en voedingspatronen. Er wordt een aantal belangrijke methodologische aspecten bediscussieerd van elke benadering, evenals de mogelijke implicaties voor de wetenschap en voor de maatschappij. Vervolgens worden de mogelijke gevolgen van de specifieke resultaten van dit proefschrift besproken voor de preventieve geneeskunde en worden aanwijzingen gegeven voor vervolgonderzoek.



Chapter 7

Appendices



Abbreviations
References
Dankwoord
PhD Portfolio

Abbreviations

AG-ratio	android/gynoid fat ratio
AMD	age-related macular degeneration
AMI	acute myocardial infarction
ANOVA	analysis of variance
BF%	body fat percentage
BMI	body mass index
BW	birth weight
CC	case-control study
CDP-choline	cytidine diphosphate-choline
CHD	coronary heart disease
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRP	c-reactive protein
CVD	cardiovascular disease
d	days
D+L	DerSimonian and Laird
DBP	diastolic blood pressure
DHD	Dutch Healthy Diet
DHDI	Dutch Healthy Diet Index
DIP	diabetes in pregnancy
DMT2	Diabetes Mellitus Type 2
DXA	dual-energy X-ray absorptiometry
ECTS	European Credit Transfer System
FFMI	fat free mass index
FFQ	food-frequency questionnaire
FMI	fat mass index
FPG	fasting plasma glucose
FUP	follow-up
g	gram
GA	gestational age
GPC	glycerylphosphorylcholine
h	hours
HbA1c	glycated hemoglobin
HDL-c	high density lipoprotein cholesterol
HIV	human immunodeficiency virus
HOMA	homeostatic model assessment

HR	hazard ratio
IFN-gamma	interferon gamma
IL	interleukin
IMT	intima-media thickness
IQ	intelligence quotient
IQR	inter-quartile range
I-V	inverse variance
JAMA	Journal of the American Medical Association
kcal	kilocalorieën
kg	kilogram
L	liter
LBW	low birth weight
LDL-c	low density lipoprotein cholesterol
LL	lower limit
LMM	linear mixed model
m	meter
MANOVA	multivariate analysis of fariance
MAP	mean arterial pressure
mcg	microgram
MeSH	Medical Subject Headings
mg	milligram
MI	myocardial infarction
min	minutes
ml	milliliter
mmol	millimole
mo	months
MRI	magnetic resonance imaging
n	number
N. Ireland	Northern Ireland
n-3	omega-3
n-6	omega-6
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NASO	Netherlands Associations Study on Obesity
NAV	The Nederlandse Academie voor Voedingswetenschappen
NIHES	The Netherlands Institute for Health Sciences
NM	not mentioned
nmol	nanomole

NR	not reported
NS	not significant
NTD	neural tube defects
NWO	Nederlandse Organisatie voor Wetenschappelijk Onderzoek
OR	odds ratio
PC	phosphatidylcholine
PCA	principal component analysis
PEMT	phosphatidylethanolamine-N-methyltransferase
PLG	post-load glucose
PPLCH	phospholipid bound choline
PWV	pulse wave velocity
Q	quartile
QS	quality score
RD	retrospective data-collection
ref	reference group
RR	relative risk
RRR	reduced-rank regression
SBP	systolic blood pressure
SCB	sugar-containing beverage
SD(S)	standard deviation (scores)
SE	standard error
SIGN-E	Special Interest Group Nutritional Epidemiology
T	tertile
TAG	triacylglycerol
TMAO	trimethylamine-N-oxide
TNF-alpha	tumor necrosis factor alpha
TV	television
UK	United Kingdom
UL	upper limit
USA	United States of America
VENA	Vrouwen binnen Erasmus MC Netwerk voor Academici
vit	vitamin
VLDL-c	very low density lipoprotein cholesterol
vs	versus
WeVo	Werkgroep Voedingsgewoonten
WHO	World Health Organisation
wk	weeks
y	years

References

1. World Health Organisation. Global satus report on noncommunicable diseases 2014. Geneva.
2. US Department of Health and Human Services. Centres for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States. Atlanta. 2014
3. Rubin RR, Peyrot M. Quality of life and diabetes. *Diabetes Metab Res Rev* 1999
4. Failde II, Soto MM. Changes in health related quality of life 3 months after an acute coronary syndrome. *BMC Public Health* 2006
5. Anderson KM, Odell PM, Wilson PWF, *et al.* Cardiovascular-Disease Risk Profiles. *Am Heart J* 1991
6. Bonow RO. Primary prevention of cardiovascular disease - A call to action. *Circulation* 2002
7. Isomaa B, Almgren P, Tuomi T, *et al.* Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001
8. Alberti KGMM, Eckel RH, Grundy SM, *et al.* Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009
9. Ford ES, Li C. Metabolic syndrome and health-related quality of life among U.S. adults. *Ann Epidemiol* 2008
10. Haslam DW, James WPT. Obesity. *Lancet* 2005
11. Sun Q, Townsend MK, Okereke OI, *et al.* Adiposity and weight change in mid-life in relation to healthy survival after age 70 in women: prospective cohort study. *Br Med J* 2009
12. de Onis M, Blossner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *Am J Clin Nutr* 2010
13. Centraal Bureau voor de Statistiek.
14. Reilly JJ, Methven E, McDowell ZC, *et al.* Health consequences of obesity. *Arch Dis Child* 2003
15. Murray CJL, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet* 1997
16. American Heart Association Nutrition C, Lichtenstein AH, Appel LJ, *et al.* Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006
17. Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: A systematic review. *Nutr Rev* 2006
18. Willett W. Nutritional epidemiology. New York: Oxford University Press. 2013
19. Malik VS, Pan A, Willett WC, *et al.* Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *Am J Clin Nutr* 2013
20. Malik VS, Popkin BM, Bray GA, *et al.* Sugar-Sweetened Beverages, Obesity, Type 2 Diabetes Mellitus, and Cardiovascular Disease Risk. *Circulation* 2010
21. Malik VS, Popkin BM, Bray GA, *et al.* Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care* 2010
22. He FJ, Nowson CA, MacGregor GA. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet* 2006
23. Li M, Fan YL, Zhang XW, *et al.* Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies. *BMJ Open* 2014
24. Carter P, Gray LJ, Troughton J, *et al.* Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *Br Med J* 2010
25. Giordano P, Scicchitano P, Locorotondo M, *et al.* Carotenoids and Cardiovascular Risk. *Curr Pharm Des* 2012
26. Hardy R, Lawlor DA, Kuh D. A life course approach to cardiovascular aging. *Future Cardiol* 2015
27. Christian P, Stewart CP. Maternal Micronutrient Deficiency, Fetal Development, and the Risk of Chronic Disease. *J Nutr* 2010
28. Craigie AM, Lake AA, Kelly SA, *et al.* Tracking of obesity-related behaviours from childhood to adulthood: A systematic review. *Maturitas* 2011
29. Franco OH, Karnik K, Osborne G, *et al.* Changing course in ageing research: The Healthy Ageing Phenotype. *Maturitas* 2009
30. Felix JF, Voortman T, van den Hooven EH, *et al.* Health in children: A conceptual framework for use in healthy ageing research. *Maturitas* 2014
31. *Quality Assessment Tool for Quantitative Studies.*: National Collaborating Centre for Methods and Tools. Hamilton, McMaster University, 2008: Updated 13 April, 2010. Retrieved from http://www.nccmt.ca/registry/view/eng/14.html.
32. Conant R, Schauss AG. Therapeutic applications of citicoline for stroke and cognitive dysfunction in the elderly: a review of the literature. *Altern Med Rev* 2004
33. McCann JC, Hudes M, Ames BN. An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring. *Neurosci Biobehav Rev* 2006
34. Jaddoe VWV, van Duijn CM, Franco OH, *et al.* The Generation R Study: design and cohort update 2012. *Eur J Epidemiol* 2012
35. Kijlstra A, Tian Y, Kelly ER, *et al.* Lutein: More than just a filter for blue light. *Prog Retin Eye Res* 2012
36. Sujak A, Gabrielska J, Grudzinski W, *et al.* Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: The structural aspects. *Arch Biochem Biophys* 1999
37. Voutilainen S, Nurmi T, Mursu J, *et al.* Carotenoids and cardiovascular health. *Am J Clin Nutr* 2006
38. Karppi J, Laukkanen JA, Makikallio TH, *et al.* Low beta-carotene concentrations increase the risk of cardiovascular disease mortality among Finnish men with risk factors. *Nutrition Metabolism and Cardiovascular Diseases* 2012
39. Fortmann SP, Burda BU, Senger CA, *et al.* Vitamin and Mineral Supplements in the Primary Prevention of Cardiovascular Disease and Cancer: An Updated Systematic Evidence Review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2013
40. Bjelakovic G, Nikolova D, Gluud LL, *et al.* Mortality in randomized trials of antioxidant supplements for primary and secondary prevention - Systematic review and meta-analysis. *Jama-Journal of the American Medical Association* 2007
41. Sommer A, Vyas KS. A global clinical view on vitamin A and carotenoids. *Am J Clin Nutr* 2012
42. Danesh J, Collins R, Appleby P, *et al.* Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease - Meta-analyses of prospective studies. *Jama-Journal of the American Medical Association* 1998
43. Chene G, Binquet C, Moreau JF, *et al.* Changes in CD4+ cell count and the risk of opportunistic infection or death after highly active antiretroviral treatment. *AIDS* 1998
44. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002
45. Ogilvie D, Fayter D, Petticrew M, *et al.* The harvest plot: A method for synthesising evidence about the differential effects of interventions. *BMC Med Res Methodol* 2008
46. Capeding R, Gepanayao CP, Calimon N, *et al.* Lutein-fortified infant formula fed to healthy term infants: evaluation of growth effects and safety. *Nutrition Journal* 2010
47. Melikian G, Mmiro F, Ndugwa C, *et al.* Relation of vitamin A and carotenoid status to growth failure and mortality among ugandan infants with human immunodeficiency virus. *Nutrition* 2001
48. Beydoun MA, Canas JA, Beydoun HA, *et al.* Serum Antioxidant Concentrations and Metabolic Syndrome Are Associated among U.S. Adolescents in Recent National Surveys. *J Nutr* 2012
49. Masters ET, Jedrychowski W, Schleicher RL, *et al.* Relation between prenatal lipid-soluble micronutrient status, environmental pollutant exposure, and birth outcomes. *Am J Clin Nutr* 2007
50. Ford ES, Giles WH. Serum vitamins, carotenoids, and angina pectoris: Findings from the National Health and Nutrition Examination Survey III. *Ann Epidemiol* 2000
51. Gey KF, Ducimetiere P, Evans A, *et al.* Low plasma retinol predicts coronary events in healthy middle-aged men: The PRIME Study. *Atherosclerosis* 2010
52. Street DA, Comstock GW, Salkeld RM, *et al.* Serum antioxidants and myocardial infarction: Are low levels of carotenoids and (alpha)-tocopherol risk factors for myocardial infarction? *Circulation* 1994
53. Hak AE, Stampfer MJ, Campos H, *et al.* Plasma Carotenoids and Tocopherols and risk of myocardial infarction in a low-risk population of US male physicians. *Circulation* 2003
54. Koh WP, Yuan JM, Wang R, *et al.* Plasma carotenoids and risk of acute myocardial infarction in the Singapore Chinese Health Study. *Nutrition Metabolism and Cardiovascular Diseases* 2011
55. Tavani A, Gallus S, Negri E, *et al.* Dietary intake of carotenoids and retinol and the risk of acute myocardial infarction in Italy. *Free Radic Res* 2006

56. Kabagambe EK, Furtado J, Baylin A, *et al.* Some dietary and adipose tissue carotenoids are associated with the risk of nonfatal acute myocardial infarction in Costa Rica. *J Nutr* 2005
57. Osganian SK, Stampfer MJ, Rimm E, *et al.* Dietary carotenoids and risk of coronary artery disease in women. *Am J Clin Nutr* 2003
58. Knekt P, Ritz J, Pereira MA, *et al.* Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am J Clin Nutr* 2004
59. Murr C, Winklhofer-Roob BM, Schroecksnadel K, *et al.* Inverse association between serum concentrations of neopterin and antioxidants in patients with and without angiographic coronary artery disease. *Atherosclerosis* 2009
60. Ruiz Rejon F, Martin-Pena G, Granado F, *et al.* Plasma status of retinol, (alpha)- and (gamma)-tocopherols, and main carotenoids to first myocardial infarction: Case control and follow-up study. *Nutrition* 2002
61. Rerksuppaphol S, Chansiri K, Rerksuppaphol L, *et al.* Correlation of low levels of serum carotenoids with increased risk of cardiovascular diseases. *Biomedicine* 2010
62. Ito Y, Suzuki K, Ishii J, *et al.* A population-based follow-up study on mortality from cancer or cardiovascular disease and serum carotenoids, retinol and tocopherols in Japanese inhabitants. *Asian Pac J Cancer Prev* 2006
63. Hak AE, Ma J, Powell CB, *et al.* Prospective study of plasma carotenoids and tocopherols in relation to risk of ischemic stroke. *Stroke* 2004
64. Hirvonen T, Virtamo J, Korhonen P, *et al.* Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers. *Stroke* 2000
65. Ascherio A, Rimm EB, Hernan MA, *et al.* Relation of consumption of vitamin E, vitamin C, and carotenoids to risk for stroke among men in the United States. *Ann Intern Med* 1999
66. Bates CJ, Hamer M, Mishra GD. Redox-modulatory vitamins and minerals that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over. *Br J Nutr* 2011
67. Mayne ST, Cartmel B, Lin HQ, *et al.* Low plasma lycopene concentration is associated with increased mortality in a cohort of patients with prior oral, pharynx or larynx cancers. *J Am Coll Nutr* 2004
68. Shardell MD, Alley DE, Hicks GE, *et al.* Low-serum carotenoid concentrations and carotenoid interactions predict mortality in US adults: the Third National Health and Nutrition Examination Survey. *Nutrition Research* 2011
69. Buijsse B, Feskens, E. J. M., Kwape, L., Kok, F. J., Kromhout, D. Both (alpha)- and (beta)-carotene, but not tocopherols and vitamin C, are inversely related to 15-year cardiovascular mortality in Dutch elderly men. *J Nutr* 2008
70. Sesso HD, Buring JE, Norkus EP, *et al.* Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in women. *Am J Clin Nutr* 2004
71. Sesso HD, Buring JE, Norkus EP, *et al.* Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in men. *Am J Clin Nutr* 2005
72. Hozawa A, Jacobs DR, Steffes MW, *et al.* Associations of serum carotenoid concentrations with the development of diabetes and with insulin concentration: Interaction with smoking - The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Am J Epidemiol* 2006
73. Kataja-Tuomola MK, Kontto JP, Mannisto S, *et al.* Intake of antioxidants and risk of type 2 diabetes in a cohort of male smokers. *Eur J Clin Nutr* 2011
74. Montonen J, Knekt, P., Jarvinen, R., Reunanen, A. Dietary Antioxidant Intake and Risk of Type 2 Diabetes. *Diabetes Care* 2004
75. Wang L, Liu SM, Pradhan AD, *et al.* Plasma lycopene, other carotenoids, and the risk of type 2 diabetes in women. *Am J Epidemiol* 2006
76. Beydoun MA, Shroff MR, Chen X, *et al.* Serum antioxidant status is associated with metabolic syndrome among U.S. adults in recent national surveys. *J Nutr* 2011
77. Coyne T, Ibiebele TI, Baade PD, *et al.* Metabolic syndrome and serum carotenoids: findings of a cross-sectional study in Queensland, Australia. *Br J Nutr* 2009
78. Sluijs I, Beulens JWJ, Grobbee DE, *et al.* Dietary Carotenoid Intake Is Associated with Lower Prevalence of Metabolic Syndrome in Middle-Aged and Elderly Men. *J Nutr* 2009
79. Sugiura M, Nakamura M, Ogawa K, *et al.* Associations of serum carotenoid concentrations with the metabolic syndrome: interaction with smoking. *Br J Nutr* 2008
80. Suzuki K, Ito Y, Inoue T, *et al.* Inverse association of serum carotenoids with prevalence of metabolic syndrome among Japanese. *Clin Nutr* 2011
81. Hozawa A, Jacobs DR, Steffes MW, *et al.* Circulating carotenoid concentrations and incident hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *J Hypertens* 2009
82. D'Odorico A, Martines D, Kiechl S, *et al.* High plasma levels of (alpha)- and (beta)-carotene are associated with a lower risk of atherosclerosisResults from the Bruneck study. *Atherosclerosis* 2000
83. Iribarren C, Folsom AR, Jacobs DR, *et al.* Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis - A case-control study. *Arteriosclerosis Thrombosis and Vascular Biology* 1997
84. Karppi J, Kurl S, Laukkanen JA, *et al.* Plasma carotenoids are related to intima - media thickness of the carotid artery wall in men from eastern Finland. *J Intern Med* 2011
85. Klipstein-Grobusch K, Launer LJ, Geleijnse JM, *et al.* Serum carotenoids and atherosclerosis - The Rotterdam Study. *Atherosclerosis* 2000
86. Nieto FJ, Iribarren C, Gross MD, *et al.* Uric acid and serum antioxidant capacity: a reaction to atherosclerosis? *Atherosclerosis* 2000
87. Dwyer JH, Navab M, Dwyer KM, *et al.* Oxygenated carotenoid lutein and progression of early atherosclerosis - The Los Angeles Atherosclerosis Study. *Circulation* 2001
88. Polidori MC, Pratico D, Parente B, *et al.* Elevated lipid peroxidation biomarkers and low antioxidant status in atherosclerotic patients with increased carotid or iliofemoral intima media thickness. *J Investig Med* 2007
89. Xu XR, Zou ZY, Huang YM, *et al.* Serum carotenoids in relation to risk factors for development of atherosclerosis. *Clin Biochem* 2012
90. Zou ZY, Xu XR, Huang YM, *et al.* High serum level of lutein may be protective against early atherosclerosis: The Beijing atherosclerosis study. *Atherosclerosis* 2011
91. Zou ZY, Xu XR, Lin XM, *et al.* Effects of lutein and lycopene on carotid intima-media thickness in Chinese subjects with subclinical atherosclerosis: a randomised, double- blind, placebo- controlled trial. *Br J Nutr* 2014
92. Tornwall ME, Virtamo J, Haukka JK, *et al.* Prospective study of diet, lifestyle, and intermittent claudication in male smokers. *Am J Epidemiol* 2000
93. Nakamura M, Sugiura M, Aoki N. High beta-carotene and beta-cryptoxanthin are associated with low pulse wave velocity. *Atherosclerosis* 2006
94. Hozawa A, Jacobs DR, Steffes MW, *et al.* Relationships of circulating carotenoid concentrations with several markers of inflammation, oxidative stress, and endothelial dysfunction: The Coronary Artery Risk Development in Young Adults (CARDIA)/Young Adult Longitudinal Trends in Antioxidants (YALTA) Study. *Clin Chem* 2007
95. Wang JW, Mark S, Henderson M, *et al.* Adiposity and glucose intolerance exacerbate components of metabolic syndrome in children consuming sugar-sweetened beverages: QUALITY cohort study. *Pediatr Obes* 2013
96. Wang Y, Chung SJ, McCullough ML, *et al.* Dietary Carotenoids Are Associated with Cardiovascular Disease Risk Biomarkers Mediated by Serum Carotenoid Concentrations. *J Nutr* 2014
97. Almushatat ASK, Talwar D, McArdle PA, *et al.* Vitamin antioxidants, lipid peroxidation and the systemic inflammatory response in patients with prostate cancer. *Int J Cancer* 2006
98. Brighenti F, Valtuena S, Pellegrini N, *et al.* Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. *Br J Nutr* 2005
99. Graydon R, Hogg RE, Chakravarthy U, *et al.* The effect of lutein- and zeaxanthin-rich foods v. supplements on macular pigment level and serological markers of endothelial activation, inflammation and oxidation: pilot studies in healthy volunteers. *Br J Nutr* 2012
100. Rowley K, Walker KZ, Cohen J, *et al.* Inflammation and vascular endothelial activation in an Aboriginal population: relationships to coronary disease risk factors and nutritional markers. *Med J Aust* 2003
101. Sundl I, Roob JM, Meinitzer A, *et al.* Antioxidant Status of Patients on Peritoneal Dialysis: Associations with Inflammation and Glycoxidative Stress. *Perit Dial Int* 2009
102. Suzuki K, Inoue T, Hashimoto S, *et al.* Association of serum carotenoids with high molecular weight adiponectin and inflammation markers among Japanese subjects. *Clin Chim Acta* 2010
103. van Herpen-Broekmans WMR, Klopping-Ketelaars IAA, Bots ML, *et al.* Serum carotenoids and vitamins in relation to markers of endothelial function and inflammation. *Eur J Epidemiol* 2004
104. D'Adamo CR, Miller RR, Shardell MD, *et al.* Higher serum concentrations of dietary antioxidants are associated with lower levels of inflammatory biomarkers during the year after hip fracture. *Clin Nutr* 2012
105. Xu XR, Zou ZY, Xiao X, *et al.* Effects of Lutein Supplement on Serum Inflammatory Cytokines, ApoE and Lipid Profiles in Early Atherosclerosis Population. *J Atheroscler Thromb* 2012
106. Ben Amara N, Tourniaire F, Maraninchi M, *et al.* Independent positive association of plasma (beta)-carotene concentrations with adiponectin among non-diabetic obese subjects. *Eur J Nutr* 2014

107. Yeon JY, Kim HS, Sung MK. Diets rich in fruits and vegetables suppress blood biomarkers of metabolic stress in overweight women. *Prev Med* 2012
108. Waters D, Clark RM, Greene CM, *et al.* Change in plasma lutein after egg consumption is positively associated with plasma cholesterol and lipoprotein size but negatively correlated with body size in postmenopausal women. *J Nutr* 2007
109. Wang MX, Jiao JH, Li ZY, *et al.* Lutein supplementation reduces plasma lipid peroxidation and C-reactive protein in healthy nonsmokers. *Atherosclerosis* 2013
110. Coyne T, Ibiebele TI, Baade PD, *et al.* Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. *Am J Clin Nutr* 2005
111. Blondin SA, Yeung EH, Mumford SL, *et al.* Serum Retinol and Carotenoids in Association with Biomarkers of Insulin Resistance among Premenopausal Women. *Isrn Nutr* 2013
112. Granado-Lorencio F, Olmedilla-Alonso B, Blanco-Navarro I, *et al.* Assessment of carotenoid status and the relation to glycaemic control in type I diabetics: a follow-up study. *Eur J Clin Nutr* 2006
113. Olea JL, Aragon JA, Zapata ME, *et al.* [Characteristics of patients with wet age-related macular degeneration and low intake of lutein and zeaxanthin] Spanish. *Arch Soc Esp Oftalmol* 2012
114. Sugiura M, Nakamura M, Ikoma Y, *et al.* The homeostasis model assessment-insulin resistance index is inversely associated with serum carotenoids in non-diabetic subjects. *J Epidemiol* 2006
115. Renzi LM, Hammond BR, Dengler M, *et al.* The relation between serum lipids and lutein and zeaxanthin in the serum and retina: results from cross-sectional, case-control and case study designs. *Lipids Health Dis* 2012
116. Ribaya-Mercado JD, Ordovas JM, Russell RM. Effect of (beta)-carotene supplementation on the concentrations and distribution of carotenoids, vitamin E, vitamin A, and cholesterol in plasma lipoprotein and non-lipoprotein fractions in healthy older women. *J Am Coll Nutr* 1995
117. Facchini FS, Hollenbeck CB, Jeppesen J, *et al.* Insulin Resistance and Cigarette-Smoking. *Lancet* 1992
118. Attvall S, Fowelin J, Lager I, *et al.* Smoking Induces Insulin Resistance - a Potential Link with the Insulin Resistance Syndrome. *J Intern Med* 1993
119. Ford ES, Mokdad AH, Giles WH, *et al.* The metabolic syndrome and antioxidant concentrations - Findings from the Third National Health and Nutrition Examination Survey. *Diabetes* 2003
120. Furukawa S, Fujita T, Shimabukuro M, *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004
121. Van Guilder GP, Hoetzer GL, Greiner JJ, *et al.* Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. *Obesity* 2006
122. Pearson TA, Mensah GA, Alexander RW, *et al.* Markers of inflammation and cardiovascular disease application to clinical and public health practice - A statement for healthcare professionals from the centres for disease control and prevention and the American Heart Association. *Circulation* 2003
123. Lagrand WK, Visser CA, Hermens WT, *et al.* C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation* 1999
124. Perez-Rodriguez L. Carotenoids in evolutionary ecology: re-evaluating the antioxidant role. *Bioessays* 2009
125. Jyonouchi H, Zhang L, Gross M, *et al.* Immunomodulating Actions of Carotenoids - Enhancement of in-Vivo and in-Vitro Antibody-Production to T-Dependent Antigens. *Nutrition and Cancer-an International Journal* 1994
126. Kim HW, Chew BP, Wong TS, *et al.* Dietary lutein stimulates immune response in the canine. *Vet Immunol Immunopathol* 2000
127. Kim HW, Chew BP, Wong TS, *et al.* Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. *Vet Immunol Immunopathol* 2000
128. McGraw KJ, Ardia DR. Carotenoids, immunocompetence, and the information content of sexual colors: An experimental test. *American Naturalist* 2003
129. Kowluru RA, Menon B, Gierhart DL. Beneficial effect of zeaxanthin on retinal metabolic abnormalities in diabetic rats. *Investigative Ophthalmology & Visual Science* 2008
130. Izumi-Nagai K, Nagai N, Ohgami K, *et al.* Macular pigment lutein is antiinflammatory in preventing Choroidal neovascularization. *Arteriosclerosis Thrombosis and Vascular Biology* 2007
131. Gruber M, Chappell R, Millen A, *et al.* Correlates of serum lutein plus zeaxanthin: Findings from the Third National Health and Nutrition Examination Survey. *J Nutr* 2004
132. Ford ES, Will JC, Bowman BA, *et al.* Diabetes mellitus and serum carotenoids: Findings from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 1999
133. Zeisel SH. Choline: An essential nutrient for humans. *Nutrition* 2000
134. Ueland PM. Choline and betaine in health and disease. *J Inherit Metab Dis* 2011
135. Olthof MR, van Vliet T, Verhoef P, *et al.* Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. *PLoS Med* 2005
136. Dalmeijer GW, Olthof MR, Verhoef P, *et al.* Prospective study on dietary intakes of folate, betaine, and choline and cardiovascular disease risk in women. *Eur J Clin Nutr* 2008
137. Corbin KD, Zeisel SH. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. *Current Opinion in Gastroenterology* 2012
138. Al Rajabi A, Castro GS, da Silva RP, *et al.* Choline supplementation protects against liver damage by normalizing cholesterol metabolism in Pemt/Ldlr knockout mice fed a high-fat diet. *J Nutr* 2014
139. Guerrerio AL, Colvin RM, Schwartz AK, *et al.* Choline intake in a large cohort of patients with nonalcoholic fatty liver disease. *Am J Clin Nutr* 2012
140. Zeisel SH. Nutrition in pregnancy: the argument for including a source of choline. *Int J Womens Health* 2013
141. Fioravanti M, Yanagi M. Cytidinediphosphocholine (CDP-choline) for cognitive and behavioural disturbances associated with chronic cerebral disorders in the elderly - art. no. CD000269.pub3. *Cochrane Database Syst Rev* 2005
142. Carmichael SL, Yang W, Shaw GM. Periconceptional Nutrient Intakes and Risks of Neural Tube Defects in California. *Birth Defects Research Part a-Clinical and Molecular Teratology* 2010
143. Shaw GM, Carmichael SL, Yang W, *et al.* Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. *Am J Epidemiol* 2004
144. Mills JL, Fan RZ, Brody LC, *et al.* Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects. *Am J Clin Nutr* 2014
145. Cheatham CL, Goldman BD, Fischer LM, *et al.* Phosphatidylcholine supplementation in pregnant women consuming moderate-choline diets does not enhance infant cognitive function: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2012
146. Villamor E, Rifas-Shiman SL, Gillman MW, *et al.* Maternal Intake of Methyl-Donor Nutrients and Child Cognition at 3 Years of Age. *Paediatr Perinat Epidemiol* 2012
147. Boeke CE, Gillman MW, Hughes MD, *et al.* Choline Intake During Pregnancy and Child Cognition at Age 7 Years. *Am J Epidemiol* 2013
148. Signore C, Ueland PM, Troendle J, *et al.* Choline concentrations in human maternal and cord blood and intelligence at 5 y of age. *Am J Clin Nutr* 2008
149. Wu BTF, Dyer RA, King DJ, *et al.* Early Second Trimester Maternal Plasma Choline and Betaine Are Related to Measures of Early Cognitive Development in Term Infants. *PLoS One* 2012
150. Strain JJ, McSorley EM, van Wijngaarden E, *et al.* Choline status and neurodevelopmental outcomes at 5 years of age in the Seychelles Child Development Nutrition Study. *Br J Nutr* 2013
151. Nurk E, Refsum, H. ,Bjelland, I. ,Drevon, C. A. ,Tell, G. S. ,Ueland, P. M. ,Vollset, S. E. ,Engedal, K. ,Nygaard, H. A. ,David Smith, A. Plasma free choline, betaine and cognitive performance: the Hordaland Health Study. *Br J Nutr* 2012
152. Poly C, Massaro JM, Seshadri S, *et al.* The relation of dietary choline to cognitive performance and white-matter hyperintensity in the Framingham Offspring Cohort. *Am J Clin Nutr* 2011
153. Sitaram N, Weingartner H, Caine ED, *et al.* Choline - Selective Enhancement of Serial-Learning and Encoding of Low Imagery Words in Man. *Life Sci* 1978
154. Ladd SL, Sommer SA, Laberge S, *et al.* Effect of Phosphatidylcholine on Explicit Memory. *Clin Neuropharmacol* 1993
155. Deuster PA, Singh A, Coll R, *et al.* Choline ingestion does not modify physical or cognitive performance. *Mil Med* 2002
156. Harris CM, Dysken MW, Fovall P, *et al.* Effect of Lecithin on Memory in Normal Adults. *Am J Psychiatry* 1983
157. Benton D, Donohoe RT. The influence on cognition of the interactions between lecithin, carnitine and carbohydrate. *Psychopharmacology (Berl)* 2004
158. Davis KL, Mohs RC, Tinklenberg JR, *et al.* Cholinomimetics and memory. The effect of choline chloride. *Arch Neurol* 1980
159. Mohs RC, Davis KL, Tinklenberg JR, *et al.* Choline Chloride Effects on Memory in the Elderly. *Neurobiol Aging* 1980
160. Sorgatz H. The effect of lecithin on the state of health and concentration in healthy adults. *Fortschr Med* 1988

161. Drachman DA, Glosser G, Fleming P, *et al.* Memory Decline in the Aged - Treatment with Lecithin and Physostigmine. *Neurology* 1982
162. Spiers PA, Myers D, Hochanadel GS, *et al.* Citicoline improves verbal memory in aging. *Arch Neurol* 1996
163. Mapstone M, Cheema AK, Fiandaca MS, *et al.* Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med* 2014
164. Jiang XY, Bar HY, Yan J, *et al.* A higher maternal choline intake among third-trimester pregnant women lowers placental and circulating concentrations of the antiangiogenic factor fms-like tyrosine kinase-1 (sFLT1). *FASEB J* 2013
165. Hogeveen M, den Heijer M, Semmekrot BA, *et al.* Umbilical choline and related methylamines betaine and dimethylglycine in relation to birth weight. *Pediatr Res* 2013
166. Ivorra C, Garcia-Vicent C, Chaves FJ, *et al.* Metabolomic profiling in blood from umbilical cords of low birth weight newborns. *J Transl Med* 2012
167. Sanz-Cortes M, Carbajo RJ, Crispi F, *et al.* Metabolomic Profile of Umbilical Cord Blood Plasma from Early and Late Intrauterine Growth Restricted (IUGR) Neonates with and without Signs of Brain Vasodilation. *PLoS One* 2013
168. Buchman AL, Sohel M, Moukarzel A, *et al.* Plasma choline in normal newborns, infants, toddlers, and in very-low-birth-weight neonates requiring total parenteral nutrition. *Nutrition* 2001
169. Kupke IR, Heller B, Weiss P, *et al.* Early Indicators for the Risk of Atherogenesis - a Field-Test of a New Methodology on Kindergarten-Children in Dusseldorf. *Atherosclerosis* 1983
170. Killgore WDS, Ross AJ, Kamiya T, *et al.* Citicoline Affects Appetite and Cortico-Limbic Responses to Images of High-Calorie Foods. *Int J Eat Disord* 2010
171. Schriewer H, Schulte H, Assmann G. Hdl Phosphatidyl Choline and Risk-Factors of Coronary Heart-Disease. *J Clin Chem Clin Biochem* 1984
172. Yan J, Winter LB, Burns-Whitmore B, *et al.* Plasma choline metabolites associate with metabolic stress among young overweight men in a genotype-specific manner. *Nutrition & Diabetes* 2012
173. Floegel A, Stefan, N. ,Yu, Z. ,Muhlenbruch, K. ,Drogan, D. ,Joost, H. G. ,Fritsche, A. ,Haring, H. U. ,Hrabe de Angelis, M. ,Peters, A. ,Roden, M. ,Prehn, C. ,Wang-Sattler, R. ,Illig, T. ,Schulze, M. B. ,Adamski, J. ,Boeing, H. ,Pischon, T. Identification of Serum Metabolites Associated With Risk of Type 2 Diabetes Using a Targeted Metabolomic Approach. *Diabetes* 2012
174. Meikle PJ, Wong G, Barlow CK, *et al.* Plasma Lipid Profiling Shows Similar Associations with Prediabetes and Type 2 Diabetes. *PLoS One* 2013
175. Ferrannini E, Natali A, Camastra S, *et al.* Early Metabolic Markers of the Development of Dysglycemia and Type 2 Diabetes and Their Physiological Significance. *Diabetes* 2013
176. Chen L, Chen YM, Wang LJ, *et al.* Higher homocysteine and lower betaine increase the risk of microangiopathy in patients with diabetes mellitus carrying the GG genotype of PEMT G774C. *Diabetes-Metabolism Research and Reviews* 2013
177. Simonsson P, Nilsson A, Akesson B. Postprandial Effects of Dietary Phosphatidylcholine on Plasma-Lipoproteins in Man. *Am J Clin Nutr* 1982
178. Wallace JMW, McCormack JM, McNulty H, *et al.* Choline supplementation and measures of choline and betaine status: a randomised, controlled trial in postmenopausal women. *Br J Nutr* 2012
179. Zeisel SH, Dacosta KA, Franklin PD, *et al.* Choline, an Essential Nutrient for Humans. *FASEB J* 1991
180. Evans M, Njike VY, Hoxley M, *et al.* Effect of soy isoflavone protein and soy lecithin on endothelial function in healthy postmenopausal women. *Menopause-the Journal of the North American Menopause Society* 2007
181. Veenema K, Solis C, Li R, *et al.* Adequate Intake levels of choline are sufficient for preventing elevations in serum markers of liver dysfunction in Mexican American men but are not optimal for minimizing plasma total homocysteine increases after a methionine load. *Am J Clin Nutr* 2008
182. Detopoulou P, Panagiotakos DB, Antonopoulou S, *et al.* Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. *Am J Clin Nutr* 2008
183. Kalhan SC, Guo LN, Edmison J, *et al.* Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism-Clinical and Experimental* 2011
184. Imajo K, Fujita K, Yoneda M, *et al.* Plasma free choline is a novel non-invasive biomarker for early-stage non-alcoholic steatohepatitis: A multi-centre validation study. *Hepatology Research* 2012
185. Shaw GM, Yang W, Carmichael SL, *et al.* One-carbon Metabolite Levels in Mid-pregnancy and Risks of Conotruncal Heart Defects. *Birth Defects Research Part a-Clinical and Molecular Teratology* 2014
186. Bertoia ML, Pai JK, Cooke JP, *et al.* Plasma homocysteine, dietary B vitamins, betaine, and choline andrisk of peripheral artery disease. *Atherosclerosis* 2014
187. Bidulescu A, Chambless, L. E. ,Siega-Riz, A. M. ,Zeisel, S. H. ,Heiss, G. Usual choline and betaine dietary intake and incident coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) Study. *BMC Cardiovasc Disord* 2007
188. Stegemann C, Pechlaner R, Willeit P, *et al.* Lipidomics Profiling and Risk of Cardiovascular Disease in the Prospective Population-Based Bruneck Study. *Circulation* 2014
189. Ried JS, Baurecht H, Stuckler F, *et al.* Integrative genetic and metabolite profiling analysis suggests altered phosphatidylcholine metabolism in asthma. *Allergy* 2013
190. Zeisel SH, Mar MH, Howe JC, *et al.* Concentrations of choline-containing compounds and betaine in common foods. *J Nutr* 2003
191. Fischer LM, daCosta KA, Kwock L, *et al.* Sex and menopausal status influence human dietary requirements for the nutrient choline. *Am J Clin Nutr* 2007
192. Fischer LM, da Costa KA, Kwock L, *et al.* Dietary choline requirements of women effects of estrogen and genetic variation. *Am J Clin Nutr* 2010
193. Holmes-McNary MQ, Cheng WL, Mar MH, *et al.* Choline and choline esters in human and rat milk and in infant formulas. *Am J Clin Nutr* 1996
194. Brion MJ, Lawlor DA, Matijasevich A, *et al.* What are the causal effects of breastfeeding on IQ, obesity and blood pressure? Evidence from comparing high-income with middle-income cohorts. *Int J Epidemiol* 2011
195. Van den Veyver IB. Genetic effects of methylation diets. *Annu Rev Nutr* 2002
196. Shaw GM, Carmichael SL, Laurent C, *et al.* Maternal nutrient intakes and risk of orofacial clefts. *Epidemiology* 2006
197. Shaw GM, Vollset SE, Carmichael SL, *et al.* Nested Case-Control Study of One-Carbon Metabolites in Mid-Pregnancy and Risks of Cleft Lip With and Without Cleft Palate. *Pediatr Res* 2009
198. Higgins JP, Flicker L. Lecithin for dementia and cognitive impairment. *Cochrane Database Syst Rev* 2003
199. Buchman AL, Ament ME, Sohel M, *et al.* Choline deficiency causes reversible hepatic abnormalities in patients receiving parenteral nutrition: Proof of a human choline requirement: A placebo-controlled trial. *Journal of Parenteral and Enteral Nutrition* 2001
200. Body R, Griffith CA, Keevil B, *et al.* Choline for diagnosis and prognostication of acute coronary syndromes in the Emergency Department. *Clin Chim Acta* 2009
201. Cavusoglu E, Chhabra S, Jiang XC, *et al.* Relation of baseline plasma phospholipid levels to cardiovascular outcomes at two years in men with acute coronary syndrome referred for coronary angiography. *Am J Cardiol* 2007
202. Danne O, Mockel M, Lueders C, *et al.* Prognostic implications of elevated whole blood choline levels in acute coronary syndromes. *Am J Cardiol* 2003
203. Senturk T, Tutuncu A, Ozdemir B, *et al.* Serum choline levels in patients with stable angina and acute coronary syndromes: relation to the severity of coronary artery disease. *Coron Artery Dis* 2010
204. Kirsch R, Clarkson V, Shephard EG, *et al.* Rodent nutritional model of non-alcoholic steatohepatitis: Species, strain and sex difference studies. *J Gastroenterol Hepatol* 2003
205. Song JN, da Costa KA, Fischer LM, *et al.* Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J* 2005
206. Clarke R, Bennett DA, Parish S, *et al.* Homocysteine and Coronary Heart Disease: Meta-analysis of MTHFR Case-Control Studies, Avoiding Publication Bias. *PLoS Med* 2012
207. Meigs JB, Jacques PF, Selhub J, *et al.* Fasting plasma homocysteine levels in the insulin resistance syndrome - The Framingham Offspring Study. *Diabetes Care* 2001
208. Hajer GR, van der Graaf Y, Olijhoek JK, *et al.* Levels of homocysteine are increased in metabolic syndrome patients but are not associated with an increased cardiovascular risk, in contrast to patients without the metabolic syndrome. *Heart* 2007
209. Wang ZN, Klipfell E, Bennett BJ, *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011
210. Tang WHW, Wang ZE, Levison BS, *et al.* Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk. *N Engl J Med* 2013
211. Sparks JD, Collins HL, Chirieac DV, *et al.* Hepatic very-low-density lipoprotein and apolipoprotein B production are increased following in vivo induction of betaine-homocysteine S-methyltransferase. *Biochem J* 2006

212. Lobstein T, Baur L, Uauy R. Obesity in children and young people: a crisis in public health. *Obes Rev* 2004
213. Singh AS, Mulder C, Twisk JWR, *et al.* Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes Rev* 2008
214. Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes* 2011
215. Barker DJP, Osmond C. Infant-Mortality, Childhood Nutrition, and Ischemic-Heart-Disease in England and Wales. *Lancet* 1986
216. Wu GY, Bazer FW, Cudd TA, *et al.* Maternal nutrition and fetal development. *J Nutr* 2004
217. Moon RJ, Harvey NC, Robinson SM, *et al.* Maternal Plasma Polyunsaturated Fatty Acid Status in Late Pregnancy Is Associated with Offspring Body Composition in Childhood. *Journal of Clinical Endocrinology & Metabolism* 2013
218. Yajnik CS, Deshpande SS, Jackson AA, *et al.* Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 2008
219. Lewis SJ, Leary S, Smith GD, *et al.* Body composition at age 9 years, maternal folate intake during pregnancy and methyltetrahydrofolate reductase (MTHFR) C677T genotype. *Br J Nutr* 2009
220. Hauner H, Much D, Vollhardt C, *et al.* Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. *Am J Clin Nutr* 2012
221. Yin J, Quinn S, Dwyer T, *et al.* Maternal diet, breastfeeding and adolescent body composition: a 16-year prospective study. *Eur J Clin Nutr* 2012
222. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002
223. Poon AK, Yeung E, Boghossian N, *et al.* Maternal Dietary Patterns during Third Trimester in Association with Birthweight Characteristics and Early Infant Growth. *Scientifica* 2013
224. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, *et al.* Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998
225. Donders-Engelen M, Heijden van der L, Hulshof KF. *Maten, gewichten en codenummers*. Wageningen: Human Nutrition of TNO and Wageningen University, 2003
226. Netherlands Nutrition Centre. *Nevo: Dutch food composition database 2006*. The Hague: Netherlands Nutrition Centre, 2006
227. Ocké MC, Hulshof KFAM, Buurma-Rethans EJM, *et al. Voedselconsumptiepeiling 2003. Samenvatting werkwijze en evaluatie*. Bilthoven: RIVM, 2004
228. Cole TJ, Bellizzi MC, Flegal KM, *et al.* Establishing a standard definition for child overweight and obesity worldwide: international survey. *Br Med J* 2000
229. Kaul S, Rothney MP, Peters DM, *et al.* Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity* 2012
230. Rubin DB, Schenker N. Multiple Imputation in Health-Care Databases - an Overview and Some Applications. *Stat Med* 1991
231. Komiya S, Eto C, Otoki K, *et al.* Sex differences in body fat of low- and high-body-mass children: relationship with body mass index. *Eur J Appl Physiol* 2000
232. Knudsen VK, Orozova-Bekkevold IM, Mikkelsen TB, *et al.* Major dietary patterns in pregnancy and fetal growth. *Eur J Clin Nutr* 2008
233. Okubo H, Miyake Y, Sasaki S, *et al.* Maternal dietary patterns in pregnancy and fetal growth in Japan: the Osaka Maternal and Child Health Study. *Br J Nutr* 2012
234. Loos RJF, Beunen G, Fagard R, *et al.* Birth weight and body composition in young adult men - a prospective twin study. *Int J Obes* 2001
235. Barker M, Robinson S, Osmond C, *et al.* Birth weight and body fat distribution in adolescent girls. *Arch Dis Child* 1997
236. Bayol SA, Farrington SJ, Stickland NC. A maternal 'junk food'diet in pregnancy and lactation promotes an exacerbated taste for 'junk food'and a greater propensity for obesity in rat offspring. *Br J Nutr* 2007
237. Deierlein AL, Siega-Riz AM, Chantala K, *et al.* The Association Between Maternal Glucose Concentration and Child BMI at Age 3 Years. *Diabetes Care* 2011
238. Lutomski JE, van den Broeck J, Harrington J, *et al.* Sociodemographic, lifestyle, mental health and dietary factors associated with direction of misreporting of energy intake. *Public Health Nutr* 2011
239. Freedman LS, Schatzkin A, Midthune D, *et al.* Dealing With Dietary Measurement Error in Nutritional Cohort Studies. *J Natl Cancer Inst* 2011
240. Sterne JAC, White IR, Carlin JB, *et al.* Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *Br Med J* 2009
241. Barker DJP, Gluckman PD, Godfrey KM, *et al.* Fetal Nutrition and Cardiovascular-Disease in Adult Life. *Lancet* 1993
242. Roseboom TJ, van der Meulen JHP, Osmond C, *et al.* Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am J Clin Nutr* 2000
243. Vanhees K, Vonhogen IGC, van Schooten FJ, *et al.* You are what you eat, and so are your children: the impact of micronutrients on the epigenetic programming of offspring. *Cell Mol Life Sci* 2014
244. Gresham E, Byles JE, Bisquera A, *et al.* Effects of dietary interventions on neonatal and infant outcomes: a systematic review and meta-analysis. *Am J Clin Nutr* 2014
245. Robinson S, Baird J, Godfrey KM. Eating for two? The unresolved question of optimal diet in pregnancy. *Am J Clin Nutr* 2014
246. Kant AK. Indexes of overall diet quality: A review. *J Am Diet Assoc* 1996
247. World Health Organisation. Preparation and use of food-based dietary guidelines. Report of a joint FAO/WHO consultation. *World Health Organ Tech Rep Ser* 1998
248. van Lee L, Geelen A, van Huysduynen EJ, *et al.* The Dutch Healthy Diet index (DHD-index): an instrument to measure adherence to the Dutch Guidelines for a Healthy Diet. *Nutrition Journal* 2012
249. Lassi ZS, Imam AM, Dean SV, *et al.* Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. *Reproductive Health* 2014
250. Kaiser HF. The varimax criterion for analytic rotation in factor analysis. *Psychometrika* 1958
251. Eisenmann JC. On the use of a continuous metabolic syndrome score in pediatric research. *Cardiovasc Diabetol* 2008
252. Voortman T, Kiefte-de Jong JC, Geelen A, *et al.* The Development of a Diet Quality Score for Preschool Children and Its Validation and Determinants in the Generation R Study. *J Nutr* 2015
253. Gillman MW. Developmental origins of health and disease. *N Engl J Med* 2005
254. Roseboom TJ, van der Meulen JHP, Ravelli ACJ, *et al.* Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol* 2001
255. Victora CG, Adair L, Fall C, *et al.* Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* 2008
256. Sanchez-Villegas A, Brito N, Doreste-Alonso J, *et al.* Methodological aspects of the study of dietary patterns during pregnancy and maternal and infant health outcomes. A systematic review. *Maternal and Child Nutrition* 2010
257. Leech RM, McNaughton SA, Timperio A. The clustering of diet, physical activity and sedentary behavior in children and adolescents: a review. *International Journal of Behavioral Nutrition and Physical Activity* 2014
258. Willett W. Nutritional Epidemiology - Issues and Challenges. *Int J Epidemiol* 1987
259. Kipnis V, Subar AF, Midthune D, *et al.* Structure of dietary measurement error: Results of the OPEN biomarker study. *Am J Epidemiol* 2003
260. Calvo MM. Lutein: A valuable ingredient of fruit and vegetables. *Crit Rev Food Sci Nutr* 2005
261. Gunanti IR, Marks GC, Al-Mamun A, *et al.* Low Serum Concentrations of Carotenoids and Vitamin E Are Associated with High Adiposity in Mexican-American Children. *J Nutr* 2014
262. Yeum KJ, Russell RM. Carotenoid bioavailability and bioconversion. *Annu Rev Nutr* 2002
263. Roodenburg AJ, Leenen R, van het Hof KH, *et al.* Amount of fat in the diet affects bioavailability of lutein esters but not of alpha-carotene, beta-carotene, and vitamin E in humans. *Am J Clin Nutr* 2000
264. Mikkila V, Rasanen L, Raitakari OT, *et al.* Consistent dietary patterns identified from childhood to adulthood: The cardiovascular risk in young finns study. *Br J Nutr* 2005
265. Kiefte-de Jong JC, de Vries JH, Bleeker SE, *et al.* Socio-demographic and lifestyle determinants of 'Western-like' and 'Health conscious' dietary patterns in toddlers. *Br J Nutr* 2013
266. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997
267. Wong SN, Sung RYT, Leung LCK. Validation of three oscillometric blood pressure devices against auscultatory mercury sphygmomanometer in children. *Blood Press Monit* 2006
268. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol* 1989

269. Govindan M, Gurm R, Mohan S, *et al.* Sex Differences in Physiologic Markers and Health Behaviors Associated With Childhood Obesity. *Pediatrics* 2013
270. Borel P, Desmarchelier C, Nowicki M, *et al.* Interindividual variability of lutein bioavailability in healthy men: characterization, genetic variants involved, and relation with fasting plasma lutein concentration. *Am J Clin Nutr* 2014
271. Mulder KA, Innis SM, Rasmussen BF, *et al.* Plasma lutein concentrations are related to dietary intake, but unrelated to dietary saturated fat or cognition in young children. *J Nutr Sci* 2014
272. Kipnis V, Freedman LS. Impact of Exposure Measurement Error in Nutritional Epidemiology. *J Natl Cancer Inst* 2008
273. Wang YC, Bleich SN, Gortmaker SL. Increasing caloric contribution from sugar-sweetened beverages and 100% fruit juices among US children and adolescents, 1988-2004. *Pediatrics* 2008
274. Reedy J, Krebs-Smith SM. Dietary Sources of Energy, Solid Fats, and Added Sugars among Children and Adolescents in the United States. *J Am Diet Assoc* 2010
275. de Ruyter JC, Olthof MR, Seidell JC, *et al.* A trial of sugar-free or sugar-sweetened beverages and body weight in children. *N Engl J Med* 2012
276. Mattes R. Fluid calories and energy balance: The good, the bad, and the uncertain. *Physiology & Behavior* 2006
277. de Ruyter JC, Katan MB, Kuijper LD, *et al.* The effect of sugar-free versus sugar-sweetened beverages on satiety, liking and wanting: an 18 month randomized double-blind trial in children. *PLoS One* 2013
278. Newby PK, Peterson KE, Berkey CS, *et al.* Beverage consumption is not associated with changes in weight and body mass index among low-income preschool children in North Dakota. *J Am Diet Assoc* 2004
279. Feunekes GIJ, Vanstaveren WA, Devries JHM, *et al.* Relative and Biomarker-Based Validity of a Food-Frequency Questionnaire Estimating Intake of Fats and Cholesterol. *Am J Clin Nutr* 1993
280. Hulshof KFAM, Breedveld B.C. Results of the study on nutrient intake in young toddlers 2002. Zeist, The Netherlands. TNO Nutrition.
281. Twisk JWR. Longitudinal data analysis. A comparison between generalized estimating equations and random coefficient analysis. *Eur J Epidemiol* 2004
282. Verbeke G, Molenberghs G. *Linear Mixed Models for Longitudinal Data*: Springer, 2009.
283. Ranawana DV, Henry CJ. Are caloric beverages compensated for in the short-term by young adults? An investigation with particular focus on sex differences. *Appetite* 2010
284. Gadah NS, Kyle LA, Rogers PJ. Sex differences in the satiety effects of sugar-containing drinks. *Appetite* 2012
285. Bagheri M, Ansari S, Sotoudeh G, *et al.* Serum ghrelin levels and sex-related indices of body composition in prepubertal children: a cross-sectional study. *Eur J Nutr* 2014
286. Taylor RW, Jones IE, Williams SM, *et al.* Body fat percentages measured by dual-energy X-ray absorptiometry corresponding to recently recommended body mass index cutoffs for overweight and obesity in children and adolescents aged 3-18 y. *Am J Clin Nutr* 2002
287. Jansen PW, Tharner A, van der Ende J, *et al.* Feeding practices and child weight: is the association bidirectional in preschool children? *Am J Clin Nutr* 2014
288. Freedman DS, Khan LK, Serdula MK, *et al.* Inter-relationships among childhood BMI, childhood height, and adult obesity: the Bogalusa Heart Study. *Int J Obes* 2004
289. van Dommelen P, de Kroon ML, Cameron N, *et al.* The Impact of Height during Childhood on the National Prevalence Rates of Overweight. *PLoS One* 2014
290. Wells JC, Cole TJ. Height, adiposity and hormonal cardiovascular risk markers in childhood: how to partition the associations? *Int J Obes* 2014
291. Caprio S. Calories from soft drinks--do they matter? *N Engl J Med* 2012
292. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. *Obes Rev* 2013
293. Levy DT, Friend KB, Wang YC. A Review of the Literature on Policies Directed at the Youth Consumption of Sugar Sweetened Beverages. *Advances in Nutrition* 2011
294. National Guideline C. Dietary guidelines for Americans, 2010. Secondary Dietary guidelines for Americans, 2010. http://www.guideline.gov/content.aspx?id=34277.
295. Raiten DJ, Raghavan R, Porter A, *et al.* Executive summary: evaluating the evidence base to support the inclusion of infants and children from birth to 24 mo of age in the Dietary Guidelines for Americans - "the B-24 Project". *Am J Clin Nutr* 2014
296. Caswell H. The role of fruit juice in the diet: An overview. *Nutrition Bulletin* 2009
297. Flood VM, Wen LM, Hardy LL, *et al.* Reliability and validity of a short FFQ for assessing the dietary habits of 2-5-year-old children, Sydney, Australia. *Public Health Nutr* 2014
298. Bennett CA, de Silva-Sanigorski AM, Nichols M, *et al.* Assessing the intake of obesity-related foods and beverages in young children: comparison of a simple population survey with 24 hr-recall. *International Journal of Behavioral Nutrition and Physical Activity* 2009
299. Vereecken C, Covents M, Maes L. Comparison of a food frequency questionnaire with an online dietary assessment tool for assessing preschool children's dietary intake. *Journal of Human Nutrition and Dietetics* 2010
300. Malik VS, Hu FB. Sweeteners and Risk of Obesity and Type 2 Diabetes: The Role of Sugar-Sweetened Beverages. *Current Diabetes Reports* 2012
301. Kell KP, Cardel MI, Brown MMB, *et al.* Added sugars in the diet are positively associated with diastolic blood pressure and triglycerides in children. *Am J Clin Nutr* 2014
302. Kosova EC, Auinger P, Bremer AA. The Relationships between Sugar-Sweetened Beverage Intake and Cardiometabolic Markers in Young Children. *Journal of the Academy of Nutrition and Dietetics* 2013
303. Leermakers ET, Felix JF, Erler NS, *et al.* Sugar-containing beverage intake in toddlers and body composition up to age 6 years: The Generation R Study. *Eur J Clin Nutr* 2015
304. Bizeau ME, Pagliassotti MJ. Hepatic adaptations to sucrose and fructose. *Metabolism-Clinical and Experimental* 2005
305. Stanhope KL, Schwarz JM, Keim NL, *et al.* Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 2009
306. Schulze MB, Manson JE, Ludwig DS, *et al.* Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *Jama-Journal of the American Medical Association* 2004
307. Tester J, Sharma S, Jasik CB, *et al.* Sex differences in prediabetes and insulin resistance among 1356 obese children in Northern California. *Diabetes Metab Syndr* 2013
308. Iyer A, Kauter K, Brown L. Sex differences in metabolic syndrome: a key research issue? *Endocr Metab Immune Disord Drug Targets* 2011
309. Hwang LC, Bai CH, Chen CJ, *et al.* Sex difference on the development of metabolic syndrome: a population-based study in Taiwan. *Eur J Epidemiol* 2007
310. Hamid N, Alam S, Mehmood R. Sex differences and prevalence in metabolic syndrome. *Journal of Medical Sciences* 2011
311. Springer KW, Stellman JM, Jordan-Young RM. Beyond a catalogue of differences: A theoretical frame and good practice guidelines for researching sex/sex in human health. *Social Science & Medicine* 2012
312. Rhee K. Childhood overweight and the relationship between parent behaviors, parenting style ' and family functioning. *Ann Am Acad Pol Soc Sci* 2008
313. Bazzano LA, Li TY, Joshipura KJ, *et al.* Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. *Diabetes Care* 2008
314. Xi B, Li SS, Liu ZL, *et al.* Intake of Fruit Juice and Incidence of Type 2 Diabetes: A Systematic Review and Meta-Analysis. *PLoS One* 2014
315. Smithers LG, Golley RK, Brazionis L, *et al.* Characterizing whole diets of young children from developed countries and the association between diet and health: a systematic review. *Nutr Rev* 2011
316. Ambrosini GL. Conference on 'Childhood Nutrition and Obesity: Current Status and Future Challenges' Symposium 3: Effects of early nutrition on later health Childhood dietary patterns and later obesity: a review of the evidence. *Proc Nutr Soc* 2014
317. Ocke MC. Evaluation of methodologies for assessing the overall diet: dietary quality scores and dietary pattern analysis. *Proc Nutr Soc* 2013
318. Paradis AM, Godin G, Perusse L, *et al.* Associations between dietary patterns and obesity phenotypes. *Int J Obes* 2009
319. Quatromoni PA, Copenhafer DL, D'Agostino RB, *et al.* Dietary patterns predict the development of overweight in women: The Framingham Nutrition Studies. *J Am Diet Assoc* 2002
320. Newby PK, Muller D, Hallfrisch J, *et al.* Food patterns measured by factor analysis and anthropometric changes in adults. *Am J Clin Nutr* 2004
321. Quatromoni PA, Pencina M, Cobain MR, *et al.* Dietary quality predicts adult weight gain: Findings from the Framingham Offspring Study. *Obesity* 2006

322. Schroder H, Marrugat J, Vila J, *et al.* Adherence to the traditional Mediterranean diet is inversely associated with body mass index and obesity in a Spanish population. *J Nutr* 2004
323. Ystrom E, Niegel S, Vollrath ME. The impact of maternal negative affectivity on dietary patterns of 18-month-old children in the Norwegian Mother and Child Cohort Study. *Maternal and Child Nutrition* 2009
324. Gao SK, Beresford SAA, Frank LL, *et al.* Modifications to the Healthy Eating Index and its ability to predict obesity: the Multi-Ethnic Study of Atherosclerosis. *Am J Clin Nutr* 2008
325. Shroff MR, Perng W, Baylin A, *et al.* Adherence to a snacking dietary pattern and soda intake are related to the development of adiposity: a prospective study in school-age children. *Public Health Nutr* 2014
326. Ambrosini GL, Emmett PM, Northstone K, *et al.* Identification of a dietary pattern prospectively associated with increased adiposity during childhood and adolescence. *Int J Obes* 2012
327. Jennings A, Welch A, van Sluijs EMF, *et al.* Diet Quality Is Independently Associated with Weight Status in Children Aged 9-10 Years. *J Nutr* 2011
328. Lazarou C, Panagiotakos DB, Matalas AL. Physical activity mediates the protective effect of the Mediterranean diet on children's obesity status: The CYKIDS study. *Nutrition* 2010
329. Tucker KL. Dietary patterns, approaches, and multicultural perspective. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme* 2010
330. Hoffmann K, Schulze MB, Schienkiewitz A, *et al.* Application of a new statistical method to derive dietary patterns in nutritional epidemiology. *Am J Epidemiol* 2004
331. Durmus B, Heppe DHM, Gishti O, *et al.* General and abdominal fat outcomes in school-age children associated with infant breastfeeding patterns. *Am J Clin Nutr* 2014
332. Wells JCK, Cole TJ, team As. Adjustment of fat-free mass and fat mass for height in children aged 8 y. *Int J Obes* 2002
333. Statistics Netherlands. *Dutch Standard Classification of Education 2003* Den Haag/Heerlen: Statistics Netherlands (Centraal Bureau voor de Statistiek) 2003
334. Centre for Research and Statistics. *http://www.rotterdam.nl/onderzoek*. Rotterdam: Centrum voor Onderzoek en Statistiek 2005
335. Bakker R, Kruithof C, Steegers EAP, *et al.* Assessment of Maternal Smoking Status During Pregnancy and the Associations With Neonatal Outcomes. *Nicotine & Tobacco Research* 2011
336. Tromp IIM, Briede S, Kiefte-de Jong JC, *et al.* Factors associated with the timing of introduction of complementary feeding: the Generation R Study. *Eur J Clin Nutr* 2013
337. Fredriks AM, Van Buuren S, Burgmeijer RJF, *et al.* Continuing positive secular growth change in the Netherlands 1955-1997. *Pediatr Res* 2000
338. American Academy of Pediatrics. American Academy of Pediatrics: Children, adolescents, and television. *Pediatrics* 2001
339. Lang P-O, Trivalle C, Vogel T, *et al.* Markers of metabolic and cardiovascular health in adults: Comparative analysis of DEXA-based body composition components and BMI categories. *J Cardiol* 2015
340. Atlantis E, Martin SA, Haren MT, *et al.* Inverse associations between muscle mass, strength, and the metabolic syndrome. *Metabolism-Clinical and Experimental* 2009
341. Srikanthan P, Karlamangla AS. Relative Muscle Mass Is Inversely Associated with Insulin Resistance and Prediabetes. Findings from The Third National Health and Nutrition Examination Survey. *Journal of Clinical Endocrinology & Metabolism* 2011
342. Golley RK, Smithers LG, Mittinty MN, *et al.* Diet quality of U.K. infants is associated with dietary, adiposity, cardiovascular, and cognitive outcomes measured at 7-8 years of age. *J Nutr* 2013
343. Meyerkort CE, Oddy WH, O'Sullivan TA, *et al.* Early diet quality in a longitudinal study of Australian children: associations with nutrition and body mass index later in childhood and adolescence. *J Dev Orig Health Dis* 2012
344. Robinson SM, Marriott LD, Crozier SR, *et al.* Variations in Infant Feeding Practice Are Associated with Body Composition in Childhood: A Prospective Cohort Study. *Journal of Clinical Endocrinology & Metabolism* 2009
345. Robinson S, Marriott L, Poole J, *et al.* Dietary patterns in infancy: the importance of maternal and family influences on feeding practice. *Br J Nutr* 2007
346. Hoffmann K, Zyriax BC, Boeing H, *et al.* A dietary pattern derived to explain biomarker variation is strongly associated with the risk of coronary artery disease. *Am J Clin Nutr* 2004
347. Wosje KS, Khoury PR, Claytor RP, *et al.* Dietary patterns associated with fat and bone mass in young children. *Am J Clin Nutr* 2010
348. Heppe DHM, Kiefte-de Jong JC, Durmus B, *et al.* Parental, fetal, and infant risk factors for preschool overweight: the Generation R Study. *Pediatr Res* 2013
349. Hu FB, Rimm E, Smith-Warner SA, *et al.* Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* 1999
350. Freedman DS, Wang J, Maynard LM, *et al.* Relation of BMI to fat and fat-free mass among children and adolescents. *Int J Obes* 2005
351. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr* 1995
352. Bettler J, Zimmer JP, Neuringer M, *et al.* Serum lutein concentrations in healthy term infants fed human milk or infant formula with lutein. *Eur J Nutr* 2010
353. Gartner LM, Morton J, Lawrence RA, *et al.* Breastfeeding and the use of human milk. *Pediatrics* 2005
354. Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002
355. Aroor AR, DeMarco VG. Oxidative Stress and Obesity: The Chicken or the Egg? *Diabetes* 2014
356. Sommerburg O, Meissner K, Nelle M, *et al.* Carotenoid supply in breast-fed and formula-fed neonates. *Eur J Pediatr* 2000
357. Ilcol YO, Ozbek R, Hamurtekin E, *et al.* Choline status in newborns, infants, children, breast-feeding women, breast-fed infants and human breast milk. *Journal of Nutritional Biochemistry* 2005
358. Yang ZY, Huffman SL. Nutrition in pregnancy and early childhood and associations with obesity in developing countries. *Maternal and Child Nutrition* 2013
359. Gaillard R, Felix JF, Duijts L, *et al.* Childhood consequences of maternal obesity and excessive weight gain during pregnancy. *Acta Obstet Gynecol Scand* 2014
360. Harder T, Rodekamp E, Schellong K, *et al.* Birth weight and subsequent risk of type 2 diabetes: A meta-analysis. *Am J Epidemiol* 2007
361. Wu TC, Chen PH. Health Consequences of Nutrition in Childhood and Early Infancy. *Pediatr Neonatol* 2009
362. World Health Organisation. Global Strategy for Infant and Young Child Feeding. Geneva. 2014
363. Schwartz C, Scholtens PAMJ, Lalanne A, *et al.* Development of healthy eating habits early in life. Review of recent evidence and selected guidelines. *Appetite* 2011
364. Northstone K, Emmett PM. Are dietary patterns stable throughout early and mid-childhood? A birth cohort study. *Br J Nutr* 2008
365. Dewey KG, Peerson JM, Brown KH, *et al.* Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. World Health Organization Working Group on Infant Growth. *Pediatrics* 1995
366. Virtanen SM, vantVeer P, Kok F, *et al.* Predictors of adipose tissue carotenoid and retinol levels in nine countries - The EURAMIC study. *Am J Epidemiol* 1996
367. Cameron AJ, Crawford DA, Salmon J, *et al.* Clustering of Obesity-Related Risk Behaviors in Children and Their Mothers. *Ann Epidemiol* 2011
368. Spiegelman D, McDermott A, Rosner B. Regression calibration method for correcting measurement-error bias in nutritional epidemiology. *Am J Clin Nutr* 1997
369. Harrison GG. Public Health interventions to combat micronutrient deficiencies. *Public Health Rev* 2010
370. Timmermans S, Jaddoe VWV, Mackenbach JP, *et al.* Determinants of folic acid use in early pregnancy in a multi-ethnic urban population in The Netherlands: The Generation R study. *Prev Med* 2008
371. World Health Organisation Food and Agricultural Organization of the United Nations. Guidelines on food fortification with micronutrients. Geneva. 2006
372. Osterhues A, Ali NS, Michels KB. The Role of Folic Acid Fortification in Neural Tube Defects: A Review. *Crit Rev Food Sci Nutr* 2013
373. Eckel RH. Egg consumption in relation to cardiovascular disease and mortality: the story gets more complex. *Am J Clin Nutr* 2008
374. Seddon JM, Ajani UA, Sperduto RD, *et al.* Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA* 1994
375. van de Gaar VM, Jansen W, van Grieken A, *et al.* Effects of an intervention aimed at reducing the intake of sugar-sweetened beverages in primary school children: A controlled trial. *International Journal of Behavioral Nutrition and Physical Activity* 2014

376. Sichieri R, Trotte AP, de Souza RA, *et al.* School randomised trial on prevention of excessive weight gain by discouraging students from drinking sodas. *Public Health Nutr* 2009
377. Tate DF, Turner-McGrievy G, Lyons E, *et al.* Replacing caloric beverages with water or diet beverages for weight loss in adults: main results of the Choose Healthy Options Consciously Everyday (CHOICE) randomized clinical trial. *Am J Clin Nutr* 2012
378. James J, Thomas P, Cavan D, *et al.* Preventing childhood obesity by reducing consumption of carbonated drinks: cluster randomised controlled trial. *BMJ* 2004
379. Estruch R, Martinez-Gonzalez MA, Corella D, *et al.* Effects of a Mediterranean-style diet on cardiovascular risk factors - A randomized trial. *Ann Intern Med* 2006
380. Willett WC, Sacks F, Trichopoulou A, *et al.* Mediterranean Diet Pyramid - a Cultural Model for Healthy Eating. *Am J Clin Nutr* 1995
381. Papadaki A, Scott JA. The impact on eating habits of temporary translocation from a Mediterranean to a Northern European environment. *Eur J Clin Nutr* 2002
382. Pan YL, Dixon Z, Himburg S, *et al.* Asian students change their eating patterns after living in the United States. *J Am Diet Assoc* 1999
383. Sharma S, Cade J, Riste L, *et al.* Nutrient intake trends among African-Caribbeans in Britain: a migrant population and its second generation. *Public Health Nutr* 1999
384. Ross RG, Hunter SK, McCarthy L, *et al.* Perinatal Choline Effects on Neonatal Pathophysiology Related to Later Schizophrenia Risk. *Am J Psychiatry* 2013
385. Simen-Kapeu A, Veugelers PJ. Should public health interventions aimed at reducing childhood overweight and obesity be sex-focused? *BMC Public Health* 2010
386. Lapidus L, Bengtsson C, Larsson B, *et al.* Distribution of Adipose-Tissue and Risk of Cardiovascular-Disease and Death - a 12 Year Follow up of Participants in the Population Study of Women in Gothenburg, Sweden. *Br Med J* 1984
387. Terry RB, Stefanick ML, Haskell WL, *et al.* Contributions of Regional Adipose-Tissue Depots to Plasma-Lipoprotein Concentrations in Overweight Men and Women - Possible Protective Effects of Thigh Fat. *Metabolism-Clinical and Experimental* 1991
388. Aucouturier J, Meyer M, Thivel D, *et al.* Effect of Android to Gynoid Fat Ratio on Insulin Resistance in Obese Youth. *Archives of Pediatrics & Adolescent Medicine* 2009
389. Kodama S, Horikawa C, Fujihara K, *et al.* Quantitative relationship between body weight gain in adulthood and incident type 2 diabetes: a meta-analysis. *Obes Rev* 2014
390. Rolland-Cachera MF, Deheeger M, Bellisle F, *et al.* Adiposity rebound in children: a simple indicator for predicting obesity. *Am J Clin Nutr* 1984
391. Rolland-Cachera MF, Deheeger M, Maillot M, *et al.* Early adiposity rebound: causes and consequences for obesity in children and adults. *Int J Obes* 2006
392. Eriksson JG, Forsen T, Tuomilehto J, *et al.* Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life. *Diabetologia* 2003
393. Plachta-Danielzik S, Bosy-Westphal A, Kehden B, *et al.* Adiposity rebound is misclassified by BMI rebound. *Eur J Clin Nutr* 2013
394. Boonpleng W, Park CG, Gallo AM. Timing of adiposity rebound: a step toward preventing obesity. *Pediatr Nurs* 2012
395. Lukaski HC, Johnson PE, Bolonchuk WW, *et al.* Assessment of Fat-Free Mass Using Bioelectrical Impedance Measurements of the Human-Body. *Am J Clin Nutr* 1985
396. Sun G, French CR, Martin GR, *et al.* Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of percentage body fat in a large, healthy population. *Am J Clin Nutr* 2005
397. Klein S, Allison DB, Heymsfield SB, *et al.* Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Diabetes Care* 2007

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Op 1 mei 2012 begon ik mijn eerste dag als PhD student, zonder data en zonder thesisplan, aan een bureau zonder computer en zonder bureaustoel, in een bibliotheek zonder ramen dat die dag benoemd werd tot kantoor. Sinds die eerste dag heb ik gewerkt in 2 gebouwen, 3 kantoren, aan 15 projecten, met 2 copromotoren, en tientallen collega's zien komen en gaan. Ook buiten kantoor gebeurde er veel; ik heb 2 keer een huis gekocht, had 9 bruiloften, 1 geregistreerd partnerschap, 3 begrafenissen, ben 2 keer tante geworden, verloor 18 kilo en werd een hele schoonfamilie rijker. Maar 1 ding bleef al die tijd hetzelfde; vanaf het allereerste uur is Trudy mijn kantoormaatje!!! Lieve *Trudy*, als ik je voor alles moet bedanken wat je voor me hebt gedaan wordt dit stuk langer dan mijn proefschrift. Is een DANK DANK DANK genoeg, of alleen als dat full-page komt? Jeetje wat zal ik moeten gaan afkicken nu ik niet meer dagelijks op kantoor zit (en niet alleen vanwege de liters koffie en kilo's paaseitjes). Al zullen Martijn en Arne wel blij zijn dat we elkaar niet meer altijd alles meteen kunnen vertellen! Er is geen twijfel over mogelijk dat jij mijn paranimf bent en naast me staat tijdens mijn verdediging. Je zou het zo van me kunnen overnemen!! Misschien dat als ik straks dokter ben, ik eindelijk ook een keer iets weet wat jij niet weet, want damn, wat weet jij veel!! Gelukkig ken ik ook je knettergekke kant (je radslag op de eerste dag zal ik niet snel meer vergeten) en ben ik niet jaloers op alle klusjes die je aangesmeerd krijgt omdat je nooit nee zegt (of.. ja DAAAG!). Ik ben blij dat er een paper van ons samen in dit boekje staat, we waren een goed team. Morgen begin ik aan onze volgende paper want ik kan je echt nog niet missen!!!

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PhD Portfolio

Summary of PhD training and teaching

Name PhD student:	Elisabeth Theodora Maria (Lisan) Leermakers
Erasmus MC Department:	Epidemiology
PhD period:	May 2012 – May 2015
Promotor(s):	Prof.dr. O.H. Franco, Prof.dr. V.W.V. Jaddoe
Co-promotor(s):	Dr. J.C. Kiefte-de Jong

TRAINING	Year	Workload
Courses and workshops		
Integrity in scientific research, Erasmus MC	2012	2.0 ECTS
Radiation hygiene and protection level 5R	2012	0.7 ECTS
Literature search, Medical Library, Erasmus MC	2012	0.3 ECTS
Endnote, Medical Library, Erasmus MC	2012	0.3 ECTS
Exposure assessment in Nutrition Research, Wageningen Unversity	2012	1.5 ECTS
Workshop Nutritional Epidemiology, ErasmusAGE	2012	0.6 ECTS
Workshop Systematic Review and Meta-analysis, ErasmusAGE	2012	0.3 ECTS
Workshop Meta-analysis, University of Warwick, UK	2013	0.6 ECTS
Nutrition and Physical Activity, Cambridge, UK	2013	1.4 ECTS
Masterclass Epidemiology, NIHES	2013	0.7 ECTS
Workshop 'De Plakkende vloer' VENA, Erasmus MC	2013	0.3 ECTS
Workshop Media contact, Erasmus MC	2013	0.3 ECTS
Advanced medical writing and editing, NIHES	2014	0.7 ECTS
Attended conferences and seminars		
Seminars, Epidemiology	2012-2015	1.0 ECTS
Research Meetings, ErasmusAGE	2012-2015	1.0 ECTS
Maternal and Child Health meetings, Generation R	2012-2015	1.0 ECTS
2020 Meetings, Epidemiology	2012-2015	1.0 ECTS
Research Meetings Nutritional Epidemiology (SIGN-E)	2012-2015	0.5 ECTS
Research Meetings, Generation R	2012-2015	0.5 ECTS
PhD day, ErasmusMC	2012	0.3 ECTS

Congress Nutrimenthe, Rotterdam	2012	0.3 ECTS
ABCD symposium, Amsterdam	2012	0.3 ECTS
Nationale Voedingscongres, Ede	2013	0.3 ECTS
DRINK symposium, VU, Amsterdam	2013	0.3 ECTS
Symposium: 'Jong geleerd, Oud gedaan', Leiden	2014	0.3 ECTS
Symposium Scientific Integrity, ErasmusMC	2014	0.3 ECTS
Symposium 'Mastering your future', ErasmusMC	2014	0.3 ECTS
WeVo meeting, Utrecht	2015	0.3 ECTS
NAV symposium, Utrecht	2015	0.3 ECTS

Conference presentations

Developmental Origins of Health and Disease, Rotterdam *poster and oral presentation*	2012	2.0 ECTS
Nutrition and growth, Barcelona, Spain *oral presentation*	2014	2.0 ECTS
NASO meeting, Oosterbeek *poster presentation*	2014	1.0 ECTS
NWO Nutritional Science Days, Deurne *oral presentation*	2014	1.0 ECTS
DIP congress, Berlin, Germany *poster presentation*	2015	2.0 ECTS

Other

Peer review of articles for scientific journals: *JAMA, European Journal of Epidemiology, PLoS ONE, British Journal of Nutrition, Pediatrics*	2014	1.0 ECTS

TEACHING

Supervising practicals

Exercise 'Public Health in Low and Middle income countries', NIHES	2012	0.3 ECTS
Introduction to Epidemiology, NIHES	2012-2014	2.0 ECTS
Practical 'How to read a scientific article', 1st year medical students	2012-2014	2.0 ECTS

Supervising students

Amira Ćerimagić, MSc thesis Medicine, University of Sarajevo, Bosnia	2012-2013	1.5 ECTS
Marion van den Broek, MSc thesis Nutrition and Health, VU University	2014	2.0 ECTS
Debora Melo van Lent, MSc thesis Clinical Epidemiology, NIHES	2014-2015	3.0 ECTS

Over de auteur

Elisabeth Theodora Maria (Lisan) Leermakers werd geboren in Eindhoven op 23 mei 1987. Ze groeide op in het pittoreske Brabantse dorpje Wintelre, samen met haar broer en twee zussen. In 2006 behaalde ze haar VWO-diploma aan het Sondervick College in Veldhoven.

Omdat ze werd uitgeloot voor de studie geneeskunde, startte ze met de opleiding Moleculaire Levenswetenschappen aan de Universiteit van Maastricht. In hetzelfde jaar nam ze deel aan de decentrale selectieprocedure voor geneeskunde aan de Erasmus Universiteit Rotterdam. Zo werd ze geselecteerd en mocht ze in 2007 alsnog starten met de studie geneeskunde. In 2008 behaalde ze haar propedeuse als een van de beste studenten van haar jaar, waardoor ze een plaats kreeg aangeboden in het programma Master of Science in Health Sciences van het Netherlands Institute of Health Sciences (NIHES). Dit combineerde ze met haar studie geneeskunde en ze volgde onder andere cursussen aan de Harvard School of Public Health in Boston, USA.

In 2011 behaalde ze haar doctoraaldiploma in de geneeskunde en vervolgens startte ze met haar onderzoeksstage bij de Generation R studie. Deze stage resulteerde in haar eerste vier wetenschappelijke publicaties. Daaropvolgend werd ze uitgenodigd om te starten met een promotietraject bij de ErasmusAGE groep (prof.dr. Oscar Franco) van de afdeling Epidemiologie van het Erasmus Universitair Medisch Centrum, in nauwe samenwerking met de Generation R studie (prof.dr. Vincent Jaddoe). Na het behalen van haar Master of Science-diploma in Clinical Epidemiology in mei 2012 startte ze met het onderzoek dat is beschreven in dit proefschrift. Eerst onder begeleiding van dr. Janine Felix en later onder begeleiding van dr. Jessica Kiefte-de Jong (copromotor).

In mei 2015 keert Lisan samen met haar vriend Arne terug naar Brabant en zal ze starten met haar coschappen in het St. Elisabeth Ziekenhuis in Tilburg. Ze verwacht af te studeren als arts in het voorjaar van 2017. Daarna wil ze zich specialiseren in de kindergeneeskunde, in het bijzonder in de zorg voor pasgeborenen, hetgeen ze graag wil combineren met haar passie voor wetenschappelijk onderzoek.