Diet, inflammation, body composition and type 2 diabetes

Insights from epidemiological studies

Niels van der Schaft

The studies described in this thesis were performed within the Rotterdam Study and the United Kingdom Biobank. I gratefully acknowledge the contribution of all participants, research staff, and health professionals who took part in these studies. Publication of this thesis was supported by the Department of Epidemiology of Erasmus University Medical Center and by Erasmus University Rotterdam.

ISBN: 978-94-6361-505-1
Layout: Niels van der Schaft and Optima Grafische Communicatie
Cover design: Niels van der Schaft and Optima Grafische Communicatie
Printing: Optima Grafische Communicatie

Diet, Inflammation, Body Composition and Type 2 Diabetes
Insights from epidemiological studies

Voeding, ontsteking, lichaamssamenstelling en type 2 diabetes
Inzichten uit epidemiologische studies

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. F.A. van der Duijn Schouten

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 16 februari 2021 om 13:00 uur

door

Niels van der Schaft

geboren te Zeist

Erasmus University Rotterdam

PROMOTIECOMMISSIE

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Voor mijn vader

So the breeze
In the boughs says
Without knowing
An imprecise
Joyful thing.

Fernando Pessoa

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MANUSCRIPTS THAT FORM THE BASIS OF THIS THESIS

1. van der Schaft N, Schoufour JD, Nano J, Kiefte-de Jong JC, Muka T, Sijbrands EJG et al. Dietary antioxidant capacity and risk of type 2 diabetes mellitus, prediabetes and insulin resistance: the Rotterdam Study. *Eur J Epidemiol* 2019; 34: 853–861.

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**Denotes equal contribution*

Chapter 1

General Introduction

INTRODUCTION

Type 2 diabetes

Type 2 diabetes, a metabolic disorder characterized by elevated serum glucose levels and reduced sensitivity to insulin, has become a worldwide public health concern. The prevalence of this disease has risen sharply during the last decades. In 2014, it was estimated that approximately 8.5% of adults suffer from type 2 diabetes globally.[1,2] Aside from symptoms directly related to disturbances in glucose metabolism, type 2 diabetes can cause severe long-term cardiovascular complications if not carefully managed.[1] These potential complications include myocardial infarction, stroke, peripheral arterial disease and blindness.[2] Due to its high prevalence and serious complications, type 2 diabetes accounts for a substantial economic and healthcare burden worldwide.[3] The healthcare costs related to type 2 diabetes are projected to have risen even further by the year 2030, in parallel with an ever increasing prevalence of the disorder in the coming decades if the present trend continues.[4,5]

Diet

The marked increase in the prevalence of type 2 diabetes is, amongst other factors, attributed to increasing rates of obesity, decreased time spent in physical activity in favor of sedentary time and the consumption of increasingly unhealthy diets.[2] The relationship between aspects of the diet and risk of type 2 diabetes appears to be especially complex. Diet may affect risk of type 2 diabetes through its effects on body weight, but dietary factors may also affect risk of the disease independently of body weight.[6] Several different approaches have been used to study the relation between diet and type 2 diabetes. For instance, at the level of individual nutrients, it has been suggested that higher intake of magnesium, vitamin C and carotenoids provide a lower risk of type 2 diabetes.[7–9] With regards to food groups, it appears that lower consumption of vegetables, fruits and whole grains and higher consumption of red meat and suger-sweetened beverages increase type 2 diabetes risk.[6,10] Considering dietary patterns as a whole, a Mediterranean-type diet, which is characterized by a high consumption of fruits, vegetables and legumes as well as moderate intake of fish and abundant use of olive oil, is associated with lower long-term risk of type 2 diabetes.[11–13] The many different approaches that have been used in studying diet as a determinant of type 2 diabetes highlight that this is a complicated field of research in which many questions remain unanswered. Notably, the mechanisms of action through which aspects of the diet may affect type 2 diabetes risk are subject to debate and may include effects on body composition and chronic low-grade inflammation.

Body composition

Given that obesity is one of the most firmly established risk factors for type 2 diabetes and its complications, one of the primary pathways through which diet may play a role in diabetes prevention is through inducing weight loss or preventing weight gain.[6,14] Although body weight is an important and frequently used parameter in this regard, more recent research has demonstrated that body weight and its simple derivatives such as body mass index (BMI) provide an incomplete picture of an individual's body composition due to the fact that BMI fails to differentiate between fat mass (adipose tissue) and lean mass (non-adipose tissues).[15,16] It has been shown that whereas higher fat mass is associated with increased risk of all-cause mortality, increases in lean mass generally confer a lower mortality risk.[17] Similarly, whereas higher lean mass is associated with lower risk of metabolic syndrome, higher fat mass is positively associated with metabolic syndrome.[18,19] The notion that body composition provides more information with regards metabolic disturbances than BMI is underlined by the observation that increased visceral fat mass is associated with increased insulin resistance, whereas increased subcutaneous fat mass may decrease insulin resistance.[20] Thus, not only the absolute quantity of fat mass but also its physical location has important metabolic implications, and BMI alone fails to capture this distinction. These differential effects of visceral and subcutaneous fat mass may be explained by differing inflammatory responses to excess adipose tissue in different locations.[21] Therefore, while the relation between obesity and type 2 diabetes may appear straightforward at first glance, much more is at play on a metabolic level. In line with this, diet may not only affect body weight but also body composition through effects on specific fat depots.[22]

Inflammation

Another pathway through which aspects of the diet may affect risk of type 2 diabetes is through systemic low-grade inflammation. Inflammation is a physiological process characterized by the release of mediators such as cytokines and chemokines in response to stressors, and is a critical feature of the immune system which helps maintain or reinstate homeostasis in the presence of tissue damage.[23] However, a persisting inflammatory response without an apparent trigger can also occur and is often regarded as detrimental to metabolic functioning.[23,24] Such an extended period of low-grade inflammation can be caused by the consumption of specific nutrients or a state of metabolic surplus as occurs in case of obesity.[25] With regards to metabolic surplus, the notion that inflammatory mediators are more abundantly expressed in obese individuals as opposed to lean individuals is commonly accepted.[26] A wide range of nutrients may have pro-inflammatory effects, although untangling the many pleiotropic effects these individual nutrients may have on inflammation in vivo has proven

challenging.[27] On a macro level, adherence to a Western-type dietary pattern (characterized by high intake of processed meat, refined grains and high-fat dairy, amongst other factors) is associated with elevated markers of inflammation.[28,29] Regardless of the exact source of the inflammatory process, inflammatory mediators such as tumor necrosis factor (TNF) may increase risk of type 2 diabetes through interfering with insulin signaling.[30] Interestingly, experimental evidence has indicated that this disruption of insulin signaling due to inflammation also takes place in the absence of overt obesity.[25] The prominent role of inflammation in the pathogenesis of obesity and insulin resistance has given rise to the idea that type 2 diabetes is, at its core, an inflammatory condition.[31] The importance of the concept of inflammation with regards to disease onset, as well as the notion that diet may be an important instigator of inflammation, emphasizes the importance of research linking diet to inflammatory processes.

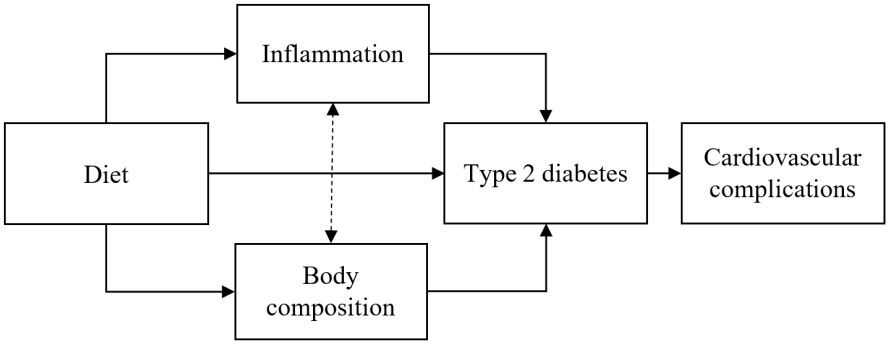

Figure 1.1.1. Proposed relation between determinants of type 2 diabetes and its eventual complications.

Thesis outline

Given that diet, body composition and inflammation are closely interwoven, disentangling how these factors interact with each other in the context of the pathogenesis of type 2 diabetes has proven no small feat. A framework for conceptualizing how they are related is displayed as Figure 1.1.1. With this thesis, I aim to further clarify how these factors are interrelated and affect risk of type 2 diabetes. The majority of the work contained in this thesis was performed within the Rotterdam Study, a large population-based cohort of approximately 15,000 participants. A number of the studies in this thesis were also performed within the United Kingdom (UK) Biobank, an open access cohort study of over half a million participants. As such, I approach the topics from an epidemiological perspective. The second chapter of this thesis is focused on dietary factors in relation to type 2 diabetes. In chapter 2.1, we investigate the relation

between total dietary antioxidant capacity and insulin resistance as well as risk of type 2 diabetes. In chapter 2.2, we examine the association between a plant-based diet and insulin resistance as well as incidence of prediabetes and type 2 diabetes. In the third chapter we discuss markers of inflammation and their relation to prediabetes and type 2 diabetes. In chapter 3.1, we examine uric acid in relation to risk of these outcomes. Following up on this, in chapter 3.2, uric acid is investigated in relation to risk of fatal and non-fatal cardiovascular events. In chapter 3.3, we study the role of C-reactive protein as a mediator in the association between coffee consumption and risk of type 2 diabetes. In the fourth chapter we address body composition and investigate its dietary determinants. In chapter 4.1, total dietary antioxidant capacity is investigated in relation to longitudinal patterns of body composition. Finally, in chapter 4.2, we explore the association between consumption of dietary advanced glycation end-products and body composition. In chapter 5, I provide an overview of the major findings from this thesis, discuss relevant methodological considerations and reflect on the implications of our work as well as potential directions for future research.

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Chapter 2

Dietary determinants of type 2 diabetes

Chapter 2.1

Dietary Antioxidant Capacity and Risk of Type 2 Diabetes Mellitus, Prediabetes and Insulin Resistance: The Rotterdam Study

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European Journal of Epidemiology, 2018

ABSTRACT

Background

Intake of individual antioxidants has been related to a lower risk of type 2 diabetes. However, the diet may contain many antioxidants with additive or synergistic effects. Therefore, we aimed to determine associations between total dietary antioxidant capacity and risk of type 2 diabetes, prediabetes and insulin resistance.

Methods

We estimated the dietary antioxidant capacity of 5,796 participants of the Rotterdam Study using a ferric reducing ability of plasma (FRAP) score. Of these participants, 4,957 had normoglycemia and 839 had prediabetes at baseline. We used covariate-adjusted proportional hazards models to estimate associations between FRAP and risk of type 2 diabetes, risk of type 2 diabetes among participants with prediabetes, and risk of prediabetes. We used linear regression models to determine the association between FRAP score and insulin resistance (HOMA-IR).

Results

We observed 532 cases of incident type 2 diabetes, of which 259 among participants with prediabetes, and 794 cases of incident prediabetes during up to 15 years of follow-up. A higher FRAP score was associated with a lower risk of type 2 diabetes among the total population (HR per SD FRAP 0.84, 95% CI 0.75; 0.95) and among participants with prediabetes (HR 0.85, 95% CI 0.73; 0.99), but was not associated with risk of prediabetes. Dietary FRAP was inversely associated with HOMA-IR (β -0.04, 95% CI -0.06; -0.03). Effect estimates were generally similar between sexes.

Conclusions

The findings of our population-based study emphasize the beneficial effects of dietary antioxidant capacity on insulin resistance and risk of type 2 diabetes.

INTRODUCTION

Oxidative stress is commonly regarded as an important contributing factor in the pathogenesis of type 2 diabetes mellitus.[1] Generally, oxidative stress is the result of an excess of reactive oxygen species (ROS), which are partially reduced forms of oxygen.[2] While ROS are considered essential for normal physiological function, an excess of ROS can lead to structural damage to important biomolecules and impairment of their function.[2,3] A biological defense mechanism against excess ROS is formed by antioxidants. These bioactive compounds may prevent the generation of ROS or scavenge free radicals.[1,2] Antioxidants can be endogenous, i.e. naturally occurring in the human body, such as uric acid and glutathione; or exogenous, in which case they are mainly derived from the diet.[2] Exogenous antioxidants, such as vitamin E and carotenoids, form an indispensable complementary component of the natural antioxidant defense system.[4]

A high dietary intake of antioxidants may lower oxidative stress and thereby lower the risk of diseases related to oxidative stress, such as type 2 diabetes. In line with this, a higher intake of certain nutrients with antioxidative properties has been associated with a lower risk of type 2 diabetes mellitus.[5,6] In addition, serum levels of certain antioxidants have been shown to be inversely related to plasma glucose levels and measures of insulin resistance.[7,8] However, the majority of previous studies on this topic have investigated individual antioxidant components only, as opposed to using a comprehensive measure of total dietary antioxidant capacity. The diet can contain many components with antioxidative properties which may have additive or synergistic effects, and intake of individual antioxidants may therefore not reflect the total antioxidant capacity of the diet.[9] The concept of total dietary antioxidant capacity aims to capture overall effects of antioxidants from dietary compounds and thereby facilitates studying the effects of antioxidants in the context of complex diets.[10] Major contributors to the overall antioxidant capacity of the diet are coffee, tea, red wine and various types of fruits (blueberries, grapes, oranges) and vegetables (cabbage species, spinach, broccoli).[11,12]

To our knowledge, only one previous study, among women only, examined the overall dietary antioxidant capacity in relation to type 2 diabetes.[13] Furthermore, dietary antioxidants have not been studied in relation to intermediate stages in the development of type 2 diabetes, such as insulin resistance or prediabetes. Therefore, we aimed to determine the association between dietary antioxidant capacity and risk of type 2 diabetes, risk of prediabetes and insulin resistance in a large population-based cohort with up to 15 years of follow-up.

METHODS

Study design and population

The general design and objectives of the Rotterdam Study have been described in detail elsewhere.[14] In brief, the Rotterdam Study (RS) is a population-based cohort which started in 1990 with the inclusion of 7,893 inhabitants of the Ommoord district in the city of Rotterdam, the Netherlands, aged 55 years or older (sub-cohort RS-I). In 2000, the cohort was extended with a second sub-cohort (sub-cohort RS-II) consisting of 3,011 participants who had moved into the Ommoord district or had become 55 years of age since the inception of the first sub-cohort. A further extension of the total cohort was initiated in 2006, when 3,932 residents of the Ommoord district aged 45-54 years were included in a third sub-cohort (sub-cohort RS-III). These participants were interviewed at home and received extensive physical examinations at the Rotterdam Study research facility at baseline, which are repeated every 3-4 years. The Rotterdam Study has received approval from the Medical Ethics Committee of Erasmus University Medical Center and from the review board of the Dutch Ministry of Health, Welfare and Sports. All participants have provided written informed consent.[14]

Population for analysis

Of the 14,926 participants in the Rotterdam Study, valid dietary data were available at the baseline examination round for each cohort for a total of 9,701 participants.[15] Among the 5,225 participants without valid dietary data, 5,141 individuals had no dietary data available, and 84 were judged to have invalid dietary data because their daily energy intake did not exceed 500 kcal or was greater than 5,000 kcal. Of the 9,701 participants with valid dietary data, 1,126 were excluded because they had prevalent cardiovascular disease (defined as a history of stroke, heart failure, myocardial infarction or revascularization procedure) and 415 were excluded because they had prevalent cancer. Of the remaining 8,160 participants, 1,682 had no information on glucose status available and 682 had prevalent type 2 diabetes. Thus, our population for analysis consisted of 5,796 individuals. Information on fasting serum glucose and insulin, used to calculate homeostatic model assessment of insulin resistance (HOMA-IR), was available for 5,422 of these individuals.

Dietary assessment

Dietary data were collected by means of a semi-quantitative food frequency questionnaire (FFQ), administered by a trained interviewer, during the baseline examination of the participants. For sub-cohorts RS-II and RS-I, a two-step approach was used in assessing dietary data. First, participants completed a self-administered checklist on which foods were consumed at least twice a month during the preceding year. The

completed checklist was used as a basis for the structured FFQ interview, performed by a trained dietician, about consumption frequencies and amounts at the Rotterdam Study research facility. The FFQ used in these sub-cohorts consisted of 170 items and was developed for and validated among the elderly.[16] For sub-cohort RS-III-I, collection of dietary data was performed by means of a single self-administered, 389-item, semi-quantitative FFQ which was based on an existing validated FFQ developed for Dutch adults.[17,18] Portion sizes in grams per day were estimated using standard household measures. Food intake data were subsequently converted into daily energy and nutrient intake using the Dutch Food Composition Tables of 1993 for RS-I-1, 2001 for RS-II-1, and 2006 for RS-III-1.

Assessment of total dietary antioxidant capacity

In order to estimate the total dietary antioxidant capacity, we used the Antioxidant Food Table published by Carlsen and colleagues, who determined the antioxidant content of over 3,100 types of food and beverages using a ferric reducing ability of plasma (FRAP) assay.[10] The FRAP assay measures the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) and has been used extensively in nutrition science.[2,19] The FRAP value of each type of food extracted from the Antioxidant Food Table (mmol/100 grams) was multiplied by its consumption frequency for every participant, and we then summed these values across all dietary sources of antioxidants to calculate a FRAP score for every participant representing the total dietary antioxidant capacity. Nutrition scientists from Wageningen University, the Netherlands, were consulted to determine the closest Dutch food equivalent for products that had different FRAP measurements listed for different manufacturers in the Antioxidant Food Table. No detailed data were available on the consumption of food supplements in our study, so we did not include food supplements in the calculation of the total dietary antioxidant capacity.

Ascertainment of normoglycemia, insulin resistance, prediabetes and type 2 diabetes mellitus

Fasting blood samples were obtained from participants during their visit to the Rotterdam Study research facility by means of venipuncture. The samples were stored at -80° Celsius in 5mL aliquots. Glucose levels were measured using the glucose hexokinase method within one week of sampling.[20] In 2008, insulin levels were measured in these samples by means of electrochemiluminescence immunoassay technology using a Roche Modular Analytics E170 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). We calculated HOMA-IR as the product of fasting serum glucose (mmol/L) and fasting serum insulin (mU/L) levels divided by 22.5. All measurements were performed at the clinical chemistry laboratory of Erasmus University Medical Center. We obtained data on the use of glucose-lowering medication through structured home

interviews as well as pharmacy dispensing records. In accordance with WHO guidelines and the Rotterdam Study protocol, we defined type 2 diabetes as a fasting plasma glucose level ≥ 7 mmol/L, a non-fasting plasma glucose level ≥ 11.1 mmol/L or the use of blood glucose lowering medication. We defined prediabetes as a fasting plasma glucose level > 6.0 mmol/L and < 7 mmol/L, or a non-fasting plasma glucose level > 7.7 mmol/L and < 11.1 mmol/L. We defined normoglycemia as a fasting plasma glucose level ≤ 6 mmol/L.[21] At baseline and throughout follow-up, we ascertained prediabetes and type 2 diabetes cases using records from general practitioners, hospital discharge letters and the glucose measurements performed as part of the Rotterdam Study.[22] Two physicians independently assessed all potential prediabetes and type 2 diabetes cases and consulted an endocrinologist in case of disagreement.[22] Serum glucose levels and incident cases of type 2 diabetes and prediabetes were recorded from the third examination round of the first cohort (RS-I-3) and the baseline examination rounds from the second and third cohort (RS-II-1 and RS-III-1) onwards. Hence, these rounds were used as the baseline for follow-up in our analyses.

Covariates

We considered the following potentially confounding variables our analyses, based on theory and previous literature: age, sex, body mass index (BMI), hypertension, dyslipidemia, highest attained level of education, degree of physical activity, smoking status, total daily energy intake, daily alcohol intake and degree of adherence to guidelines for a healthy diet. Anthropomorphic characteristics were recorded during participants' visits to the Rotterdam Study research facility. We calculated BMI as weight in kilograms divided by squared height in meters. We defined hypertension as the use of antihypertensive medication, having a systolic blood pressure ≥ 140 mmHg or having a diastolic blood pressure ≥ 90 mmHg. Blood pressure was recorded as the mean value of two blood pressure readings at the right upper arm in sitting position, separated by two minutes, using a random-zero sphygmomanometer. We defined dyslipidemia as a serum total cholesterol level > 6.5 mmol/L or use of lipid-lowering medication. Serum total cholesterol was determined in fasting blood samples using the CHOD-PAP method (Monotest Cholesterol kit, Boehringer Mannheim Diagnostica, Germany).[23] We determined the use of antihypertensive and lipid-lowering drugs through home interviews and consulting pharmacy dispensing records. Smoking status and the highest attained level of education were also ascertained during home interviews. We categorized participants as never smokers, former smokers or current smokers. Education level was split into four categories: primary education, lower or intermediate general education or lower vocational education, intermediate vocational education or higher general education and higher vocational education or university education. We calculated total daily energy intake (kcal/day) and daily

alcohol intake (grams/day) from data obtained from the FFQs. The overall dietary pattern was taken into account using a diet quality score reflecting adherence to dietary guidelines. This dietary pattern index, described by Voortman et al.[15], reflected intake of 14 food groups, including fruits and vegetables, whole grains and whole grain products, legumes, nuts, dairy, fish, tea, unsaturated fats and oils, red and processed meat, sugar-containing beverages and salt. The final index was a score ranging from 0 to 14 with a higher score reflecting a higher diet quality. The degree of physical activity was assessed by means of the LASA Physical Activity Questionnaire and a modified version of the Zutphen Study Physical Activity Questionnaire, and was expressed as metabolic equivalent of task (MET) hours per week based on time spent in light, moderate and vigorous activity.[24] To account for the use of two different questionnaires, we divided participants into quartiles of physical activity based on questionnaire-specific standard deviation scores.

Statistical analysis

Cox proportional hazards regression was performed with total dietary antioxidant capacity as the primary independent variable and incident prediabetes or incident type 2 diabetes as the response variable. The time scale in these models is follow-up time in years to either clinical endpoint, death, loss-to-follow-up or January 1st 2012 – whichever came first. As main analysis, we first investigated associations of FRAP score with incident type 2 diabetes. Subsequently, we analyzed this trajectory in more detail by investigating incident prediabetes among normoglycemic individuals and incident type 2 diabetes among individuals with prediabetes. We used multivariable linear regression models to assess the association between FRAP score and HOMA-IR. In these linear regression models, HOMA-IR was transformed using the natural logarithm to better approximate a normal distribution. For all outcomes, we constructed models adjusted only for age, sex and cohort (model 1), models adjusted additionally for BMI, hypertension, dyslipidemia, highest level of education attained, physical activity and smoking status (model 2), and models further adjusted for degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake (model 3). We accounted for potential non-linear relations between the independent and dependent variables by including three-knot natural cubic splines in our regression models when their use resulted in a significantly better model fit. Potential effect modification by age, sex or smoking status was investigated by introducing the product of these variables and the total dietary antioxidant capacity to our regression models. We ran separate models if the interaction terms were statistically significant at the $p < 0.10$ level. As a sensitivity analysis, we repeated our analyses with a modified FRAP score calculated without the contribution of coffee because some discussion remains on the bioavailability of the antioxidants found in coffee, and we also performed our

analyses excluding the first year of follow-up.[13] Five-fold multiple imputation using predictive mean matching was performed to account for missing values of covariates (ranging from 0% to 4.3%). Our results are presented as pooled hazard ratios (HRs) with 95% confidence intervals (95% CIs) obtained after multiple imputation for a standard deviation increment in total dietary antioxidant capacity. Statistical analyses were performed using R version 3.4.1 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The baseline characteristics of the total study population (n = 5,796) and the subgroups of men (n = 2,266) and women (n = 3,530) are displayed in Table 2.1.1. The major contributors to FRAP in our study were intake of coffee, fruit, vegetables, tea and chocolate. A comparison between participants who were and were not included in the analysis of this study based on missing data is presented in Supplementary Table 2.1.1. Because we observed statistical interactions between FRAP score and sex on risk of prediabetes (p-value for interaction 0.06) and on HOMA-IR (p-value for interaction 0.01), we stratified all our analyses by sex. The mean (SD) FRAP score was 24.0 (9.0) for the total population, 25.1 (9.8) for men and 23.2 (8.4) for women.

Of all 5,796 individuals eligible for analysis, 532 developed type 2 diabetes over a mean follow-up duration of 8.1 years (incidence rate 11.4 per 1,000 person-years). We observed an association between a higher FRAP score and a lower risk of type 2 diabetes, which remained statistically significant after adjusting for metabolic and socio-economic factors in model 2 (HR 0.85, 95% CI 0.76; 0.95) and further adjustment for dietary factors in model 3 (HR 0.84, 95% CI 0.75; 0.95). For incident type 2 diabetes there was no statistical interaction between dietary antioxidant capacity and sex, and indeed we observed similar effect estimates among men (HR 0.84, 95% CI 0.71; 1.00) and women (HR 0.83, 95% CI 0.70; 0.99) after adjustment for all covariates. (Table 2.1.2).

Of the 839 individuals with prediabetes at baseline, 259 developed type 2 diabetes over a mean follow-up duration of 7.4 years (incidence rate 41.5 per 1,000 person-years). We also found a significant association between FRAP score and incident type 2 diabetes in this subgroup (model 3; HR 0.85, 95% CI 0.73; 0.99), with similar effect estimates among men and women (p-value for interaction 0.90) (Table 2.1.2).

Table 2.1.1. Baseline characteristics of the study population.

	Overall (n = 5,796)	Men (n = 2,266)	Women (n = 3,530)
Age (years)	64.2 (9.2)	63.4 (8.7)	64.6 (9.5)
Body Mass Index (kg/m^2)	26.9 (4.1)	26.6 (3.3)	27.1 (4.5)
Dyslipidemia			
No	3,818 (65.9%)	1,640 (72.4%)	2,178 (61.7%)
Yes	1,978 (34.1%)	626 (27.6%)	1,352 (38.3%)
Hypertension			
No	2,394 (41.3%)	940 (41.5%)	1,454 (41.2%)
Yes	3,402 (58.7%)	1,326 (58.5%)	2,076 (58.8%)
Physical Activity (metabolic Equivalents of Task- hours/week)[1]			
-RS-I / RS-II (LASA questionnaire)	81.8 (57.5)	70.6 (56.2)	88.5 (57.2)
-RS-III (Zutphen Questionnaire)	45.0 (64.7)	38.7 (55.8)	52.4 (69.1)
-Total	71.2 (63.8)	59.8 (58.9)	77.8 (62.5)
Education			
Primary	650 (11.2%)	183 (8.1%)	467 (13.2%)
Lower	2,398 (41.4%)	625 (27.6%)	1,773 (50.2%)
Intermediate	1,660 (28.6%)	827 (36.5%)	833 (23.6%)
Higher	1,088 (18.8%)	631 (27.8%)	457 (12.9%)
Smoking			
Never	1,932 (33.3%)	397 (17.5%)	1,535 (43.5%)
Former	2,527 (43.6%)	1,242 (54.8%)	1,285 (36.4%)
Current	1,337 (23.1%)	627 (27.7%)	710 (20.1%)
Dietary Guideline Score	6.8 (1.9)	6.3 (1.8)	7.1 (1.9)
Alcohol consumption (g/day)[1]	6.6 (18.1)	13.0 (23.4)	3.44 (12.3)
Daily energy intake (kcal/day)	2,143.8 (622.4)	2,436.3 (633.3)	1,955.9 (537.1)
FRAP score	24.0 (9.0)	25.1 (9.8)	23.2 (8.4)

Variables are presented as mean (SD) unless otherwise indicated. [1]Variable is presented as median (interquartile range) because it did not follow a normal distribution. The statistics reported above represent the dataset after multiple imputation.

Over a mean follow-up duration of 7.7 years, 794 of the 4,957 individuals with normoglycemia at baseline developed prediabetes (incidence rate 20.9 per 1,000 person-years). FRAP score was not significantly associated with incident prediabetes (model 3; HR 0.93, 95% CI 0.84; 1.02). However, after stratification by sex (p-value for interaction 0.06), we observed a significant inverse association among men (model 3; HR 0.84, 95% CI 0.72; 0.98) whereas among women, we observed no association (HR 0.99, 95% CI 0.87; 1.12) (Table 2.1.2).

Table 2.1.2. Associations between total dietary antioxidant capacity, risk of type 2 diabetes, risk of type 2 diabetes among prediabetics and risk of prediabetes.

	Incident Type 2 Diabetes					
	Total population (n = 5,796, n cases = 532)	P-value	Men (n = 2,266, n cases = 218)	P-value	Women (n = 3,530, n cases = 314)	P-value
Model 1[1]	0.86 (0.76; 0.96)	0.01	0.85 (0.72; 1.00)	0.05	0.87 (0.74; 1.02)	0.09
Model 2[2]	0.85 (0.76; 0.95)	0.004	0.82 (0.70; 0.97)	0.02	0.86 (0.73; 1.01)	0.07
Model 3[3]	0.84 (0.75; 0.95)	0.01	0.84 (0.71; 1.00)	0.06	0.83 (0.70; 0.99)	0.03
	Incident Type 2 Diabetes among Participants with Prediabetes					
	Total population (n = 839, n cases = 259)	P-value	Men (n = 398, n cases = 114)	P-value	Women (n = 441, n cases = 145)	P-value
Model 1[1]	0.84 (0.73; 0.97)	0.02	0.85 (0.70; 1.04)	0.11	0.82 (0.66; 1.04)	0.10
Model 2[2]	0.85 (0.73; 0.98)	0.03	0.83 (0.69; 1.01)	0.06	0.85 (0.68; 1.07)	0.18
Model 3[3]	0.85 (0.73; 0.99)	0.03	0.86 (0.70; 1.05)	0.13	0.81 (0.63; 1.04)	0.10
	Incident Prediabetes					
	Total population (n = 4,957, n cases = 794)	P-value	Men (n = 1,868, n cases = 297)	P-value	Women (n = 3,089, n cases = 497)	P-value
Model 1[1]	0.94 (0.86; 1.03)	0.17	0.85 (0.74; 0.98)	0.02	1.01 (0.90; 1.14)	0.85
Model 2[2]	0.92 (0.84; 1.01)	0.09	0.83 (0.72; 0.95)	0.01	1.00 (0.89; 1.13)	0.99
Model 3[3]	0.93 (0.84; 1.02)	0.13	0.84 (0.72; 0.98)	0.02	0.99 (0.87; 1.12)	0.87

Results are presented as hazard ratio (95% confidence interval) for a standard deviation increment in FPAP score. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + body mass index, hypertension, dyslipidaemia, highest level of education attained, physical activity and smoking status. [3]Model 3: model 2 + degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake.

Finally, in the multivariable linear regression models, we observed that FRAP score was significantly inversely associated with HOMA-IR after adjustment for age, sex and cohort (model 1; regression coefficient (β) -0.04, 95% CI -0.06; -0.03). This association remained significant after adjusting for all covariates (model 3; β -0.04, 95% CI -0.06; -0.03). In the analysis stratified for sex (p-value for interaction 0.01), the association between FRAP score and HOMA-IR was significant among both men (β -0.03, 95% CI -0.06; -0.01) and women (β -0.05, 95% CI -0.07; -0.03), although slightly stronger among women (Table 2.1.3).

In sensitivity analyses, we observed that upon exclusion of participants with less than one year of follow-up, the associations between dietary antioxidant capacity and incident type 2 diabetes remained significant (HR 0.86, 95% CI 0.76; 0.98) (Supplementary Table 2.1.2). However, among individuals with prediabetes, the association was no

longer significant (HR 0.90, 95% CI 0.76; 1.05). Exclusion of participants with less than one year of follow-up did not change our conclusion with regards to incident prediabetes, which remained significantly associated with dietary antioxidant capacity only among men (HR 0.82, 95% CI 0.70; 0.97). After excluding coffee from the calculation of the FRAP score, the associations observed previously attenuated and FRAP score was no longer significantly associated with any of the outcomes (Supplementary Tables 2.1.3-2.1.4). Finally, in stage-specific analyses of HOMA-IR, we observed similar associations of dietary antioxidant capacity with HOMA-IR among participants with normoglycemia (β -0.04, 95% CI -0.05; -0.02) and participants with prediabetes (β -0.03, 95% CI -0.07; 0.002) (Supplementary Table 2.1.5).

Table 2.1.3. Associations between total dietary antioxidant capacity and homeostatic model assessment of insulin resistance (HOMA-IR).

	Total population (n = 5,422)	P-value	Men (n = 2,135)	P-value	Women (n = 3,287)	P-value
Model 1[1]	-0.04 (-0.06; -0.03)	< 0.001	-0.03 (-0.06; -0.01)	0.005	-0.06 (-0.08; -0.03)	< 0.001
Model 2[2]	-0.04 (-0.05; -0.03)	< 0.001	-0.03 (-0.05; -0.01)	0.001	-0.05 (-0.07; -0.03)	< 0.001
Model 3[3]	-0.04 (-0.06; -0.03)	< 0.001	-0.03 (-0.06; -0.01)	0.002	-0.05 (-0.07; -0.03)	< 0.001

Results are presented as regression coefficient (95% confidence interval) for a standard deviation increment in FPAP score. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + body mass index, hypertension, dyslipidaemia, highest level of education attained, physical activity and smoking status. [3]Model 3: model 2 + degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake.

DISCUSSION

In this population-based cohort, we observed that a higher total dietary antioxidant capacity is associated with a lower risk of type 2 diabetes, both in the total population and among those with prevalent prediabetes. In further stage-specific analyses, we found that a higher total dietary antioxidant capacity is also associated with lower risk of incident prediabetes among men, but not among women, and with a lower HOMA-IR among both men and women.

Our results are in line with the findings of previous studies which have investigated individual antioxidant components in relation to type 2 diabetes.[5,6,25] Montonen and colleagues demonstrated that various types of tocopherols were associated with a reduced risk of type 2 diabetes over 23 years of follow-up.[5] Similarly, Salonen and colleagues observed that low vitamin E levels predispose individuals to developing type

2 diabetes.[25] Sluijs and colleagues found that carotenoid intake was inversely related to risk of type 2 diabetes.[6] Furthermore, our findings confirm previous studies which have found associations between dietary antioxidant capacity and measures of insulin resistance.[7,8] Only one previous study has examined the total dietary antioxidant capacity in relation to type 2 diabetes.[13] In line with our findings, this study observed a strongly significant inverse association, but was performed among women only and did not investigate dietary antioxidant capacity in relation to stage-specific transitions from normoglycemia to type 2 diabetes. Thus, our study is the first that investigated total dietary antioxidant capacity among both men and women in relation to incident type 2 diabetes, including intermediate endpoints such as prediabetes and insulin resistance to capture the full trajectory from normoglycemia to type 2 diabetes.

Dietary antioxidants may directly affect glucose homeostasis in multiple ways. It has been hypothesized that oxidative stress activates the NF-κB pathway and various protein kinase pathways.[26] Activation of these pathways may inhibit signaling between insulin receptors and the glucose transport system, which contributes to the development of insulin resistance.[26,27] Through suppressing the formation of ROS, and thereby lowering oxidative stress, dietary antioxidants may improve insulin sensitivity. Furthermore, it has been demonstrated in animal models that antioxidants can suppress apoptosis of pancreatic β-cells induced by oxidative stress.[28] Therefore, dietary antioxidants may also help in sustaining β-cell function and preventing damage to these cells.

We found that dietary antioxidant capacity was not significantly associated with risk of prediabetes in the total study population. However, we did find significant associations between dietary antioxidant capacity and incident type 2 diabetes and HOMA-IR among both participants with normoglycemia and those with prediabetes. Because the relative contribution of pancreatic β-cell dysfunction to the pathogenesis of type 2 diabetes increases as hyperglycemia worsens, dietary antioxidants may more strongly affect risk of type 2 diabetes among individuals with prediabetes trough preserving β-cell function rather than attenuating insulin resistance.[29] These findings also suggest that a diet with a high antioxidant capacity will exert its protective effects against type 2 diabetes regardless of whether or not prediabetes is already present. It could therefore be hypothesized that the mechanisms underlying the protective effects of dietary antioxidants are related to both early-phase phenomena in the pathogenesis of type 2 diabetes (such as insulin resistance) and later-phase phenomena (such as β-cell dysfunction). However, the exact nature of these mechanisms is currently unclear, and further research is necessary to confirm our findings.

We observed significant modification of our effect estimates by sex for some of the analyses. However, sex differences were not consistent among outcomes: the association between total dietary antioxidant capacity and incident prediabetes was significant among men, but not among women, whereas associations with insulin resistance were slightly stronger among women compared to men. The latter observation is in line with findings reported by Okubo and colleagues.[8] Potential sex differences in associations of dietary antioxidant capacity with earlier stages in the development of type 2 diabetes could be caused by differences in visceral fat mass between men and women, because visceral fat mass is positively associated with the degree of oxidative stress and differs according to sex.[30,31] However, further research into the nature of potential sex differences is warranted, especially because we report for the first time that these appear to be stage-specific.

Our effect estimates decreased in magnitude when the contribution of coffee was excluded from the total dietary antioxidant capacity, suggesting that part of the association is explained by coffee intake. Coffee is commonly regarded as a major constituent of the total dietary antioxidant capacity. A recent study found that coffee intake captured 54% of the variation in total antioxidant intake among Norwegian women.[12] Likewise, in our study population, coffee constituted on average 49% of the total dietary antioxidant capacity. The fact that coffee forms an integral component of the total dietary antioxidant capacity probably accounts for the significant attenuation we observed in our effect estimates when coffee intake was excluded from the FRAP score. In relation to this, coffee intake has also been shown to be inversely related to risk of type 2 diabetes.[32–34] Disregarding coffee, the most important contributors to total dietary antioxidant capacity in our study were fruit and vegetables. Indeed, it has been demonstrated that increased fruit and vegetable consumption is associated with a lower risk of type 2 diabetes.[35] The findings of our study therefore further underline the putative beneficial health effects of coffee, fruit and vegetable consumption. With regards to tea and chocolate consumption, both of these food groups have also been associated with lower risk of type 2 diabetes.[36,37]

The main strengths of our study include its prospective design, the large sample size and the long-duration of follow-up. This enabled us to study the association between total dietary antioxidant capacity and various endpoints in the pathway from normoglycemia to type 2 diabetes with a large pool of validated cases. We were also able to adjust for an extensive set of socio-economic, metabolic and dietary confounders, including a measure of overall diet quality, to minimize the chance of residual confounding influencing our results. However, approximately 95% of our study population was of Caucasian ethnicity, and all participants were aged 45 years and older.

Therefore, caution should be taken in generalizing our results to other populations. Furthermore, we calculated the total dietary antioxidant capacity based on an antioxidant food database developed in Norway. We cannot rule out the possibility that differences between Norway and the Netherlands with regards to the geographical origin of food may have introduced error in our estimates of the true antioxidant capacity. In addition, we had no information on the cooking methods that participants used, which may also affect the antioxidant content of food. It is also conceivable that the use of different FFQ's and different food composition tables in our study caused differences between participants in the assessment of their FRAP score. However, regarding the use of different FFQ's, since the use of these different questionnaires coincided with the start of a new study cohort, and "cohort" was included in our analyses as a confounder, our analyses should to a large degree be adjusted for this effect. Finally, we were unable to account for the use of food supplements in our study, which may have led us to underestimate the actual total dietary antioxidant capacity.

In conclusion, total dietary antioxidant capacity was related to a lower risk of type 2 diabetes, but not risk of prediabetes, and was inversely associated with insulin resistance in this population-based cohort of individuals aged 45 years and older. Our findings emphasize the beneficial health effects of a diet rich in antioxidants with regards to the prevention of type 2 diabetes. Further studies could contribute to a better understanding of the stage-specific associations we have observed and unravel potential underlying mechanisms.

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Supplementary Table 2.1.1. Baseline characteristics of the study population, stratified by whether or not participants were included in the analysis of this study.

	Included participants (n = 5,796)	Excluded participants (n = 9,130)
Age (years)	64.2 (9.2)	66.7 (10.8)
Body Mass Index (kg/m^2)	26.9 (4.1)	27.7 (4.5)
Dyslipidemia		
No	3,656 (63.1%)	2,713 (79.7%)
Yes	1,922 (33.2%)	1,832 (20.1%)
Hypertension		
No	2,328 (40.2%)	1,535 (16.8%)
Yes	3,370 (58.1%)	5,690 (62.3%)
Physical Activity (metabolic Equivalents of Task- hours/week)[1]		
-RS-I/RS-II (Zutphen Questionnaire)	82.0 (57.4)	67.3 (57.4)
-RS-III (LASA Questionnaire)	42.9 (63.2)	36.0 (61.6)
-Total	71.7 (63.8)	63.5 (60.4)
Education		
Primary	645 (11.1%)	2,072 (22.7%)
Lower	2,386 (41.2%)	3,384 (37.1%)
Intermediate	1,645 (28.4%)	2,233 (24.5%)
Higher	1,084 (18.7%)	1,055 (11.6%)
Smoking		
Never	1,925 (33.2%)	2,894 (31.7%)
Former	2,514 (43.4%)	3,659 (40.1%)
Current	1,329 (22.9%)	2,192 (24.0%)
Alcohol consumption (g/day)[1]	6.6 (18.1)	3.2 (15.6)

Variables are presented as mean (SD) unless otherwise indicated. [1]Variable is presented as median (interquartile range) because it did not follow a normal distribution. Differences between men and women were assessed using Student's T-tests in the case of normally distributed continuous variables, χ^2-tests in the case of categorical variables and Mann-Whitney U tests in the case of non-normally distributed continuous variables. Included participants are those who had valid dietary data available, did not have cancer or a history of cardiovascular disease and had information on glucose status available at baseline. The statistics presented above stem from an unimputed dataset.

Supplementary Table 2.1.2. Associations between total dietary antioxidant capacity and risk of type 2 diabetes, type 2 diabetes among participants with prediabetes and prediabetes, excluding participants with less than one year of follow-up.

	Incident Type 2 Diabetes					
	Total population (n = 5,738, n cases = 505)	P-value	Men (n = 2,236, n cases = 203)	P-value	Women (n = 3,502, n cases = 302)	P-value
Model 1[1]	0.88 (0.78; 1.00)	0.04	0.89 (0.75; 1.05)	0.17	0.89 (0.75; 1.04)	0.15
Model 2[2]	0.87 (0.78; 0.98)	0.03	0.86 (0.72; 1.02)	0.08	0.88 (0.74; 1.04)	0.13
Model 3[3]	0.86 (0.76; 0.98)	0.02	0.87 (0.72; 1.04)	0.12	0.85 (0.71; 1.01)	0.06
	Incident Type 2 Diabetes among Participants with Prediabetes					
	Total population (n = 821, n cases = 244)	P-value	Men (n = 389, n cases = 106)	P-value	Women (n = 432, n cases = 138)	P-value
Model 1[1]	0.89 (0.77; 1.04)	0.15	0.94 (0.77; 1.15)	0.54	0.85 (0.67; 1.07)	0.17
Model 2[2]	0.92 (0.75; 1.16)	0.53	0.91 (0.75; 1.12)	0.38	0.88 (0.69; 1.12)	0.29
Model 3[3]	0.90 (0.76; 1.05)	0.18	0.93 (0.75; 1.15)	0.52	0.83 (0.64; 1.08)	0.17
	Incident Prediabetes					
	Total population (n = 4,888, n cases = 753)	P-value	Men (n = 1,837, n cases = 280)	P-value	Women (n = 3,051, n cases = 473)	P-value
Model 1[1]	0.93 (0.85; 1.02)	0.13	0.83 (0.72; 0.97)	0.02	1.01 (0.89; 1.14)	0.92
Model 2[2]	0.91 (0.83; 1.00)	0.06	0.81 (0.70; 0.94)	0.01	0.99 (0.88; 1.12)	0.89
Model 3[3]	0.91 (0.83; 1.01)	0.08	0.82 (0.70; 0.97)	0.02	0.97 (0.85; 1.11)	0.68

Results are presented as hazard ratio (95% confidence interval) for a standard deviation increment in FPAP score. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + body mass index, hypertension, dyslipidaemia, highest level of education attained, physical activity and smoking status. [3]Model 3: model 2 + degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake.

Supplementary Table 2.1.3. Associations between total dietary antioxidant capacity and risk of type 2 diabetes, type 2 diabetes among participants with prediabetes and prediabetes, excluding the contribution of coffee.

	Incident Type 2 Diabetes					
	Total population (n = 5,796, n cases = 532)	P-value	Men (n = 2,266, n cases = 218)	P-value	Women (n = 3,530, n cases = 314)	P-value
Model 1[1]	0.89 (0.80; 0.99)	0.03	0.88 (0.74; 1.04)	0.13	0.90 (0.79; 1.03)	0.12
Model 2[2]	0.97 (0.87; 1.07)	0.52	0.82 (0.70; 0.97)	0.02	0.99 (0.87; 1.13)	0.91
Model 3[3]	0.96 (0.85; 1.09)	0.54	0.95 (0.79; 1.15)	0.61	0.96 (0.82; 1.12)	0.57
	Incident Type 2 Diabetes among Participants with Prediabetes					
	Total population (n = 839, n cases = 259)	P-value	Men (n = 398, n cases = 114)	P-value	Women (n = 441, n cases = 145)	P-value
Model 1[1]	1.00 (0.87; 1.14)	0.98	0.99 (0.81; 1.22)	0.96	1.01 (0.84; 1.20)	0.96
Model 2[2]	1.04 (0.91; 1.18)	0.60	0.99 (0.81; 1.21	0.91	1.06 (0.88; 1.28)	0.52
Model 3[3]	1.07 (0.92; 1.25)	0.38	1.04 (0.84; 1.29)	0.71	1.07 (0.85; 1.34)	0.57
	Incident Prediabetes					
	Total population (n = 4,957, n cases = 794)	P-value	Men (n = 1,868, n cases = 297)	P-value	Women (n = 3,089, n cases = 497)	P-value
Model 1[1]	0.89 (0.82; 0.97)	0.01	0.90 (0.78; 1.04)	0.17	0.89 (0.80; 0.99)	0.03
Model 2[2]	0.96 (0.88; 1.04)	0.32	0.96 (0.83; 1.11)	0.60	0.96 (0.86; 1.07)	0.46
Model 3[3]	0.96 (0.87; 1.06)	0.44	1.00 (0.85; 1.17)	0.99	0.94 (0.83; 1.06)	0.30

Results are presented as hazard ratio (95% confidence interval) for a standard deviation increment in FPAP score. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + body mass index, hypertension, dyslipidaemia, highest level of education attained, physical activity and smoking status. [3]Model 3: model 2 + degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake.

Supplementary Table 2.1.4. Associations between total dietary antioxidant capacity and HOMA-IR, excluding the contribution of coffee.

	Total population (n = 5,422)	P-value	Men (n = 2,135)	P-value	Women (n = 3,287)	P-value
Model 1[1]	-0.04 (-0.06; -0.03)	< 0.001	-0.05 (-0.85; -0.023)	< 0.001	-0.03 (-0.05; -0.01)	0.001
Model 2[2]	-0.02 (-0.03; -0.002)	0.03	-0.03 (-0.05; -0.01)	0.004	-0.01 (-0.02; 0.01)	0.57
Model 3[3]	-0.01 (-0.03; 0.01)	0.21	-0.03 (-0.06; -0.01)	0.02	0.003 (-0.02; 0.02)	0.74

Results are presented as regression coefficient (95% confidence interval) for a standard deviation increment in FPAP score. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + body mass index, hypertension, dyslipidaemia, highest level of education attained, physical activity and smoking status. [3]Model 3: model 2 + degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake.

Supplementary Table 2.1.5. Associations between total dietary antioxidant capacity and HOMA-IR among participants with normoglycaemia and prediabetes.

	Participants with normoglycaemia					
	Total population (n = 4,614)	P-value	Men (n = 1,752)	P-value	Women (n = 2,862)	P-value
Model 1*	-0.04 (-0.05; -0.02)	< 0.001	-0.03 (-0.05; -0.004)	0.02	-0.05 (-0.07; -0.03)	< 0.001
Model 2†	-0.04 (-0.05; -0.02)	< 0.001	-0.03 (-0.05; -0.01)	0.01	-0.05 (-0.06; -0.03)	< 0.001
Model 3‡	-0.04 (-0.05; -0.02)	< 0.001	-0.03 (-0.05; -0.01)	0.01	-0.04 (-0.06; -0.02)	< 0.001
	Participants with prediabetes					
	Total population (n = 808)	P-value	Men (n = 383)	P-value	Women (n = 425)	P-value
Model 1*	-0.03 (-0.07; 0.004)	0.08	-0.03 (-0.07; 0.02)	0.30	-0.06 (-0.12; -0.001)	0.05
Model 2†	-0.03 (-0.06; 0.01)	0.10	-0.03 (-0.07; 0.01)	0.15	-0.04 (-0.01; 0.01)	0.13
Model 3‡	-0.03 (-0.07; 0.002)	0.06	-0.03 (-0.08; 0.01)	0.18	-0.05 (-0.11; 0.004)	0.07

Results are presented as regression coefficient (95% confidence interval) for a standard deviation increment in FPAP score. *Model 1: adjusted for age, sex and Rotterdam Study cohort. †Model 2: model 1 + body mass index, hypertension, dyslipidaemia, highest level of education attained, physical activity and smoking status. ‡Model 3: model 2 + degree of adherence to dietary guidelines, total daily energy intake and daily alcohol intake.

Chapter 2.2

Plant versus Animal-based Diets and Insulin Resistance, Prediabetes and Type 2 Diabetes: the Rotterdam Study

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European Journal of Epidemiology, 2018

ABSTRACT

Background

Vegan or vegetarian diets have been suggested to reduce risk of type 2 diabetes (T2D). However, not much is known on whether variation in the degree of having a plant-based versus animal-based diet may be beneficial for the prevention of T2D. We aimed to investigate whether level of adherence to a diet high in plant-based foods and low in animal-based foods is associated with insulin resistance, prediabetes, and T2D.

Methods

Our analysis included 6,798 participants (mean age 62.7 years, SD 7.8) from the Rotterdam Study, a prospective population-based cohort in the Netherlands. Dietary intake data were collected with food-frequency questionnaires at baseline of three Rotterdam Study sub-cohorts (RS-I-1: 1989-1993, RS-II-1: 2000-2001, RS-III-1: 2006-2008). We constructed a continuous plant-based dietary index (range 0-92) expressing adherence to a plant-based versus animal-based diet. Insulin resistance at baseline and follow-up was assessed using homeostatic model assessment of insulin resistance (HOMA-IR). Information on prediabetes and T2D were collected from general practitioners' records, pharmacies' databases, and follow-up examinations in our research center up to 2012. We used multivariable linear mixed models to examine associations of the index with longitudinal HOMA-IR and multivariable proportional hazards regression models to examine associations of the index with risk of prediabetes and T2D.

Results

We documented 928 cases of prediabetes and 642 cases of T2D, during a mean duration of follow-up of 5.7 and 7.3 years, respectively. After adjusting for sociodemographic and lifestyle factors, a higher score on the plant-based dietary index was associated with lower insulin resistance (per 10 units higher score: β -0.09, 95% CI -0.10; -0.08), lower prediabetes risk (HR 0.89, 95% CI 0.81; 0.98), and lower T2D risk (HR 0.82, 95% CI 0.73; 0.92). After additional adjustment for BMI, associations attenuated and remained statistically significant for longitudinal insulin resistance (β -0.05, 95% CI -0.06; -0.04) and T2D risk (HR 0.87, 95% CI 0.79; 0.99), but no longer for prediabetes risk (HR 0.93, 95% CI 0.85; 1.03).

Conclusions

A more plant-based and less animal-based diet may lower risk of insulin resistance, prediabetes and T2D. These findings strengthen recent dietary recommendations to adopt a more plant-based diet.

INTRODUCTION

Diet is an important modifiable lifestyle determinant in the development of type 2 diabetes (T2D).[1] Among dietary components, consumption of several plant-based foods such as root vegetables, green leafy vegetables, whole grains, nuts and peanut butter has been associated with a lower risk of T2D.[2–5] In contrast, consumption of several animal-based foods, including red meat, processed meat and eggs, has been associated with an increased risk of T2D.[4,6,7]

Although multiple food groups seem to influence the risk of T2D, humans generally do not consume single food items or food groups, and the role of diet in health may be better described by overall dietary patterns.[8] Previous studies have observed that vegan or vegetarian diets are associated with improved glycemic control and lower T2D risk.[9,10] However, these previous studies dichotomously classified participants, and only defined diets as vegetarian or vegan versus non-vegetarian diets. A dichotomous classification of vegans or vegetarians versus their non-vegetarian counterparts might not be an optimal approach in understanding the effect of a plant-based diet in Western countries, because it does not reflect dietary patterns of a large proportion of the population. From a public health perspective, it is interesting to know whether a more plant-based and less animal-based diet may also influence insulin resistance and risk of prediabetes and T2D beyond strict adherence to a vegetarian or vegan diet. To our knowledge, only one previous study, a large prospective cohort study in the US, examined associations between variations in the degree of adherence to plant-based versus animal-based diets with T2D risk and observed that a more plant-based diet was associated with a lower T2D risk.[11] Studies on the associations of such plant-based dietary patterns with T2D risk in other populations are needed. In addition, the association of such plant-based dietary patterns with intermediate risk factors for T2D, such as insulin resistance and prediabetes remain unknown.

Therefore, we aimed to investigate whether adherence to a more plant-based, and less animal-based diet is associated with insulin resistance, and risk of prediabetes and T2D in a Dutch middle-aged and older general population.

METHODS

Study population

This study was performed within three sub-cohorts of the Rotterdam Study, a prospective cohort study of adult aged 45 years and older living in the well-defined

district of Ommoord in Rotterdam, the Netherlands. A detailed description of the Rotterdam Study methodology has been provided elsewhere.[12] Briefly, recruitment of participants for the first sub-cohort (RS-I) started in the period of 1989-1993 among inhabitants aged ≥ 55 years (n = 7,983). In 2000-2001, the study was extended with a second sub-cohort (RS-II) of new individuals (n = 3,011) who had become 55 years of age or moved into the study area after 1990. In 2006-2008, a third sub-cohort (RS-III) was recruited with new individuals aged 45 years and older (n = 3,932). By the end of 2008, the overall study population contained 14,926 participants. Upon entering the study, participants underwent home interviews and a series of examinations in our research center every 3-5 years. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports. The approval has been renewed every 5 years. All participants gave informed consent.

Population for current analyses

For the current study, we used data from all three sub-cohorts. Of the 14,926 participants, we excluded 5,225 participants without valid dietary data (no dietary data, unreliable dietary intake according to a trained nutritionist or an estimated energy intake of < 500 or > 5000 kcal/day[13]) at baseline (RS-I-1: starting 1989, RS-II-1: starting 2000, RS-III-1: starting 2006), and 2,903 participants without information on T2D status or with prevalent T2D at baseline, leaving 6,798 participants included as main population for analysis.

From this group of 6,798 participants, 6,514 participants had data on HOMA-IR before onset of T2D and were included in the longitudinal HOMA-IR analyses. For the analyses on prediabetes risk, we excluded those with prevalent prediabetes at baseline (n = 1,005) or without follow-up for prediabetes (n = 25), leaving 5,768 participants. In the analyses assessing risk of T2D, we excluded participants without follow-up of T2D (n = 28), leaving 6,770 participants. The flow-diagram of the included participants is presented in Figure 2.2.1.

Dietary assessment

Dietary intake was assessed at baseline in all three sub-cohorts using semi-quantitative food-frequency questionnaires (FFQ) as described in more detail elsewhere.[13] We used an FFQ with 170 food items to assess dietary intake at baseline of RS-I and RS-II; at baseline of RS-III, we used an FFQ with 389 food items.[14,15] The 170-item FFQ was validated in a subsample of the Rotterdam Study (n = 80) against fifteen 24-h food records and four 24h urinary urea excretion samples,[14] and the 389-item FFQ was previously validated in other Dutch population against measurement of biomarkers, against a

9-day dietary record, and against a 4 week dietary history.[16] In general, the validation studies demonstrated that the FFQs were able to adequately rank participants according to their intake.[13] Food intake data were converted to energy and nutrient intake based on Dutch Food Composition tables (NEVO).

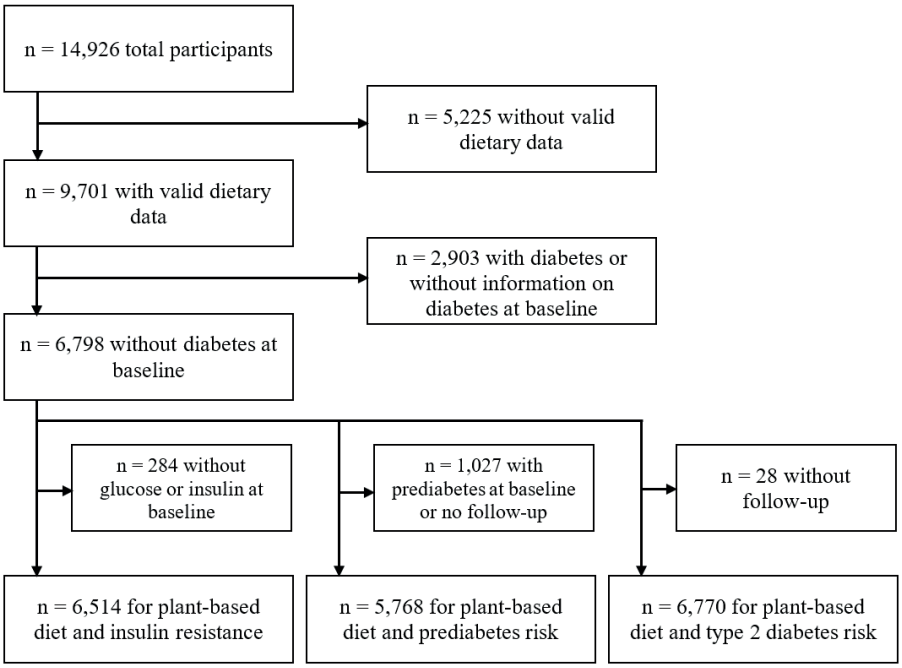

Figure 2.2.1. Selection of the study population.

Plant-based dietary index

We constructed an overall plant-based dietary index, which was a modified version of two previously created indices.[11,17] More specifically, our index is similar to the provegetarian food pattern of Martínez-Gonzáles et al.[17] and to the overall plant-based diet index of Satija et al.,[11] but was adapted to include slightly different types and numbers of food categories. First, the food items as measured by the FFQs were divided into 23 food categories (Supplemental Table 2.2.1), on the basis of the main food groups in the Dutch diet and the Dutch food-based dietary guidelines.[18,19] Twelve of the categories were plant-based and eleven were animal-based. Food items that were not clearly animal-based or plant-based, such as pizza, as well as dietary supplements, were not included in the food categories for the index. Dietary intake for each of the 23 food categories was calculated for each participant in grams per day. Subsequently, for each category, the intake was divided into cohort-specific quintiles. Each quintile

was assigned a value between 0 and 4. For the twelve plant-based food categories, consumption within the highest quintile was scored a 4, consumption within the second highest quintile was scored a 3, and so on, ending with consumption within the lowest quintile receiving a score of 0. The eleven animal-based food categories were scored in reverse: consumption within the highest quintile was scored a 0, consumption within the second highest quintile was scored a 1, ending with consumption within the lowest quintile receiving a score of 4. Furthermore, we ensured that all participants with null consumption were given the score belonging to the lowest quintile by re-scoring when necessary. Finally, these category quintile-scores were added up per participant to create their overall score on the plant-based dietary index. The resulting index yielded a score for each participant that measures adherence to a plant-based versus animal-based diet on a continuous scale, with a lowest possible score of 0 (low adherence to a plant-based diet) and a highest possible score of 92 (high adherence to a plant-based diet). Information on intake of each food category across quintiles of scores on the plant-based dietary index is shown in Supplemental Table 2.2.2.

Assessment of insulin resistance

Information on prediabetes and T2D was collected from general practitioners' records, pharmacies' databases, and follow-up examinations in our research center. Data on prediabetes and T2D in our analyses were collected until January 1, 2012. Prediabetes and T2D were identified according to WHO criteria: prediabetes was defined as a fasting blood glucose concentration of > 6.0 and < 7.0 mmol/L, or a non-fasting blood glucose concentration of > 7.7 mmol/L and < 11.1 mmol/L; T2D was defined as a fasting blood glucose concentration of ≥ 7.0 mmol/L, a non-fasting blood glucose concentration of ≥ 11.1 mmol/L (when fasting samples were unavailable), the use of blood glucose-lowering medication or dietary treatment, or registration of the diagnosis T2D. All possible cases of prediabetes and T2D were formally judged by two independently working study physicians or, in case of disagreement, by an endocrinologist.[20]

Assessment of covariates

Information on age, sex, smoking status, educational level, medication use, food supplement use and family history of diabetes was obtained from questionnaires at baseline. Information on physical activity was obtained using the adapted version of the Zutphen Physical Activity Questionnaire at RS-I-3 and RS-II-1 and using the LASA Physical Activity Questionnaire at RS-III-1. Physical activities were weighted according to intensity with Metabolic Equivalents of Task (MET), from the Compendium of Physical Activities version 2011. To account for differences between the two questionnaires, questionnaire-specific z-scores of MET-hours per week were calculated. At our

research center at baseline, body weight was measured using a digital scale and body height was measured using a stadiometer, while participants wore light clothing and no shoes, and BMI was calculated (kg/m^2). Information on hypertension, hypercholesterolemia, coronary heart disease (CHD), cancers, and stroke was obtained from general practitioners, pharmacies' databases, Nationwide Medical Register, or follow-up examinations in our research center.

Data analysis

To obtain a normal distribution for HOMA-IR, we applied a natural-log transformation. Non-linearity of associations of score on the plant-based dietary index with all outcomes were explored using three-knot natural cubic splines. As no indication for non-linear associations was found in the main model, all primary analyses were performed using models assuming linearity. We examined the association between score on the plant-based dietary index with longitudinal HOMA-IR using linear mixed models, with a random-effects structure including a random intercept and slope (for time of repeated measurements of HOMA-IR). We examined the association between score on the plant-based dietary index and risk of prediabetes and risk of T2D using proportional hazards regression. Hazard ratios (HRs) and regression coefficients (βs) are presented per 10 units higher score on the plant-based dietary index, along with the corresponding 95% confidence intervals (CIs). All analyses were performed in participants of the three sub-cohorts combined and in the three sub-cohorts separately.

All analyses were adjusted for energy intake, age, sex and RS sub-cohort in model. For the analyses of longitudinal HOMA-IR we additionally adjusted for the time of repeated measurements of HOMA-IR. In model 2, we additionally adjusted for smoking status, educational level, physical activity, food supplement use, and family history of diabetes. Baseline BMI was added in model 3 to examine its potential mediating effect. We examined effect modification by including interactions of the plant-based index with age, sex, or BMI for all outcomes in model 2.

Several sensitivity analyses were performed based on model 2. First, to check whether the associations were driven by any specific component of the plant-based dietary index, we repeated our main analyses by excluding each one of the 23 components from the plant-based dietary index one by one at a time, and additionally adjusting for the excluded component. Second, to check if the associations were mainly driven by plant-based beverages combined, we examined the associations by excluding all plant-based beverages combined (the categories coffee and tea, alcoholic beverages, and sugary beverages) from the plant-based dietary index at a time, and additionally adjusting for them. Third, we examined the associations by excluding less healthy

plant-based foods combined (the categories sweets, sugary beverages, potatoes and refined grains) from the plant-based dietary index at a time, and additionally adjusting for them. To further examine whether these less healthy plant foods contributed to the association of the plant-based dietary index, we created a less healthy plant foods score in which positive scores were given to these four types of less healthy plant-based food groups. In calculating this score, reverse scores were given to healthy plant food groups and animal food groups.[21] Fourth, to examine if potential associations of the plant-based dietary score with outcomes were independent of overall quality of the diet based on adherence to dietary guidelines, we examined the correlation between the plant-based dietary score and the dietary guidelines score, and we repeated analyses with additional adjustment for dietary guidelines score. Fifth, we additionally adjusted for hypertension and hypercholesterolemia. Sixth, we excluded the participants with chronic diseases at baseline, such as participants with coronary heart disease, cancers, or stroke, to exclude the possibility of a significant change of diet and lifestyle at follow-up. Last, we excluded the participants who developed prediabetes and T2D in the first 2 years of follow-up in the analyses for risk of prediabetes and T2D, respectively. Missing values in covariates (ranging from 0.3% to 3.9%) were accounted for using ten-fold multiple imputation. We used SPSS version 21 (IBM Corp., Armonk, NY, USA) and R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) to perform these analyses.

RESULTS

Baseline characteristics of the study population are shown in Table 2.2.1. In our population of 6,798 participants, baseline scores on the plant-based dietary index (with a theoretical range from 0 to 92) ranged from 24 to 75, with a mean ± SD score of 49.3 ± 7.1. Mean age of the study population was 62.0 ± 7.8 years and 41.3% of the participants were male. Mean BMI was 26.6 ± 3.9 kg/m^2. Characteristics were similar before and after multiple imputation (Supplemental Table 2.2.3). Supplemental Table 2.2.4 shows baseline characteristics of the participants not included in our analyses.

After adjustment for confounders in model 2, a higher score on the plant-based dietary index was associated with lower longitudinal HOMA-IR (per 10 units higher score on the index: β -0.09, 95% CI -0.10; -0.08) (Table 2.2.2). Additional adjustment for BMI in model 3 attenuated the association, but it remained statistically significant (β -0.05, 95% CI -0.06; -0.04).

Table 2.2.1. Baseline characteristics of study participants (n = 6,798)

Characteristic	Mean (SD), median (IQR), or %
Age (years)	62.0 (7.8)
Sex (% male)	41.3 %
BMI (kg/m^2)	26.6 (3.9)
Smoking (%)	
Never	32.2 %
Ever	45.1 %
Current	22.7 %
Physical activity[1] (MET-hours/week)	
RS-I and RS-II (Zutphen Questionnaire, n = 4,393)	86.7 (44.7)
RS-III (LASA Questionnaire, n = 2,194)	58.4 (55.8)
Hypertension (%)	42.3 %
Hypercholesterolemia (%)	45.4 %
Family history of diabetes (%)	10.8 %
Highest level of education (%)	
Primary	11.8 %
Lower	40.9 %
Intermediate	29.0 %
Higher	18.3 %
Current food supplement use (%)	16.5 %
Total energy intake (kcal/day)	2,134 (615)
Plant-based food category intake (grams/day)[2]	
Fruit	212.2 (115.5, 332.3)
Vegetables	209.1 (147.9, 286.87)
Whole grains	105.7 (61.3, 152.5)
Nuts	3.9 (0.0, 12.0)
Legumes	4.1 (0.0, 19.4)
Potatoes	99.7 (61.4, 148.2)
Vegetable oils	19.7 (9.2, 30.0)
Tea and coffee	758.9 (580.4, 1000)
Sugary beverages	46.3 (0.0, 139.6)
Refined grains	50.7 (23.9, 102.1)
Sweets	63.8 (37.1, 97.4)
Alcoholic beverages	56.4 (4.9, 159.8)
Animal-based food category intake (grams/day)[2]	
Low-fat milk	82.3 (0.0, 232.3)
Full-fat milk	0.0 (0.0, 0.0)
Low-fat yoghurt	56.1 (0.0, 164.6)
Full-fat yoghurt	0.0 (0.0, 4.9)

Table 2.2.1. Baseline characteristics of study participants (n = 6,798) (continued)

Characteristic	Mean (SD), median (IQR), or %
Cheese	30.8 (20.0, 47.1)
Unprocessed lean meat	10.7 (4.3, 18.1)
Fish	15.9 (3.9, 30.7)
Eggs	14.3 (7.1, 19.6)
Animal fat	0.0 (0.0, 0.9)
Desserts/dairy with sugars	14.1 (0.0, 54.6)
Processed meat/red meat	86.8 (60.4, 118.9)
Plant-based dietary index (score)	49.3 (7.1)

Plant-based dietary index: a higher score indicates a higher adherence to a plant-based diet (theoretical range from 0 to 92). Values shown are based on pooled results of imputed data. [1]Values shown for MET-hours are un-imputed; imputation was performed on z-scores of physical activity. [2]Variable expressed as median (IQR). Abbreviations: MET, metabolic equivalent of task; SD, standard deviation.

Table 2.2.2. Associations of the plant-based dietary index with longitudinal insulin resistance (HOMA-IR), risk of prediabetes, and risk of type 2 diabetes

	HOMA-IR	Prediabetes	Type 2 diabetes
	(n = 6,514)	(n = 5,768)	(n = 6,770)
	β (95% CI)	HR (95% CI)	HR (95% CI)
Model 1	-0.09 (-0.10; -0.08)***	0.88 (0.80; 0.97)**	0.82 (0.73; 0.92)***
Model 2	-0.09 (-0.10; -0.08)***	0.89 (0.81; 0.98)*	0.82 (0.73; 0.92)**
Model 3	-0.05 (-0.06; -0.04)***	0.93 (0.85; 1.03)	0.87 (0.79; 0.99)*

Effect estimates are regression coefficients (β) for ln HOMA-IR or hazard ratios (HRs) for incidence of prediabetes or type 2 diabetes with their 95%-confidence intervals (95% CIs), per 10 units higher score on the plant-based dietary index. Estimates are based on pooled results of imputed data. Model 1 is adjusted for energy intake, sex, age and RS sub-cohort (RS-I, RS-II, or RS-III); and only for the HOMA analyses additionally for the time measurements of longitudinal HOMA. Model 2 is additionally adjusted for education, smoking status, family history of diabetes, physical activity and food supplement use. Model 3 is additionally adjusted for BMI. *p < 0.05; ** p < 0.01; ***p < 0.001. Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; MET, metabolic equivalent of task; RS, Rotterdam Study.

During 43,773 person-years of follow-up among 5,768 participants (median follow-up 5.7 years), 928 participants developed prediabetes. After adjustment for confounders in model 2, a higher score on the plant-based dietary index was associated with a lower incidence of prediabetes (per 10 units higher score on the index: HR 0.89, 95% CI 0.81; 0.98). After additional adjustment for BMI in model 3 the association attenuated and was no longer statistically significant (HR 0.93, 95% CI 0.85; 1.03).

During 54,024 person-years of follow-up amongst 6,770 participants (median follow-up 7.3 years), 642 participants developed T2D. In model 2, a higher score on the plant-based dietary index was associated with a lower incidence of T2D (per 10 units higher score on the index: HR 0.82, 95% CI 0.73; 0.92). Additional adjustment for BMI in model 3 attenuated this association, but it remained statistically significant (HR 0.87, 95% CI 0.79; 0.99). The associations between the plant-based dietary index with longitudinal insulin resistance and risk of prediabetes and T2D were similar in three sub-cohorts (Supplemental Tables 2.2.5-2.2.7). Associations did not differ by age, sex or baseline BMI (p-values for all interaction terms were > 0.05).

Exclusion of each one of 23 foods from the index one by one at a time did not substantially change the estimates (Supplemental Table 2.2.8). Excluding all plant-based beverages combined at a time (coffee and tea, alcoholic beverages and sugary beverages) did not substantially change the estimates for insulin resistance (per 10 units higher score on the index: β -0.06, 95% CI -0.10; -0.03), risk of prediabetes (HR 0.93, 95% CI 0.84; 1.02) or risk of T2D (HR 0.85, 95% CI 0.80; 0.96). The estimates also remained similar after excluding these less healthy plant-based foods (sweets, sugary beverages, potatoes, and refined grains) combined at a time (per 10 units higher score on the index: insulin resistance: β -0.09, 95% CI -0.10; -0.07, prediabetes risk: HR 0.90, 95% CI 0.84; 0.98, T2D risk: HR 0.83, 95% CI 0.74; 0.94), but the less healthy plant foods score was not associated with insulin resistance, risk of prediabetes or type 2 diabetes. The Pearson's correlation coefficient between the plant-based dietary score with the dietary guidelines score was 0.16 ($p < 0.05$), and additionally controlling for the dietary guidelines score did not substantially affect the estimates. Additional adjustment for hypertension and hypercholesterolemia did not change effect estimates substantially. Estimates also remained similar after excluding participants with chronic diseases at baseline. Finally, excluding participants who developed T2D or prediabetes in the first 2 years of follow-up modestly attenuated the associations for risk of prediabetes (per 10 units higher score on the index, HR 0.91, 95% CI 0.83; 1.01) and risk of T2D (HR 0.82, 95% CI 0.73; 0.92).

DISCUSSION

In this large population-based cohort, we observed that a diet higher in plant-based foods and lower in animal-based foods was associated with lower insulin resistance as well as a lower risk of prediabetes and T2D, suggesting a protective role of a more plant-based as opposed to a more animal-based diet in the development to T2D, beyond strict adherence to a vegetarian or vegan diet.

The inverse association between plant-based diets and T2D risk is in agreement with previous research showing lower T2D risk for vegans or vegetarians compared to non-vegetarians.[10] Moreover, the associations we observed confirmed previous observations by Satija and colleagues in a US sample, which is the only other prospective study examining adherence to plant-based diets in a continuous graduation with risk of T2D[11]. We extend upon these previous findings by also showing associations between plant-based diets and earlier stages in the development of T2D (insulin resistance and prediabetes) in a European population.

Our results imply a beneficial effect of adherence to a diet higher in plant-based foods and lower in animal-based foods on the development of T2D, irrespective of general healthiness of the specific plant-based and animal-based foods. With these results, we provide a different view on what a healthy diet may entail. However, we acknowledge that our plant-based diet included positive scoring for some components that are not necessarily healthy choices for prevention of T2D, or a healthy diet in general. Sugary beverages, for example, have been associated with adverse effects for T2D in other studies.[22,23] To further clarify whether these less healthy plant foods contributed to the observed associations, we examined the associations between a less healthy plant-based diet score with insulin resistance and risks of prediabetes and T2D in sensitivity analyses, and observed null associations; suggesting beneficial associations were mainly driven by higher intake of healthy plant-based food groups and lower intake of animal-based food groups. This emphasizes that it is important to also consider the quality of plant-based foods consumed, which has important public health implications. Furthermore, the estimates for the plant-based dietary index remained similar after excluding these plant-based beverages combined, or after excluding the less healthy plant-based foods combined, meaning that our results were stable in diverse versions of plant-based diets and thus increasing our confidence in the validity of the findings. We also observed that excluding each one of 23 components one by one at a time resulted in similar associations as observed for the total plant-based index, indicating that the associations were not mainly explained by any one specific food group, which supports the importance of recognizing overall plant-based diet. Finally, we extended our analyses to examine if adherence to a plant-based diet was independent of adherence to current Dutch dietary guidelines. In line with results from the large prospective cohort study in the US which examined if adherence to a plant-based diet was independent of general healthy dietary patterns that have been linked to prevention of T2D, such as the Mediterranean diet, the alternative Healthy Eating Index (aHEI), and the Dietary approaches to stop hypertension (DASH) diet,[24–26] we observed that associations of the plant-based dietary index with outcomes remained

similar after additional adjustment for adherence to current Dutch dietary guidelines. This lends support to the novelty of the plant-based dietary index.

Taken together, a more plant-based, less animal-based diet may help prevent the development of T2D. Still more important, a more plant-based diet does not require a radical change in diet in terms of total elimination of meat or animal products but instead can be achieved in various ways, increasing the potential for population-wide health recommendations. For example, if a participant in our cohort would increase fruits intake from 95 grams per day to 200 grams per day, increase vegetables intake from 100 grams to 260 grams, and at the same time decrease red meat intake from 129 grams per day to 55 grams per day, this would improve the plant-based dietary index by 10 units, which may decrease risk of T2D by 13%, assuming other covariates remain stable.

Several mechanisms may underlie the observed associations. On the one hand, a plant-based diet usually contains more fiber, chlorogenic acids, certain types of amino acids, unsaturated fatty acids and antioxidants. For example, vegetables and fruits are the main sources of fiber, antioxidants, and chlorogenic acids; nuts are rich in poly-unsaturated fatty acids; soy and beans are main sources of plant protein; whole grains are rich in fiber and plant protein; and coffee and tea are rich in antioxidants and chlorogenic acid. These beneficial components may influence the development of T2D through affecting intermediate conditions in the pathogenesis of this disorder, such as obesity and inflammation. Fiber is known to lower gastric emptying and thereby glycemic responsiveness and might also have beneficial effects on inflammation and obesity.[27–30] Chlorogenic acids can improve inflammation, glucose tolerance and glucose levels, and improve increasing insulin secretion.[31] Soy protein contains high amounts of the amino acids arginine and glycine, which have been associated with a decrease in cholesterol levels.[32] High intake of unsaturated fatty acids has also been associated to lower inflammation and less obesity.[28,33] Phenol chlorogenic acid was reported to reduce insulin resistance.[34] On the other hand, a plant-based diet usually has less animal protein, saturated fatty acids, and heme iron. Animal protein is rich in branched-chain amino acids and aromatic amino acids and may impair glucose metabolism and increase T2D risk;[35–38] animal protein is also rich in heme iron, which has been suggested to increase risk of cardiometabolic disease.[39–41] Higher saturated fatty acids have been suggested to be associated with higher inflammation as well as higher risk of obesity and T2D.[33,42,43] Furthermore, other nutrients contained in processed red meat, such as sodium and nitrites, may also increase risk of cardio-metabolic disease.[41] More research is needed to explore whether the mechanisms underlying the observed associations also involve an effect of plant-based foods on gut

microbiome. Finally, these different mechanisms may influence each other because of interrelations between different food components. This also highlights the relevance of examining overall diets in addition to isolated food items, as this enables capturing the combined effects of the suggested pathways.

This study has several strengths. First, to our knowledge, we are the first to investigate the associations between plant-based diets and longitudinal insulin resistance and prediabetes, for which we had longitudinal data available with a long period of follow-up. Studying these early risk stages helps to minimize reverse causation in understanding how a plant-based diet influences the development of T2D. Second, we observed that the potential beneficial effect of a more plant-based diet was independent of less healthy plant-based foods, such as sweets, sugary beverages and refined grains, emphasizing the importance of considering the quality of plant-based foods consumed. We also observed associations of the plant-based dietary score independent of overall adherence to dietary guidelines, indicating that the plant-based diet score may reflect more than only a healthy dietary pattern as reflected by current dietary guidelines. Other strengths also included the population-based nature of the study, the detailed and thorough data collected on the outcomes and the assessment of the extent to which diets were plant-based and animal based, based upon overall dietary intake patterns of the general population.

Nevertheless, there are several limitations we should consider. First, the assessment of a plant-based diet with this index has its limitations as several, sometimes arbitrary, decisions had to be made. One such decision was, for example, to add up food items within categories based on the intake in grams per day. As a result, products that were high in water-content will have contributed less energy or nutrients compared to products containing less water in the same category. However, using grams per day reflects intake of foods as they are consumed and recommended.[19] Also, decisions had to be made for the categorization of foods and the number of categories. We chose categories reflecting those used in the Dutch dietary guidelines, which are based on similarities of the food items in (botanical) origin, nutrient composition, and nutrient density;[18] thereby reducing nutritional differences between food items within one category. Furthermore, in our main analyses, we treated all plant-based foods equally by giving all plant-based foods positive scores, and all animal-based foods equally by giving all animal-based foods reverse scores, irrespective of their nutrient-density or previous evidence for a role in T2D prevention and general health. For example, less healthy plant-based foods, such as sugary beverages and refined grains, were included as positive scores, although sugary beverages,[23] and refined grains[44] have been linked to higher T2D risk; by contrast, healthy animal-based foods, such as dairy

and fish, were included as reverse scores, although dairy[45] and fish[46] have been linked to lower T2D risk or mortality risk. This is because our study aimed to emphasize an overall plant-based diet accounting for the possibilities of increased plant-based foods consumption as well as decreased animal-based foods consumption, which could increase the potential for population-wide recommendation. However, in our sensitivity analyses, excluding any one of alcoholic beverages, sugary beverages, sweets, potatoes, refined grains, fish, and dairy did not substantially change our estimates. In addition to the choices we had to make in the construction of the index, this study has some other limitations. First, dietary data were derived from self-reported diet measured with FFQs, making measurement error likely. However, because we used relative scores (quintiles) of intake and the FFQs were shown in several validation studies to adequately rank subjects according to intake,[13–16] we do not expect this measurement error to have affected our results to a large extent. Second, we did not have dietary data for many of the participants of the original cohort, which might have resulted in sampling bias if associations of plant-based diets with T2D risk differed in those included and those not included in our current analyses. Third, we assumed that diets remained stable over time. However, the estimates were similar after excluding the participants who were likely to change their diet during follow-up, such as participants with CHD, stroke, or cancer at baseline. Lastly, our results may be generalizable only to people of similar age and ethnicity.

In conclusion, in this large population-based cohort, higher adherence to an overall plant-based diet is associated with lower longitudinal insulin resistance, and lower risk of prediabetes and T2D, indicating a protective role of diets high in plant-based foods and low in animal-based foods in the development to T2D beyond strict adherence to a vegetarian or vegan diet. These promising findings call for further exploration of overall plant-based dietary recommendations aimed at T2D prevention.

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Supplemental Table 2.2.1. Food categories used for the plant-based diet index and examples of food items included in each of the food categories

Plant-based food categories	
Fruits	Apple, banana, pear, orange, strawberry, grapes, other fruits
Vegetables	Cauliflower, broccoli, spinach, carrots, onion, lettuce, tomato, cabbage, cooked vegetables
Whole grains	Whole grain bread, dark bread, rye bread, whole grain breakfast oats, whole grain pasta, brown rice
Nuts	Peanuts, walnuts, other nuts, peanut butter
Legumes	Legumes, tofu, soybeans, other soy products
Potatoes	Potatoes, fries
Vegetable oils	Olive oil, vegetable oils used for cooking, and all margarines
Tea and coffee	Black tea, green tea, herbal tea, coffee
Sugary beverages	Carbonated beverages with sugar, non-carbonated beverages with sugar, orange juice, fruit juice
Refined grains	Cornflakes, white bread, croissants, raisin bread, white pasta, white rice
Sweets	Sugar, cookies, cake, chocolate, candy-bars, honey, sweets, chocolate toppings, other sweet toppings
Alcoholic beverages	Red wine, white wine, beer, liquor, Dutch-eggnog
Animal-based food categories	
Low-fat Yoghurt	Skimmed yoghurt, semi-skimmed yoghurt, skimmed quark, buttermilk
Full-fat Yoghurt	Full-fat yoghurt, semi-skimmed quark, full quark
Low-fat milk	Skimmed milk, semi-skimmed milk, skimmed coffee creamer, semi-skimmed coffee creamer
Full-fat milk	Full-fat milk, cream, coffee-cream
Cheese	Full fat cheese, low fat cheese, cheese fondue, other cheese
Fish	Salmon, tuna, trout, herring, mussels, other fish
Eggs	Boiled eggs, fried eggs
Animal fat	Butter on bread, butter used for cooking, lard
Desserts and sugary dairy	Custard, cream, ice cream, mousse, cream, chocolate milk, fruit yoghurt, yoghurt drinks
Unprocessed lean meat	Chicken
Processed and red meat	Beef, pork, meatballs, sate, bacon, liver, processed meats

Supplemental Table 2.2.2. Baseline intake of 23 food categories of participants (grams/day) in quintiles of the plant-based dietary index

Plant-based dietary index	score ≤ 43	43 < score ≤ 47	47 < score ≤ 51	51 < score ≤ 55	score > 55
	n = 1,417	n = 1,311	n = 1,559	n = 1,226	n = 1,285
Fruits	168.0 (83.4, 274.5)	197.4 (104.0, 320)	215.7 (115.2, 340.3)	226.7 (127.3, 351.9)	258.5 (161.1, 395.1)
Vegetables	181.6 (128.0, 252.9)	199.4 (143.9, 277.1)	205.2 (146.4, 283.3)	216.9 (156.4, 297.7)	241.3 (180.4, 331.4)
Whole grains	88.3 (46.6, 125.0)	99.5 (50.0, 140.6)	108.3 (63.0, 151.1)	114.7 (67.6, 160.0)	135.0 (80.0, 188.0)
Legumes	0.0 (0.0, 8.9)	0.0 (0.0, 16.9)	4.1 (0.0, 18.0)	7.8 (0.0, 24.0)	13.5 (0.0, 35.6)
Nuts	13.5 (0.0, 6.0)	2.1 (0.0, 8.8)	3.6 (0.0, 11.8)	5.6 (0.4, 14.1)	9.0 (2.7, 19.2)
Vegetable oils	12.0 (3.3, 21.4)	16.6 (7.2, 26.0)	20.6 (10.4, 30.0)	24.0 (13.3, 32.6)	27.7 (18.1, 38.5)
Tea and coffee	705.4 (500.0, 875.0)	750.0 (525.0, 937.5)	767.9 (597.1, 1000.0)	812.5 (625.0,1044.6)	900.0 (705.4, 1125.0)
Refined grains	37.7 (17.1, 76.8)	50.0 (22.7, 97.6)	50.6 (23.5, 101.3)	60.0 (30.4, 115.6)	61.2 (30.9, 122.2)
Potato	83.6 (45.9, 122.0)	88.2 (57.0, 131.0)	97.9 (61.7, 142.5)	108.3 (71.2, 163.1)	126.0 (85.5, 178.1)
Sweets	50.3 (26.6, 81.7)	57.2 (32.6, 87.5)	64.2 (38.2, 95.6)	71.3 (43.5, 105.2)	71.3 (43.5, 105.2)
Sugary beverages	15.0 (0.0, 89.6)	40.0 (0.0, 139.3)	42.9 (0.0, 139.6)	42.9 (0.0, 139.6)	59.8 (1.2, 152.6)
Alcoholic beverages	31.8 (2.5, 124.7)	47.7 (3.6, 155.3)	58.8 (4.9, 160.3)	65.4 (8.4, 167.9)	81.9 (14.2, 189.3)
Low-fat yoghurt	82.3 (5.4, 192.9)	64.1 (0.0, 166.1)	60.0 (0.0, 164.5)	53.6 (0.0, 162.0)	32.1 (0.0, 149.6)
Full-fat yoghurt	0.0 (0.0, 34.8)	0.0 (0.0, 13.4)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Low-fat milk	111.0 (1.9, 278.6)	100.8 (0.88, 263.6)	91.0 (0.0, 224.4)	59.0 (0.0, 224.4)	48.0 (0.0, 196.5)
Full-fat milk	0.0 (0.0, 7.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Cheese	32.9 (21.3, 47.1)	32.6 (20.3, 50.0)	30.3 (20.0, 46.6)	28.4 (18.2, 44.6)	29.9 (17.8, 47.0)
Fish	21.4 (7.1, 33.8)	18.9 (5.9, 33.0)	14.6 (4.2, 30.2)	14.4 (2.4, 28.6)	11.0 (0.0, 25.9)
Eggs	14.3 (8.9, 21.4)	14.3 (7.1, 21.4)	14.3 (7.1, 17.9)	14.3 (7.1, 17.1)	10.7 (7.1, 17.1)
Animal fat	0.7 (0.0, 12.0)	0.0 (0.0, 2.3)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Desserts / sugary dairy	21.4 (1.5, 63.9)	18.4 (0.4, 60.5)	14.9 (0.0, 59.6)	10.2 (0.0, 48.1)	6.4 (0.0, 35.8)
Unprocessed lean meat	14.3 (6.9, 21.4)	14.3 (7.1, 21.4)	11.4 (4.3, 18.6)	10.7 (4.3, 17.8)	7.6 (0.0, 14.9)
Processed/red meat	93.2 (65.4, 127.5)	89.3 (63.4, 127.5)	86.9 (60.0, 118.0)	85.5 (60.4, 117.9)	80.0 (52.5, 112.3)

Supplemental Table 2.2.3. Baseline characteristics of participants in original and multiple imputed dataset.

Characteristics	Original data (mean (SD) or %)	After imputation (mean (SD) or %)
Age (years)	62.0 (7.8)	NI
Missing (%)	-	-
Gender (% male)	41.3 %	NI
Missing (%)	-	-
BMI (kg/m^2)	26.6 (3.9)	26.6 (3.9)
Missing (%)	1.3%	-
Smoking (%)		
Never	32.2%	32.2%
Ever	45.1%	45.1%
Current	22.7%	22.7%
Missing (%)	0.5%	-
Physical activity[1] (MET-hours/week)		
RS-I / RS-II (Zutphen Questionnaire, n = 4,393)	86.7 (44.7)	86.7 (44.7)
RS-III (LASA Questionnaire, n = 2,194)	58.4 (55.8)	58.4 (55.8)
Missing (%)	3.9%	-
Hypertension (%)	42.3 %	42.3 %
Missing (%)	0.9 %	-
Hypercholesterolemia (%)	45.6 %	45.4 %
Missing (%)	1.6%	-
Family history of type 2 diabetes (%)	10.8 %	NI
Missing (%)	-	-
Education level (%)		
Primary	11.8%	11.8%
Lower	40.9%	40.9%
Intermediate	29.0%	29.0%
Higher	18.3%	18.3%
Missing (%)	0.6%	-
Current food supplement use (%)	16.5 %	16.5 %
Missing (%)	0.3%	-
Total energy intake (kcal/day)	2134 (615)	NI
Missing (%)	-	-
Food category intake[2] (grams/day)		
Fruits	212.2 (115.5, 332.3)	NI
Vegetables	209.1 (147.9, 286.9)	NI
Whole Grains	105.7 (61.3, 152.5)	NI
Nuts	3.9 (0.0, 12.0)	NI
Legumes	4.1 (0.0, 19.4)	NI

Supplemental Table 2.2.3. Baseline characteristics of participants in original and multiple imputed dataset. (continued)

Characteristics	Original data (mean (SD) or %)	After imputation (mean (SD) or %)
Potatoes	99.7 (61.4, 148.2)	NI
Vegetable oils	19.7 (9.2, 30.0)	NI
Tea and coffee	758.9 (580.4, 1000)	NI
Sugary beverages	46.3 (0.0, 139.6)	NI
Refined grains	50.7 (23.9, 102.1)	NI
Sweets	63.8 (37.1, 97.4)	NI
Alcoholic beverages	56.4 (4.9, 159.8)	NI
Low-fat milk	82.3 (0.0, 232.3)	NI
Full-fat milk	0.0 (0.0, 0.0)	NI
Low-fat yoghurt	56.1 (0.0, 164.6)	NI
Full-fat yoghurt	0.0 (0.0, 4.9)	NI
Cheese	30.8 (20, 47.1)	NI
Unprocessed lean meat	10.7 (4.3, 18.1)	NI
Fish	15.9 (3.9, 30.7)	NI
Eggs	14.3 (7.1, 19.6)	NI
Animal fat	0.0 (0.0, 0.9)	NI
Desserts / sugary diary	14.1 (0.0, 54.6)	NI
Processed / red meat	86.8 (60.4, 118.9)	NI
Plant-based dietary index (score)	49.3 (7.1)	NI

Plant-based dietary index: a higher score indicates a higher adherence to a plant-based diet (theoretical range from 0 to 92). [1]Values shown are un-imputed; imputation was performed on z-scores of physical activity. [2]Variables expressed as median (IQR) because of their skewed distributions. Abbreviations: MET, metabolic equivalent of task; NI, not imputed.

Supplemental Table 2.2.4. Non-response analyses.

	Participants without valid dietary data (n = 5,225)	Participants with valid dietary data (n = 9,701)	P value
	Mean (SD) or %	Mean (SD) or %	T-test or χ^2 test
Age (years)	64.9 (12.7)	62.0 (7.8)	P < 0.05
Sex (%)			
Female	59.0%	41.8%	P < 0.05
Male	38.8%	58.0%	
BMI (kg/m^2)	27.0 (4.4)	26.6 (3.9)	P < 0.05
Physical activity (MET-hours/week)			
RS-I and RS-II (Zutphen Questionnaire)	72.4 (42.5)	83.5 (44.6)	P < 0.05
RS-III (LASA Questionnaire)	65.3 (43.5)	59.3	
Education level (%)			
Primary	25.0%	11.8%	P > 0.05
Lower	37.2%	40.9%	
Intermediate	24.4%	29.0%	
Higher	13.3%	18.4%	
Smoking status (%)			
Never	35.0%	32.2%	P > 0.05
Ever	39.0%	45.1%	
Current	25.6%	22.7%	
Current food supplement use (%)			
Yes	16.9%	16.5%	P > 0.05
No	83.1%	83.2%	
Family history of diabetes (%)			
Yes	9.0%	10.8%	P > 0.05
No	39.8%	45.8%	
Unknown	51.3%	43.4%	

Supplemental Table 2.2.4. Non-response analyses. (continued)

	Participants not included in analyses (n = 8,128)	Included participants in analyses (n = 6,798)	P value
	Mean (SD) or %	Mean (SD) or %	T-test or χ^2 test
Age (years)	69.3 (11.4)	62.0 (7.8)	P < 0.05
Sex (%)			
Female	59.5%	57.0%	P > 0.05
Male	40.1%	41.3%	
BMI (kg/m^2)	27.1 (4.3)	26.6 (3.9)	P < 0.05
Physical activity (MET-hours/week)			
RS-I and RS-II (Zutphen Questionnaire)	72.1 (42.5)	86.7 (44.7)	P < 0.05
RS-III (LASA Questionnaire)	61.6 (79.9)	58.4 (55.8)	
Education level (%)			
Primary	23.6%	11.8%	P > 0.05
Lower	37.0%	40.9%	
Intermediate	23.6%	29.0%	
Higher	11.1%	18.3%	
Smoking status (%)			
Never	32.5%	32.2%	P > 0.05
Ever	38.4%	45.1%	
Current	24.3%	22.7%	
Current food supplement use (%)			
Yes	14.6%	16.5%	P < 0.05
No	84.6%	83.5%	
Family history of diabetes (%)			
Yes	13.9%	45.8%	P > 0.05
No	49.1%	10.8%	
Unknown	36.9%	43.4%	

T-tests were performed for continuous variables, and χ^2 tests were performed for categorical variables.

Supplemental Table 2.2.5. Associations of the plant-based dietary index with longitudinal insulin resistance (HOMA-IR) for the three sub-cohorts separately.

	β for HOMA-IR (95% CI)		
	RS-I (n = 2,892)	RS-II (n = 1,389)	RS-III (n = 2,233)
Model 1	-0.09 (-0.10; -0.08)***	-0.07 (-0.11; -0.03)***	-0.11 (-0.14; -0.07)***
Model 2	-0.09 (-0.10; -0.08)***	-0.06 (-0.10; -0.02)**	-0.10 (-0.13; -0.07)***
Model 3	-0.05 (-0.07; -0.03)*	-0.01 (-0.05; 0.02)	-0.06 (-0.09; -0.03)***

Effect estimates are βs for ln-transformed HOMA-IR per 10 units higher score on the plant-based dietary index and are based on pooled results of the imputed dataset. Model 1 is adjusted for energy intake, sex, age and time of repeated measurements of longitudinal insulin resistance. Model 2 is additionally adjusted for education, smoking status, family history of diabetes, physical activity and food supplement use. Model 3 is additionally adjusted for BMI (kg/m^2). *p < 0.05; **p < 0.01; ***p < 0.001. Abbreviations: CI, confidence interval; HOMA-IR, homeostasis model assessment for insulin resistance; MET, metabolic equivalent of task; RS, Rotterdam-Study.

Supplemental Table 2.2.6. Associations of the plant-based dietary index with incidence of prediabetes for the three sub-cohorts separately.

	HR (95% CI) for prediabetes		
	RS-I (n = 2,492)	RS-II (n = 1,151)	RS-III (n = 2,125)
Model 1	0.93 (0.82; 1.05)	0.94 (0.78; 1.14)	0.65 (0.51; 0.84)***
Model 2	0.94 (0.83; 1.06)	0.94 (0.78; 1.14)	0.66 (0.52; 0.85)**
Model 3	0.96 (0.85; 1.09)	1.00 (0.83; 1.21)	0.70 (0.54; 0.90)**

Effect estimates are HRs (95% CIs) for incidence of prediabetes per 10 units higher score on the plant-based dietary index and are based on pooled results of the imputed dataset. Model 1 is adjusted for energy intake, sex and age. Model 2 is additionally adjusted for education, smoking status, family history of diabetes, physical activity and food supplement use. Model 3 is additionally adjusted for BMI (kg/m^2). *p < 0.05; **p < 0.01; ***p < 0.001. Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; MET, metabolic equivalent of task; RS, Rotterdam-Study.

Supplemental Table 2.2.7. Associations of the plant-based dietary index with incidence of type 2 diabetes for the three sub-cohorts separately.

	HR (95% CI) for type 2 diabetes		
	RS-I (n = 2,975)	RS-II (n = 1,411)	RS-III (n = 2,384)
Model 1	0.85 (0.73; 0.98)*	0.82 (0.65; 1.02)	0.74 (0.54; 1.02)
Model 2	0.86 (0.74; 1.00)*	0.86 (0.69; 1.07)	0.75 (0.54; 1.04)
Model 3	0.91 (0.78; 1.05)	0.93 (0.74; 1.16)	0.80 (0.58; 1.12)

Effect estimates are HRs (95% CIs) for incidence of type 2 diabetes per 10 units higher score on the plant-based dietary index and are based on pooled results of the imputed dataset. Model 1 is adjusted for energy intake, sex and age. Model 2 is additionally adjusted for education, smoking status, family history of diabetes, physical activity (z-score of MET-hours/week); and food supplement use (yes or no). Model 3 is additionally adjusted for BMI (kg/m2). *p<0.05; **p<0.01; ***p<0.001. Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; MET, metabolic equivalent of task; RS, Rotterdam-Study.

Supplemental Table 2.2.8. Associations of the plant-based dietary index with longitudinal insulin resistance (HOMA-IR), risk of prediabetes and type 2 diabetes (T2D) after excluding each one of 23 components one by one at a time, and additionally adjusting for the excluded one.

Plant-based dietary index with 22 components instead of 23 components	β (95% CI) for HOMA-IR	HR (95% CI) for prediabetes risk	HR (95% CI) for T2D risk
	n = 6,514	n = 5,768	n = 6,770
Excluding fruits	-0.08 (-0.10; -0.07) ***	0.89 (0.81; 0.98) *	0.82 (0.73; 0.92) **
Excluding vegetables	-0.09 (-0.10; -0.09) ***	0.89 (0.81; 0.98) *	0.81 (0.72; 0.92) **
Excluding whole grains	-0.09 (-0.10; -0.09) ***	0.89 (0.81; 0.98) *	0.81 (0.73; 0.92) **
Excluding nuts	-0.07 (-0.09; -0.06) ***	0.91 (0.81; 1.00)	0.84 (0.76; 0.95) **
Excluding legumes	-0.08 (-0.10; -0.07) ***	0.90 (0.82; 0.99) *	0.83 (0.74; 0.92) **
Excluding vegetable oils	-0.08 (-0.10; -0.07) ***	0.90 (0.82; 0.99) *	0.82 (0.73; 0.92) **
Excluding tea and coffee	-0.07 (-0.09; -0.06) ***	0.91 (0.83; 0.99) *	0.84 (0.75; 0.95) **
Excluding potatoes	-0.09 (-0.10; -0.09) ***	0.89 (0.81; 0.98) *	0.82 (0.73; 0.92) **
Excluding sugary beverages	-0.09 (-0.10; -0.08) ***	0.89 (0.81; 0.98) *	0.82 (0.72; 0.92) **
Excluding refined grains	-0.09 (-0.10; -0.08) ***	0.89 (0.81; 0.98) *	0.82 (0.73; 0.92) **
Excluding sweets	-0.08 (-0.10; -0.08) ***	0.90 (0.82; 0.99) *	0.81 (0.73; 0.92) **
Excluding alcoholic beverages	-0.08 (-0.10; -0.06) ***	0.89 (0.82; 0.98) *	0.83 (0.71; 0.95) **
Excluding red and processed meat	-0.07 (-0.08; -0.07) ***	0.93 (0.84; 0.99) *	0.84 (0.76; 0.95) **
Excluding unprocessed lean meat	-0.07(-0.08; -0.07) ***	0.90 (0.82; 0.99) *	0.84 (0.76; 0.95) **
Excluding fish	-0.08 (-0.10; -0.07) ***	0.90 (0.81; 0.99) *	0.84 (0.74; 0.94) **
Excluding eggs	-0.09 (-0.10; -0.08) ***	0.89 (0.80; 0.98) *	0.82 (0.73; 0.92) **
Excluding animal fat	-0.08 (-0.10; -0.08) ***	0.89 (0.79; 0.99) *	0.83 (0.70; 0.95) **
Excluding cheese	-0.08 (-0.10; -0.07) ***	0.91 (0.82; 0.99) *	0.84 (0.75; 0.94) **
Excluding low-fat milk	-0.08 (-0.10; -0.06) ***	0.86 (0.79; 0.95) *	0.81 (0.72; 0.92) **
Excluding full-fat milk	-0.08 (-0.10; -0.07) ***	0.90 (0.82; 0.99) *	0.83 (0.72; 0.93) **
Excluding low-fat yoghurt	-0.08 (-0.10; -0.07) ***	0.89 (0.81; 0.98) *	0.82 (0.74; 0.92) **
Excluding full-fat yoghurt	-0.09 (-0.10; -0.09) ***	0.86 (0.78; 0.94) *	0.80 (0.70; 0.90) **
Excluding desserts/dairy with sugars	-0.08 (-0.10; -0.08) ***	0.90 (0.81; 0.99) *	0.83 (0.71; 0.94) **

Effect estimates are regression coefficients (β) for ln HOMA-IR or hazard ratios (HRs) for incidence of prediabetes or type 2 diabetes with their 95%-confidence intervals (95%CIs), per 10 units higher score on the plant-based dietary index by excluding one of 23 foods at a time and additionally adjusting for the excluded food group. Estimates are adjusted for total energy, age, sex, RS subcohort, education, smoking status, family history diabetes, physical activity, and food supplement use (only for the HOMA analyses additionally for the time measurements of longitudinal HOMA), based on pooled results of imputed data. *p < 0.05; ** p < 0.01; ***p < 0.001

Chapter 3

Markers of inflammation and risk of type 2 diabetes

Chapter 3.1

The Association between Serum Uric Acid and the Incidence of Prediabetes and Type 2 Diabetes Mellitus: the Rotterdam Study

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PloS One, 2017

ABSTRACT

Background

Limited evidence is available about the association between serum uric acid and sub-stages of the spectrum from normoglycemia to type 2 diabetes. We aimed to investigate the association between serum uric acid and risk of prediabetes and type 2 diabetes.

Methods

Eligible participants of the Rotterdam Study (n = 8,367) were classified into mutually exclusive subgroups of normoglycemia (n = 7,030) and prediabetes (n = 1,337) at baseline. These subgroups were followed up for incident prediabetes (n = 1,071) and incident type 2 diabetes (n = 407), respectively. We used Cox proportional hazard models to determine hazard ratios (HRs) for incident prediabetes among individuals with normoglycemia and incident type 2 diabetes among individuals with prediabetes.

Results

The mean duration of follow-up was 7.5 years for incident prediabetes and 7.2 years for incident type 2 diabetes. A standard deviation increment in serum uric acid was significantly associated with incident prediabetes among individuals with normoglycemia (HR 1.10, 95% confidence interval (CI) 1.01; 1.18), but not with incident type 2 diabetes among individuals with prediabetes (HR 1.07, 95% CI 0.94; 1.21). Exclusion of individuals who used diuretics or individuals with hypertension did not change our results. Serum uric acid was significantly associated with incident prediabetes among normoglycemic women (HR 1.13, 95% CI 1.02; 1.25) but not among normoglycemic men (HR 1.08, 95% CI 0.96; 1.21). In contrast, serum uric acid was significantly associated with incident type 2 diabetes among prediabetic men (HR 1.23, 95% CI 1.01; 1.48) but not among prediabetic women (HR 1.00, 95% CI 0.84; 1.19).

Conclusions

Our findings agree with the notion that serum uric acid is more closely related to early-phase mechanisms in the development of type 2 diabetes than late-phase mechanisms.

INTRODUCTION

Uric acid is generated during nucleotide and adenosine triphosphate (ATP) metabolism and comprises the end product of human purine metabolism.[1] We have previously demonstrated in a large population-based cohort study that elevated serum levels of uric acid are associated with increased risk of type 2 diabetes independently of other risk factors.[2] This association has since then been replicated in many other prospective studies and subsequent meta-analyses.[3–6] In addition, serum uric acid has been associated with various cardiovascular and metabolic conditions such as hypertension, obesity, heart failure and atrial fibrillation in large population-based studies. [7]

Prediabetes is a disorder of glucose homeostasis characterized by impaired glucose tolerance or impaired fasting glucose. These are both reversible stages of intermediate hyperglycemia that provide an increased risk of type 2 diabetes.[8] Prediabetes can therefore be regarded as an important reversible stage that could lead to type 2 diabetes, and early identification of prediabetes might contribute to the prevention of type 2 diabetes. Despite its established association with incident type 2 diabetes, serum uric acid has not been studied extensively in relation to incident prediabetes in individuals with normoglycemia or incident type 2 diabetes in individuals with established prediabetes.

Therefore, the objective of the present study is to determine whether serum uric acid is associated with incident prediabetes among normoglycemic individuals and type 2 diabetes among prediabetic individuals. This study is performed within the framework of the Rotterdam Study, a large population-based prospective cohort study of participants aged 45 years and older.[9]

MATERIALS AND METHODS

The Rotterdam Study

The methodology of the Rotterdam Study has been outlined extensively elsewhere.[9] Briefly, the study initially consisted of 7,983 residents of the Ommoord district aged 55 years and over in the city of Rotterdam, the Netherlands (RS-I). Following extension of the cohort in 2000 (RS-II), when individuals who had become 55 years of age or moved into the district since the study start were added to the cohort, and 2006 (RS-III), when individuals aged 45-54 years also became eligible for participation, the total number of subjects was 14,926 by the end of 2008.[9] These participants undergo physical examinations at the Rotterdam Study research facility and home interviews

every 3-4 years. Data is collected on health status, risk factors for various diseases common in the elderly, anthropometric characteristics, incident disease and cause-specific mortality.[9] The Medical Ethics Committee of the Erasmus Medical Centre Rotterdam and the review board of the Dutch Ministry of Health, Welfare and Sport have approved this population-based cohort study, and all participants have provided written informed consent. For the purposes of this analysis, we combined data from cohorts RS-I (using the third visit in 1997-1999 as baseline), RS-II (baseline visit 2000-2001) and RS-III (baseline visit 2006-2009) of the Rotterdam Study.

Definition of type 2 diabetes mellitus, prediabetes and normoglycemia

As per the Rotterdam Study protocol and WHO guidelines, type 2 diabetes was defined as having a fasting plasma glucose level ≥ 7.0 mmol/L, a non-fasting plasma glucose ≥ 11.1 mmol/L, the use of oral anti-diabetic medication or insulin, treatment by diet with type 2 diabetes as an indication, or being registered with a general practitioner as having type 2 diabetes.[10,11] Prediabetes was defined as a fasting plasma glucose level 6.0-6.9 mmol/L or a non-fasting plasma glucose level 7.7-11.1 mmol/L, in addition to absence of all type 2 diabetes criteria. Normoglycemia was defined as a fasting plasma glucose level ≤ 6.0 mmol/L and absence of any of the above criteria for prediabetes and type 2 diabetes. Fasting blood samples were obtained by means of venipuncture at the Rotterdam Study research facility. The samples were stored at −80°C in 5 mL aliquots. Within one week of sampling, glucose levels were measured by means of the glucose hexokinase method.[12] All measurements were performed at the clinical chemistry laboratory of Erasmus University Medical Center, Rotterdam.

Measurement of serum uric acid

Serum uric acid was determined in non-fasting blood samples, centrifuged for 10 minutes at 3,000 RPM and then stored for one week at -20°C. Uric acid activity was determined using a Kone Diagnostica reagent kit and a Kone auto-analyzer. After every 10 samples, 3 control samples were included to check calibration. If the average values of the control samples were not within 2.5% of the true value in each run of 100 samples, this run was repeated. Day-by-day variation had to be within 5% of this average value.

Covariates

In our study, the following covariates are considered: age, sex, body mass index (BMI), smoking status, daily alcohol intake, total serum cholesterol, serum HDL (high-density lipoprotein) cholesterol, systolic blood pressure, serum insulin, serum glucose, hypertension (defined as having a systolic blood pressure > 140 mmHg, a diastolic blood pressure > 100 mmHg or receiving blood-pressure lowering medication with hypertension

as an indication), physical activity, use of diuretics and estimated glomerular filtration rate (eGFR). Data on serum glucose, total serum cholesterol, serum HDL cholesterol, serum insulin, blood pressure and eGFR were obtained at baseline by means of venipuncture, performed during participants' visits to the Rotterdam Study research facility. Anthropometric characteristics were also recorded at the Rotterdam Study research facility. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[13] The disease status with respect to type 2 diabetes and prediabetes was ascertained through follow-up using general practitioners' records and hospital discharge letters, collected as part of the Rotterdam Study. Physical activity was assessed at baseline by means of a modified version of the Zutphen Study Physical Activity Questionnaire and the LASA Physical Activity Questionnaire.[9] Metabolic equivalents of task (MET) hours per week were calculated based on time spent in light, moderate and vigorous activity. Data concerning the use of medication, alcohol consumption and smoking at baseline was obtained through Rotterdam Study home interviews and, for medication, consulting pharmacy dispensing records.

Statistical analysis

To determine the association between serum uric acid and risk of incident prediabetes or incident type 2 diabetes, Cox proportional hazards regression was performed with serum uric acid as the primary independent variable and either incident prediabetes or incident type 2 DM as the response variable. The timescale in these models is follow-up time in years from baseline to either of the clinical endpoints, death, loss-to-follow-up or January 1st, 2012. Models adjusted only for age, sex and cohort as well as multivariable-adjusted Cox models were designed. The confounders BMI, smoking status, daily alcohol intake, total serum cholesterol, serum HDL cholesterol, systolic blood pressure, serum insulin, serum glucose, hypertension status, physical activity, use of diuretics and eGFR, selected based on previous literature, were added to the models adjusted for age, sex and cohort incrementally. The covariates serum insulin level, serum glucose level, daily alcohol intake and physical activity were log-transformed in the analyses because they displayed non-normality. Non-linearity was accounted for by inclusion of polynomial terms in the regression models if they significantly improved model fit. Interaction of uric acid with age and sex was investigated by introducing the product of the variables age and sex with uric acid to the regression models. Five-fold multiple imputation was performed to account for missing values. The results of our analyses are presented as hazard ratios (HR) with corresponding 95% confidence intervals (95% CI). A p-value < 0.05 was considered statistically significant. Analyses were performed using SPSS Statistics version 21 (IBM Corp., Armonk, New York, USA) and R version 3.2.4 (The R foundation for Statistical Computing, Vienna, Austria).

RESULTS

The total study population eligible for analysis (n = 8,367) was divided into two mutually exclusive subgroups: a subgroup with normoglycemia at baseline (n = 7,030) and a subgroup with prevalent prediabetes at baseline (n = 1,337). The selection procedure of our study population and the subgroups is outlined in Figure 3.1.1. Baseline characteristics of the study population are presented in Table 3.1.1.

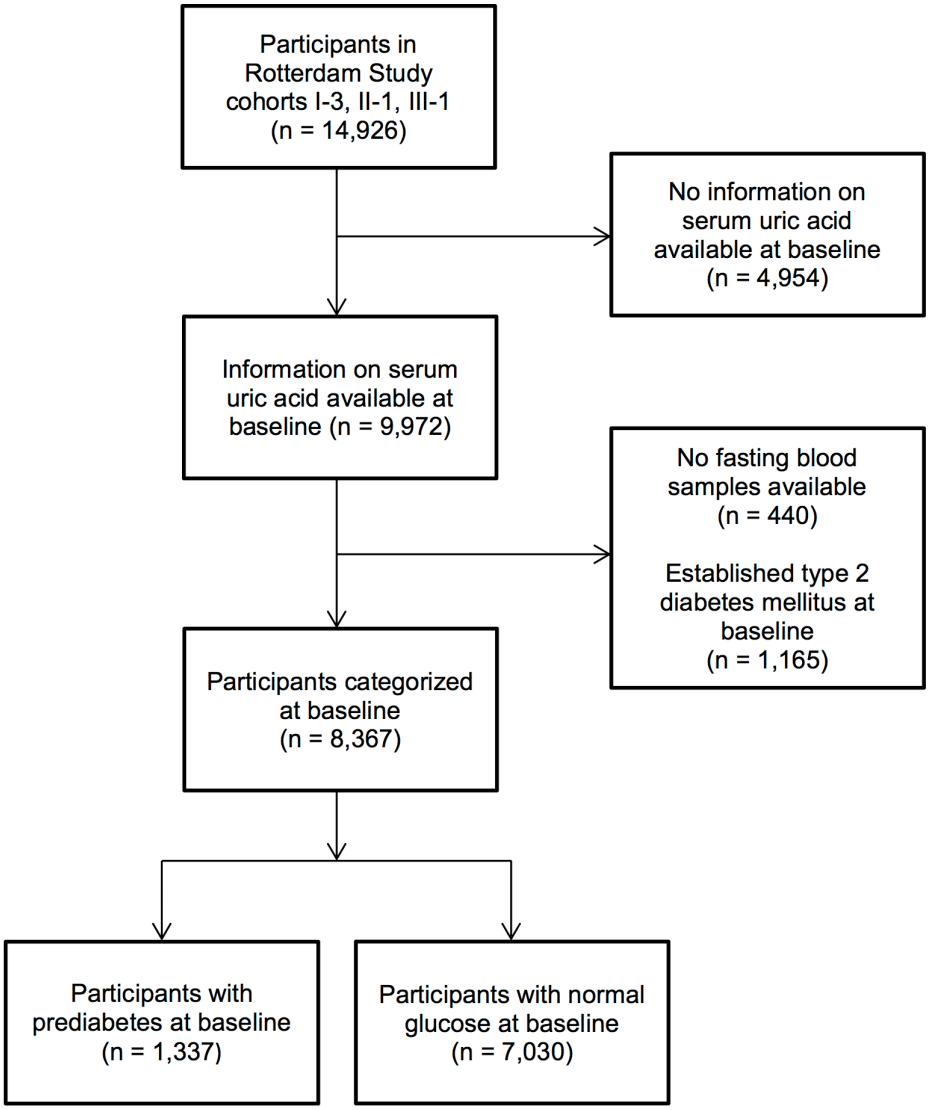

Figure 3.1.1. Selection of the study population.

Table 3.1.1. Baseline characteristics of the study population.

	Normoglycaemia at baseline (n = 7,030)	Missing data (%)	Prediabetes at baseline (n = 1,337)	Missing data (%)
Age (years)	64.2 (9.7)	0.0%	66.6 (9.4)	0.0%
Sex		0.0%		0.0%
Male	2,890 (41.1%)		665 (49.7%)	
Female	4,140 (58.9%)		672 (50.3%)	
Body Mass Index	26.7 (3.9)	0.7%	28.5 (4.4)	0.6%
Serum Total Cholesterol (mmol/L)	5.8 (1.0)	0.0%	5.8 (1.0)	0.2%
Serum HDL (mmol/L)	1.4 (0.4)	0.6%	1.3 (0.4)	0.8%
Systolic Blood Pressure (mmHg)	137.2 (20.5)	0.5%	145.4 (20.8)	0.2%
Serum Insulin (pmol/L)[1]	66.0 (44.0)	0.2%	93.0 (67.0)	0.1%
Alcohol Consumption (g/day)[1,2]	10.1 (12.6)	30.6%	13.7 (17.7)	35.2%
Current	6,008 (85.2%)	0.7%	1,154 (86.3%)	0.5%
Former or never	975 (14.1%)		176 (13.2%)	
Smoking		0.7%		0.4%
Current	1,236 (17.6%)		237 (17.7%)	
Former or never	5,794 (82.4%)		1,100 (82.3%)	
Hypertension[3]		1.3%		0.7%
Yes	3,981 (56.6%)		1,000 (74.8%)	
No	3.049 (43.4%)		337 (25.2%)	
Use of diuretics		2.9%		3.1%
Yes	581 (8.3%)		199 (14.9%)	
Not	6,449 (91.7%)		1,138 (85.1%)	
Serum Glucose (mmol/L)[1]	5.3 (0.6)	0.0%	6.3 (0.4)	0.2%
Estimated Glomerular Filtration Rate (mL/min)	79.9 (15.7)	1.2%	77.4 (16.1)	0.5%
Metabolic Equivalents of Task (hours/week)[1]	71.6 (64.5)	11.6%	68.7 (64.0)	10.2%
Serum Uric Acid (mmol/L)	0.31 (0.07)	n/a	0.35 (0.08)	n/a

Variables are presented as mean (standard deviation) unless otherwise indicated. [1]Variable is presented as median (interquartile range). [2]Median alcohol consumption applies only to active drinkers. [3]Hypertension is defined as having a systolic blood pressure > 140 mmHg, a diastolic blood pressure > 100 mmHg or receiving blood-pressure lowering medication.

Over a mean follow-up time of 7.5 years, 1,071 individuals with normoglycemia at baseline developed prediabetes (incidence rate 20.2 per 1,000 person-years). In this analysis, the percentage of individuals who were lost to follow up was 0.6% (40 out of 7,030 individuals). The results of our analysis of the association between serum uric acid and incident prediabetes are presented in Table 3.1.2. We found a significant association between serum uric acid and incident prediabetes within individuals who were normoglycemic at baseline in a model adjusted only for age, sex and cohort

(HR 1.31 per SD increment, 95% CI 1.23; 1.40). This association was attenuated but remained significant in the multivariable-adjusted model (HR 1.10, 95% CI 1.01; 1.18). Performing separate analyses for men and women, we found that the association between serum uric acid and incident prediabetes was present in both men (HR 1.28, 95% CI 1.16; 1.41) and women (HR 1.34, 95% CI 1.23; 1.45) in models adjusted for age and cohort (Table 3.1.3). After multivariable adjustment, serum uric acid was significantly associated with incident prediabetes among women (HR 1.13, 95% CI 1.02; 1.25) but not among men (HR 1.08, 95% CI 0.96; 1.21). Exclusion of individuals who use diuretics or individuals with hypertension did not substantially change our findings (Table 3.1.4). The association was no longer statistically significant upon exclusion of individuals with a BMI ≥ 25 (HR 1.14, 95% CI 0.98; 1.33). In the multivariable-adjusted model we also analyzed serum uric acid in quartiles, providing quartile-specific HRs relative to the first quartile (Figure 3.1.2).

Table 3.1.2. The association between serum uric acid and incidence of prediabetes and type 2 diabetes mellitus.

	Incident prediabetes in normoglycaemic individuals	P-value	Incident type 2 DM in prediabetic individuals	P-value
Model 1[1]	1.31 (1.23; 1.40)	< 0.001	1.17 (1.06; 1.30)	0.002
Model 2[2]	1.30 (1.21; 1.40)	< 0.001	1.21 (1.08; 1.35)	0.001
Model 3[3]	1.10 (1.01; 1.18)	0.022	1.07 (0.94; 1.21)	0.330

Results are presented as Hazard Ratio (95% confidence interval) for a standard deviation increment in serum uric acid. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + hypertension status, serum total cholesterol, eGFR, MET-hours per week, systolic blood pressure and use of diuretics. [3]Model 3: model 2 + daily alcohol intake, serum HDL, smoking status, BMI, serum glucose and serum insulin.

A total of 407 individuals with prediabetes at baseline developed type 2 DM over a mean follow-up time of 7.2 years (incidence rate 42.4 per 1,000 person-years). In this analysis, the percentage of individuals who were lost to follow up was 0.4% (6 out of 1,337 individuals). Serum uric acid was significantly associated with incident type 2 diabetes in individuals with prediabetes in a model adjusting only for age, sex and cohort (HR 1.17, 95% CI 1.06; 1.30), but this association weakened and was not statistically significant in the multivariable-adjusted model (HR 1.07, 95% CI 0.94; 1.21) (Table 3.1.2). In sex-specific analyses, the association was significant among men (HR 1.19, 95% CI 1.01; 1.40), and women (HR 1.18, 95% CI 1.03; 1.35) in models adjusted for age and cohort (Table 3.1.3). After multivariable adjustment, serum uric acid was significantly associated with incident type 2 diabetes among men (HR 1.23, 95% CI 1.01; 1.48) but not among women (HR 1.00, 95% CI 0.84; 1.19). Exclusion of diuretic users, individuals with hypertension or individuals with a BMI ≥ 25 did not change

our findings (Table 3.1.4). No significant difference was observed in any serum uric acid quartile compared to the first quartile (Fig 2).

Table 3.1.3. The association between serum uric acid and incidence of prediabetes and type 2 diabetes mellitus, stratified by gender.

	Incident prediabetes in normoglycaemic individuals	P-value	Incident type 2 diabetes in prediabetic individuals	P-value
Men				
Model 1[1]	1.28 (1.16; 1.41)	< 0.001	1.19 (1.01; 1.40)	0.038
Model 2[2]	1.26 (1.13; 1.40)	< 0.001	1.30 (1.09; 1.56)	0.004
Model 3[3]	1.08 (0.96; 1.21)	0.216	1.23 (1.01; 1.48)	0.039
Women				
Model 1[1]	1.34 (1.23; 1.45)	< 0.001	1.18 (1.03; 1.35)	0.015
Model 2[2]	1.35 (1.23; 1.48)	< 0.001	1.19 (1.02; 1.38)	0.027
Model 3[3]	1.13 (1.02; 1.25)	0.024	1.00 (0.84; 1.19)	0.877

Results are presented as Hazard Ratio (95% confidence interval) for a standard deviation increment in serum uric acid. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: model 1 + hypertension status, serum total cholesterol, eGFR, MET-hours per week, systolic blood pressure and use of diuretics. [3]Model 3: model 2 + daily alcohol intake, serum HDL, smoking status, BMI, serum glucose and serum insulin.

Table 3.1.4. Subgroup analyses for the association between serum uric acid and incident prediabetes and incident type 2 diabetes mellitus.

	Incident prediabetes in normoglycaemic individuals	P-value	Incident type 2 DM in prediabetic individuals	P-value
Exclusion of participants who use diuretics	1.11 (1.02; 1.21)	0.016	1.05 (0.92; 1.21)	0.497
Exclusion of participants with hypertension	1.16 (1.00; 1.34)	0.045	1.14 (0.84; 1.56)	0.412
Exclusion of participants with a BMI ≥ 25	1.14 (0.98; 1.33)	0.097	1.10 (0.75; 1.61)	0.647

Results are presented as multivariable-adjusted Hazard Ratios (95% confidence interval) for a standard deviation increment in serum uric acid, adjusted for age, sex, Rotterdam Study cohort, hypertension status, serum total cholesterol, eGFR, MET-hours per week, systolic blood pressure, use of diuretics, daily alcohol intake, serum HDL, smoking status, BMI, serum glucose and serum insulin.

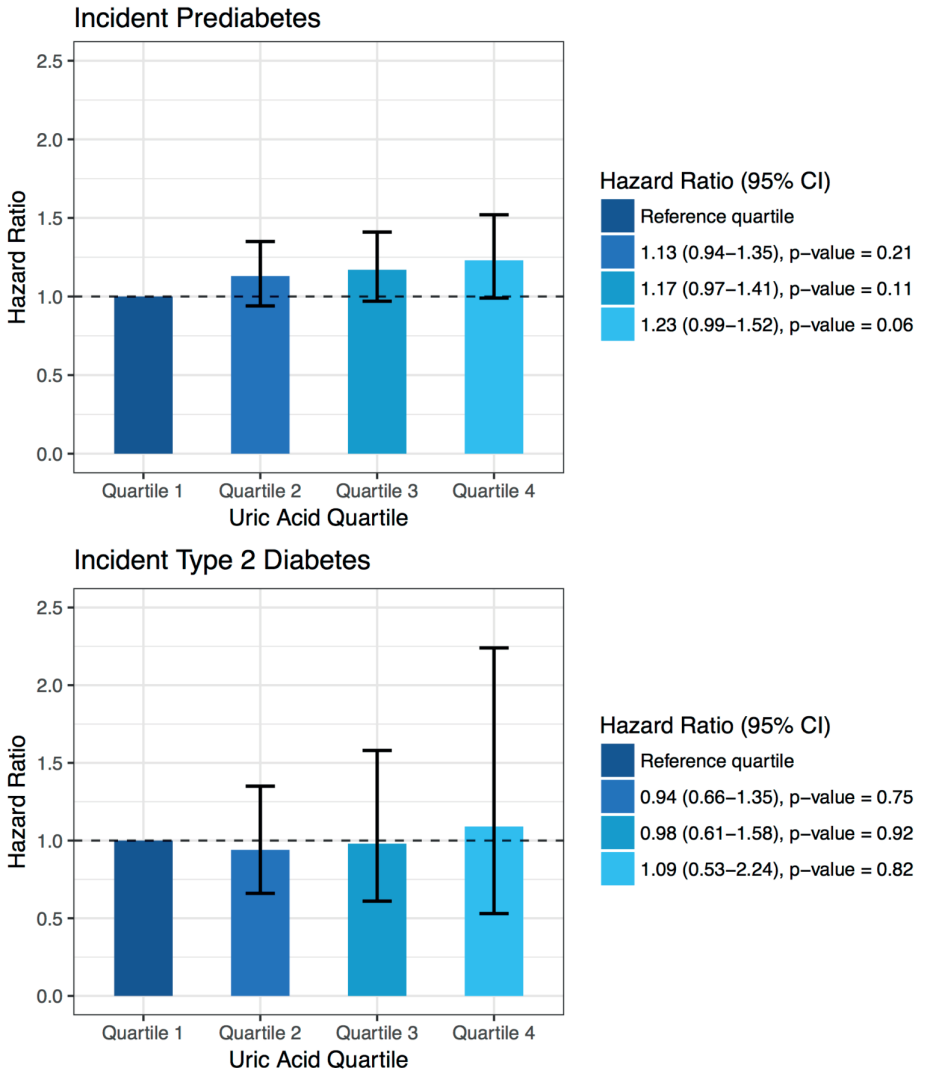

Figure 3.1.2. Quartile-specific hazard ratios for serum uric acid in association with incident prediabetes and incident type 2 diabetes mellitus.

DISCUSSION

We have found that higher serum uric acid levels are associated with an increased risk of incident prediabetes in individuals with normoglycemia aged 45 years or over, independently of confounders. No significant association was observed between serum uric acid and incident type 2 diabetes in individuals with prediabetes after multivariable adjustment.

The result with relation to incident prediabetes is consistent with previous research on this subject using impaired fasting glucose as an endpoint.[14–17] This could indicate that serum uric acid is more closely associated with early-phase rather than late-phase mechanisms that play a role in the development of type 2 diabetes. Typically, insulin resistance impairs pancreatic β-cell physiology and compensatory mechanisms, thereby inducing β-cell dysfunction as a consequence.[18] Insulin resistance could therefore be regarded as a reflection of early mechanisms that contribute to the development of type 2 diabetes, whereas β-cell dysfunction reflects the influence of late-stage mechanisms.[19] Currently, not much evidence is available concerning the relation between serum uric acid and pancreatic β-cell function. Tang and colleagues found an independent positive association between serum uric acid levels and residual pancreatic β-cell function.[20] In their cross-sectional analysis of 1,021 individuals with type 2 diabetes, they observed that patients with higher serum uric acid had greater insulin secretion ability in early disease stages, but their residual β-cell function decayed more quickly. The authors suggest that this increased insulin secretion might be a compensatory mechanism to overcome initial insulin resistance. In addition, Shimodaira and colleagues observed a significant negative association between serum uric acid and disposition index, a measure of pancreatic β-cell function, in a cross-sectional analysis among non-diabetic Japanese women after adjustment for age, BMI, systolic blood pressure, HbA1c, serum triglyceride level, serum HDL and use of antihypertensive or antilipidemic drugs.[21] However, no definitive conclusions regarding the association between serum uric acid and pancreatic β-cell function can be drawn at this point. Further population-based, prospective studies investigating this association are warranted. Although the association between serum uric acid and incident prediabetes was not significant among individuals with BMI < 25, this finding is most likely due to a lack of statistical power, because individuals with a BMI ≥ 25 constitute over half of our sample size in this subgroup.

Serum uric acid has been investigated in relation to incident type 2 diabetes in individuals with impaired fasting glucose by Kramer and colleagues, who found a significant association (OR 1.75, 95% CI 1.1; 2.9) after adjusting for various confounders in study population with characteristics similar to ours.[22] We were not able to replicate this finding in our analysis, in which we had a considerably larger sample available and were able to adjust for a more comprehensive set of confounding variables. It is possible that residual confounding in the previous study could account for this difference, because Kramer and colleagues were unable to adjust for smoking status and serum HDL. These covariates were particularly impactful in our multivariable-adjusted model. Excluding these covariates from the model yields an increase in the effect estimate (HR 1.13, 95% CI 1.00; 1.27) compared to the model which includes them (HR

1.07, 95% CI 0.94; 1.21). We also observe a steep decrease in the estimated hazard ratio for incident type 2 diabetes between model 2 and 3 in our analysis. The variable that is responsible for most of this decrease is serum HDL. It has been demonstrated that serum HDL is associated with plasma glucose levels and that it is strongly inversely associated with serum uric acid levels.[23,24] Therefore, serum HDL can be regarded as a particularly strong confounder of this association.

Conflicting results have been reported in the literature about a possible sex-specific nature of the association between serum uric acid and impaired fasting glucose.[16,17] In our study, we observe that serum uric acid is significantly associated with incident prediabetes among normoglycemic women, but not among normoglycemic men. Several studies report that the association between serum uric acid and glucose-related endpoints is especially pronounced among women.[15,16,25,26] The difference between men and women with relation to incident prediabetes in our study can possibly be attributed to residual confounding. We also have fewer events among men (n = 439) than among women (n = 632) in this analysis, which might lead to more imprecision in our estimated hazard ratio for men.

In contrast to this finding relating to incident prediabetes, serum uric acid was significantly associated with incident type 2 diabetes among men with prediabetes, but not among women with prediabetes in our study after multivariable adjustment. This observation was despite the fact that the number of events was higher among women (n = 222) than among men (n = 185) in this analysis. No other study has investigated the relation between serum uric acid and type 2 diabetes specifically among men with established glucose intolerance. Our result might suggest that serum uric acid affects women more strongly in the early stages of glucose intolerance development, whereas it affects men more strongly in more advanced stages. Potential biological mechanisms underlying this phenomenon have not yet been investigated in the literature, and further research is warranted.

Our findings build on the conclusion of a report by Kodama and colleagues, who performed a meta-analysis on the association between serum uric acid and incident type 2 diabetes in populations not stratified by glucose tolerance status (normoglycemia or prediabetes) at baseline.[27] They conclude that serum uric acid is significantly associated with incident type 2 diabetes across 11 cohort studies, and that their result should encourage other studies to identify sub-populations for which the association might be especially important. We report that serum uric acid appears to be most strongly associated with the early stages of the development of type 2 diabetes. A similar meta-analysis by Jia and colleagues also found a positive association between

serum uric acid and a combined endpoint of incident impaired fasting glucose and incident type 2 diabetes.[5] Our results further characterize the association between serum uric acid and glucose intolerance by treating incident prediabetes and incident type 2 diabetes as separate endpoints.

The strengths of our study include its prospective nature, which minimizes the chance of reverse causation, its long follow-up time and our ability to adjust for a large set of confounders. We provide a comprehensive overview of the relation between serum uric acid and different sub-stages on the spectrum between normoglycemia and type 2 diabetes. However, our study population consisted of mainly elderly individuals and roughly 95% of our participants were of Caucasian ethnicity. Therefore, our results cannot be generalized to populations with a different composition without further consideration. Finally, we cannot exclude the possibility of residual confounding, although the fact that we adjusted for many covariates should minimize the chance of this type of bias.

In conclusion, serum uric acid was independently and positively associated with incident prediabetes in individuals with normoglycemia but not with incident type 2 diabetes in individuals with prediabetes in a large population-based cohort of individuals aged 45 years and over. Our results indicate that serum uric acid might be more closely associated with early-phase pathogenic mechanisms that contribute to the development of type 2 diabetes rather than late-phase mechanisms.

ACKNOWLEDGMENTS

The dedication, commitment, and contribution of inhabitants, general practitioners, and pharmacists of the Ommoord district to the Rotterdam Study are gratefully acknowledged. We thank Symen Ligthart, Layal Chaker and Jolande Verkroost-van Heemst of Erasmus University Medical Center for their invaluable contribution to the collection and organization of the diabetes data.

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Chapter 4

Diet and body composition

Chapter 4.1

Total Dietary Antioxidant Capacity and Longitudinal Trajectories of Body Composition

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Antioxidants, 2020

ABSTRACT

Background

Although there is some evidence that total dietary antioxidant capacity (TDAC) is inversely associated with the presence of obesity, no longitudinal studies have been performed investigating the effect of TDAC on comprehensive measures of body composition over time.

Methods

In this study, we included 4,595 middle-aged and elderly participants from the Rotterdam Study, a population-based cohort. We estimated TDAC among these individuals by calculating a ferric reducing ability of plasma (FRAP) score based on data from food-frequency questionnaires. Body composition was assessed by means of dual X-ray absorptiometry at baseline and every subsequent 3–5 years. From these data, we calculated fat mass index (FMI), fat-free mass index (FFMI), android-to-gynoid fat ratio (AGR), body fat percentage (BF%) and body mass index (BMI). We also assessed hand grip strength at two time points and prevalence of sarcopenia at one time point in a subset of participants. Data were analyzed using linear mixed models or multinomial logistic regression models with multivariable adjustment.

Results

We found that higher FRAP score was associated with higher FFMI (0.091 kg/m^2 per standard deviation higher FRAP score, 95% CI 0.031; 0.150), lower AGR (−0.028, 95% CI −0.053; −0.003), higher BMI (0.115, 95% CI 0.020; 0.209) and lower BF% (−0.223, 95% CI −0.383; −0.064) across follow-up after multivariable adjustment. FRAP score was not associated with hand grip strength or prevalence of sarcopenia. Additional adjustment for adherence to dietary guidelines and exclusion of individuals with comorbid disease at baseline did not change our results.

Conclusions

Dietary intake of antioxidants may positively affect the amount of lean mass and overall body composition among the middle-aged and elderly.

INTRODUCTION

Dietary intake of antioxidants, a group of compounds that are capable of mitigating oxidative stress, has been shown to lower the risk of diseases such as type 2 diabetes, myocardial infarction and cancer.[1–3] Examples of such dietary antioxidants include vitamins C and E, polyphenols and carotenoids, and foods that are generally regarded as rich in antioxidants include fruits, vegetables, tea, coffee, spices and herbs.[4,5] Because multiple antioxidants may have synergistic effects, it is important to study the total dietary antioxidant capacity (TDAC) comprehensively rather than considering the effects of individual compounds.[6] Although the exact intermediate pathways through which the beneficial health effects of TDAC occur are not precisely known, there is some evidence that higher TDAC is inversely associated with the presence of obesity and age-related muscle loss.[7–9]

In recent years, advances in imaging technology have allowed for more thorough assessment of body composition in population studies than was previously possible. In particular, the use of dual X-ray absorptiometry (DXA) allows for accurate estimation of body composition at low cost and negligible radiation exposure.[10,11] DXA not only provides information about total body fat mass and fat-free mass, but also about fat distribution (i.e., android or gynoid type fat distribution) within a given individual. Investigating these detailed measures of body composition as opposed to more simple measures such as body mass index (BMI) is of importance because fat mass and fat-free mass differentially affect risk of several different health outcomes.[12] Changes in body composition are especially relevant in elderly individuals, in whom loss of muscle mass and function is commonly observed.[13] Such losses in muscle mass are associated with reduced functional outcomes over time.[14]

Most studies on antioxidants and body composition so far have been of cross-sectional design with relatively small sample sizes and have only investigated a small number of antioxidants. A systematic review reported that although a number of cross-sectional studies found a significant inverse association between TDAC and waist circumference, issues relating to the design or statistical power of these studies made inference on this association difficult.[15] No studies thus far have investigated a comprehensive measure of TDAC in relation to more detailed body composition measurements. For these reasons, we aimed to investigate the relationship between TDAC and longitudinal profiles of body composition derived by means of DXA, as well as muscle strength and sarcopenia, in the context of a large population-based cohort study among middle-aged and elderly individuals.

MATERIALS AND METHODS

The Rotterdam Study

The general design of the Rotterdam Study has been outlined extensively elsewhere.[16] In short, this prospective cohort study was initiated in 1990 in the district of Ommoord, Rotterdam, the Netherlands. All inhabitants of this district aged 55 years or older (n = 10,215) were invited to participate, and 7,983 participants were included for a response rate of 78% (subcohort RS-I). In 2000, a second subcohort of participants who had moved into the study district or had become 55 years of age since the start of the Rotterdam Study was included in the study; 4,472 were invited and 3,011 participated (response rate 67%) (subcohort RS-II). A third subcohort was added in 2006 with the inclusion of 3,932 participants, out of 6,057 invited (response rate 65%), aged 45–54 years (subcohort RS-III). Together, these subcohorts account for a total number of 14,926 participants at baseline. Participants underwent home interviews and an extensive set of physical examinations at baseline and every subsequent 3–4 years. The Medical Ethics Committee of Erasmus University Medical Center (registration number MEC 02.1015) and the review board of the Dutch Ministry of Health, Welfare and Sports (Population Screening Act WBO, license number 1071272-159521-PG) have approved the Rotterdam Study. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR) and into the WHO International Clinical Trials Registry Platform (ICTRP) under shared catalogue number NTR6831. All participants have provided written informed consent to participate in the study and to have their information obtained from treating physicians[17].

Assessment of total dietary antioxidant capacity

Assessment of dietary intake was performed at the fifth examination round for the first subcohort (RS-I-5; 2009–2011), the first examination round of the second subcohort (RS-II-1; 2000–2001), the third examination round of the second cohort (RS-II-3; 2011–2012) and the first examination round of the third subcohort (RS-III-1; 2006–2008) (Supplementary Figure 4.1.1). Semi-quantitative food frequency questionnaires (FFQs) were used to assess dietary intake at baseline. We used two versions: a 170-item FFQ for the measurements of RS-II-1 in 2000 and an updated 389-item FFQ for the later examination rounds, as described in detail elsewhere[18]. Food intake data from all cohorts was converted into daily nutrient and energy intake (in kcal) using Dutch Food Composition Tables corresponding to the years of dietary assessment. Both FFQs were developed to assess diet in a Dutch population and both FFQs have been validated against other assessment methods, which showed that the FFQs are able to adequately rank participants according to nutrient intakes. The 170-item FFQ was validated against fifteen twenty-four-hour food records and four twenty-four-hour

urea excretion samples among 80 participants of the Rotterdam Study. Pearson's correlations between the FFQ and the food records ranged between 0.44 and 0.85, and Spearman's correlation for estimated protein intake with urea excretion samples was 0.67.[19] The 389-item FFQ was validated among two other Dutch populations using a 9-day dietary record and a 4-week dietary history, with Pearson's correlations ranging between 0.40 and 0.86.[20,21]

The TDAC was calculated for each participant using the Antioxidant Food Table published by Carlsen et al., who used a ferric reducing ability of plasma (FRAP) assay to estimate the antioxidant content of over 3,100 types of food.[22] This assay measures absorption changes that occur when ferric ion (Fe^{3+}) is reduced to ferrous ion (Fe^{2+}) in the presence of antioxidants from different food samples. The measured value is the antioxidant capacity for a given type of food expressed in mmol per 100 g. We multiplied these values by the consumption of the different types of food in our FFQs and then summed across all food types for every participant. The resulting value is a FRAP score that represents the total dietary antioxidant intake in mmol per day. Because the Antioxidant Food Table lists different antioxidant capacities for the same types of food produced by different manufacturers, we consulted nutrition scientists from Wageningen University, the Netherlands, to determine the closest Dutch food equivalent for food types with multiple listings. Due to lack of data, food supplements were not included in the calculation of TDAC.

Measurement of body composition

Body composition was measured by means of Dual X-ray Absorptiometry (DXA; Prodigy and iDXA devices, GE Healthcare, Chicago, United States). From these DXA data, we calculated fat mass index (FMI) as total fat mass in kilograms divided by height in meters squared, fat-free mass index (FFMI) as total lean mass (excluding bone mineral content) in kilograms divided by height in meters squared, android-to-gynoid fat ratio (AGR) as android fat mass in kilograms divided by gynoid fat mass in kg and total body fat percentage (BF%) by expressing total fat mass in kilograms as a percentage of total body weight in kilograms. Weight was recorded with a digital scale with the participant wearing light clothing and height was recorded with the participant in a standing position without shoes. BMI was calculated as total body weight in kilograms divided by height in meters squared.

Sarcopenia was defined according to the updated European Working Group on Sarcopenia in Older People (EWGSOP2) criteria.[23,24] According to these criteria, sarcopenia is defined as the combination of low muscle strength and low muscle quantity or quality with or without low physical performance, and probable sarcopenia is defined

as isolated low muscle strength. We defined low muscle strength as a peak hand grip strength < 27 kg (for men) or < 16 kg (for women) over three attempts as measured at the Rotterdam Study research center. Low muscle quantity was defined as appendicular skeletal muscle mass index (ASMI; appendicular skeletal muscle mass divided by height squared) < 7.0 kg/m^2 (for men) or < 5.5 kg/m^2 (for women). Appendicular skeletal muscle mass was assessed by DXA and was calculated as the sum of the muscle masses of all four limbs. Low physical performance was defined as a gait speed ≤ 0.8 m/s.

Population for analysis

Data availability in the different examination rounds is outlined in Supplementary Figure 4.1.1. Full-body DXA measurements were performed from 2009 (RS-I-5), 2004 (RS-II-2) and 2006 (RS-III-1) onward, which constitute the baseline of our current study for a total of 8,547 participants. Dietary data were available for 5,791 of these 8,457 individuals, of which 309 were excluded for having invalid dietary data (reported energy intake < 500 or > 5000 kcal per day). Of the remaining 5,663 participants, 4,971 underwent DXA at least once. Another 375 individuals were excluded because their body mass index (BMI) was greater than 35 kg/m^2. Such individuals typically exceed the surface area limitations of the DXA-scanner, which would compromise image accuracy and therefore produce biased estimations of body composition[25]. Thus, our final population for analysis consisted of 4,595 individuals, of whom 3,065 had more than one DXA measurement available.

Data on hand grip strength were available for 4,193 individuals from the total of 4,595, measured from 2009 (RS-I-5), 2011 (RS-II-3) and 2006 (RS-III-1) onward. Sufficient data to assess the prevalence of sarcopenia was only available for the fifth visit round of the first cohort (RS-I-5) and the third visit round of the second cohort (RS-II-3) for a total of 2,001 participants. For the second cohort (RS-II), we used dietary data from the first examination round (RS-II-1) for the DXA outcomes and dietary data from the third examination round (RS-II-3) for the analyses pertaining to hand grip strength and prevalent sarcopenia, to minimize the time between assessment of FRAP score and the respective outcomes (Supplementary Figure 4.1.1).

Covariates

The following variables were considered as potential confounders in our analyses: age, sex, Rotterdam Study cohort, hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education, total daily energy intake, overall diet quality and serum glucose level. Potential confounders were selected based on general knowledge of their association with exposure and outcomes or on the basis of previous literature. We used directed

acyclic graph (DAG) modeling to help theorize which variables would potentially be relevant to include in our analyses as confounders.[26] Participant height and weight were recorded at every center visit. Participants were considered to have dyslipidemia if their total serum cholesterol was > 6.5 mmol/L or if they used lipid-lowering medication. Serum cholesterol was determined in blood samples taken at baseline using a CHOD-PAP method (Monotest Cholesterol kit, Boehringer Mannheim Diagnostica, Mannheim, Germany).[27] Hypertension was defined as having a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication. We performed two blood pressure readings at the right upper arm using a random-zero sphygmomanometer. Information on use of lipid-lowering or antihypertensive drugs was obtained during home interviews and by consulting pharmacy dispensing records. Smoking status (never, former or current user of tobacco products) and highest attained level of education were assessed during home interviews. Energy intake (kcal per day) and alcohol consumption (glasses per day) were derived from the FFQ data. To assess physical activity, we used the LASA physical activity questionnaire and a modified version of the Zutphen Study Physical Activity Questionnaire to estimate activity in metabolic equivalent of task (MET) hours.[28,29] Because different questionnaires were used, we calculated cohort-specific standard deviation (SD) scores for physical activity. Finally, as a measure of overall healthiness of diet, we used a diet quality score which describes the degree of adherence to the Dutch Dietary Guidelines[18]. Data on comorbid disease (coronary heart disease, heart failure, stroke, type 2 diabetes and cancer) were collected by consulting general practitioners' records and hospital discharge data and using measurements in our research center.[30–33]

Statistical analysis

In order to assess the association between baseline FRAP score and longitudinal changes in body composition measures and hand grip strength, we used a linear mixed model approach. We used the residual method to adjust FRAP score for energy intake.[34] We did this in each of the cohorts separately to account for the use of different FFQs and we used the standardized residuals as exposure in our analyses. For every regression model, we investigated whether non-linear terms (polynomials or three-knot natural cubic splines) for the variables age and time significantly ($p < 0.05$) improved model fit by performing likelihood ratio tests with the models fitted under maximum likelihood. Using the same procedure, we tested whether interaction between FRAP score and time, age or sex significantly improved the model fit. If this was the case, the non-linear or interaction terms were kept in the model. For the random effects structure of these models, we specified random intercepts and random slopes (for time between repeated measurements). In order to investigate the association between FRAP score and prevalence of probable sarcopenia or sarcopenia, we fitted

multinomial logistic regression models. To provide insight into how the covariates influence the association between FRAP score and body composition parameters, these covariates were introduced into the models in a stepwise process. Model 1 was adjusted for age, sex, Rotterdam Study cohort and time difference between exposure and outcome measurement (where applicable) in years. Model 2 was additionally adjusted for hypertension, dyslipidemia, alcohol consumption (natural log-transformed), physical activity, smoking, education and serum glucose. In model 3, we also included diet quality. Missing values were accounted for by the use of ten-fold multiple imputation with chained equations. All statistical analyses were performed using R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria), using the *mice* package (version 3.8.0) for multiple imputation and the *nlme* package (version 3.1-140) for designing the linear mixed models.[35,36] As sensitivity analysis, we repeated our analyses excluding participants with comorbidities (as defined previously) at baseline.

RESULTS

The characteristics of the study population are presented in Table 4.1.1. Overall, the mean FRAP score was 25.2 (SD 10.3) mmol/day. For the different measures of body composition, population averages at the first measurement were 9.3 (2.9) kg/m^2 for FMI, 17.5 (2.1) kg/m^2 for FFMI, 0.6 (0.2) for AGR, 34.20 (7.79) % for BF% and 26.8 (3.4) kg/m^2 for BMI. The food groups that contributed most to FRAP in our study were coffee, fruit, vegetables and tea. For those participants with more than one DXA measurement (n = 3,065), the average follow-up duration was 6.6 years (6.1 years for the 2,705 participants with two measurements and 10.9 years for the 360 participants with 3 measurements).

The results of our main analyses are displayed in Table 4.1.2. We observed an inverse association between FRAP score and FMI during follow-up in model 1, but this association was explained by the metabolic and lifestyle factors in model 2 and by overall diet quality in model 3 (model 3: −0.018 kg/m^2 per SD higher FRAP score, 95% CI −0.089; 0.053). Furthermore, we found a positive association between FRAP score and FFMI during follow-up in model 1, for which the effect estimates hardly changed and remained statistically significant after adjustment for covariates (model 3: 0.091, 95% CI 0.031; 0.150). We observed an inverse association of FRAP score with AGR, which was also persistent across models (model 3: −0.028, 95% CI −0.053; −0.003). FRAP score was not significantly associated with BMI during follow-up in the first model, but we did observe a positive association in model 3 (0.115, 95% CI 0.020; 0.209). Finally, we found that FRAP score was inversely associated with BF% during follow-up, with some attenuation after adjustment for covariates (model 3: −0.223, 95% CI −0.383; −0.064).

Table 4.1.1. Baseline characteristics of the total study population (n = 4,595).

Characteristic	Mean (SD) or n (%)
Age (Years)	65.1 (10.8)
Sex	
Female	2581 (56.2%)
Male	2014 (43.8%)
Highest level of education (%)	
Primary	372 (8.1%)
Lower/intermediate general or lower vocational	1782 (38.8%)
Intermediate vocational or higher general	1063 (23.1%)
Higher vocational or university	1063 (23.1%)
Hypertension (%)	
No	1668 (36.3%)
Yes	2927 (63.7%)
Dyslipidemia (%)	
No	2644 (57.5%)
Yes	1951 (42.5%)
Alcohol intake (glasses/day)[1]	0.9 [1.1]
Smoking (%)	
Never smoker	1429 (31.1%)
Former smoker	2293 (49.9%)
Current smoker	873 (19.0%)
Physical activity (MET-hours/week)[1]	54.3 [67.7]
Energy intake (kcal/day)	2199 (676)
Dietary guideline score	6.8 (1.9)
Fasting serum glucose (mmol/L)	5.6 (1.2)
Height (cm)[2]	168.6 (9.3)
Weight (kg)[2]	76.4 (12.6)
Body mass index (kg/m^2)[2]	26.8 (3.4)
Fat mass index (kg/m^2)[2]	9.3 (2.9)
Fat-free mass index (kg/m^2)[2]	17.5 (2.1)
Android-to-gynoid fat ratio[2]	0.6 (0.2)
Total body fat percentage (%)[2]	34.0 (7.9)
FRAP score (mmol/day)	25.2 (10.3)

[1]Median (interquartile range). The presented statistics represent the data after ten-fold multiple imputation. [2]Variable is presented for the individuals who participated in the baseline DXA measurement round (n = 3,770), i.e., RS-I-5, RS-II-2 or RS-III-1.

Table 4.1.2. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and fat mass index, fat-free mass index, android-to-gynoid fat ratio, body mass index and body fat percentage.

	Fat Mass Index (kg/m^2)	p-value	Fat-Free Mass Index (kg/m^2)	p-value	Android-To-Gynoid Fat Ratio	p-value	Body Mass Index (kg/m^2)	p-value	Body Fat %	p-value
Model 1[1]	−0.083 (−0.156; −0.010)	0.026	0.076 (0.016; 0.135)	0.013	−0.028 (−0.054; −0.002)	0.033	0.039 (−0.058; 0.137)	0.426	−0.365 (−0.527; −0.202)	<0.001
Model 2[2]	−0.037 (−0.108; 0.033)	0.301	0.088 (0.028; 0.147)	0.004	−0.029 (−0.054; −0.004)	0.025	0.092 (−0.002; 0.186)	0.055	−0.266 (−0.425; −0.107)	0.001
Model 3[3]	−0.018 (−0.089; 0.053)	0.619	0.091 (0.031; 0.150)	0.003	−0.028 (−0.053; −0.003)	0.026	0.115 (0.020; 0.209)	0.017	−0.223 (−0.383; −0.064)	0.006

Sample size for analysis of n = 4,595. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for time interval, age, sex and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score. At the baseline examination round (cohorts RS-I-5, II-2 and III-1), n = 3,770 participants underwent a DXA measurement; during the second round (cohorts I-6, II-3 and III-2), n = 3,492 participants were measured and, during the final examination round (cohort II-4), n = 718 participants were measured. Of the n = 4,595 total participants, n = 1,530 participants were measured once during the study period, n = 2,705 were measured twice and n = 360 were measured at all three time points.

The results for subsequent analyses on hand grip strength and sarcopenia are presented in Tables 4.1.3 and 4.1.4. FRAP score was not associated with hand grip strength across follow-up after multivariable adjustment (model 3: 0.177, 95% CI −0.135; 0.488) (Table 4.1.3). Within the subgroup of individuals with data on sarcopenia, we identified 314 cases of probable sarcopenia and 104 cases of sarcopenia. FRAP score was not associated with probable sarcopenia (model 3: OR 0.95, 95% CI 0.81; 1.12) (Table 4.1.4). Although higher FRAP score was associated with lower probability of sarcopenia in model 1 (OR 0.77; 95% CI 0.60; 0.99), after adjustment for covariates this association slightly attenuated and was no longer statistically significant (model 3: OR 0.81, 95% CI 0.62; 1.05).

Table 4.1.3. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and hand grip strength.

	Hand Grip Strength (kg)	p-value
Model 1[1]	0.232 (−0.078; 0.541)	0.142
Model 2[2]	0.182 (−0.129; 0.493)	0.251
Model 3[3]	0.177 (−0.135; 0.488)	0.267

Sample size for analysis of n = 4,193. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for time interval, age and sex. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Table 4.1.4. Associations between Ferric Reducing Ability of Plasma (FRAP) score and prevalence of (probable) sarcopenia.

	Probable Sarcopenia (n cases = 314)	p-value	Sarcopenia (n cases = 104)	p-value
Model 1[1]	0.93 (0.79; 1.08)	0.342	0.77 (0.60; 0.99)	0.045
Model 2[2]	0.95 (0.81; 1.11)	0.504	0.80 (0.62; 1.04)	0.098
Model 3[3]	0.95 (0.81; 1.12)	0.564	0.81 (0.62; 1.05)	0.110

Sample size for analysis of n = 2,001. Results are presented as odds ratio (OR) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for age, sex and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Excluding participants with one or more comorbidities at baseline left 3,327 individuals for analysis. Repeating our analyses in this subgroup did not substantially change our conclusions with regard to the association between TDAC and body composition, although we did observe some attenuation of the association between FRAP score and AGR (Supplemental Tables 4.1.1 - 4.1.3). We observed no significant interaction between FRAP score and follow-up time for any of the body composition outcomes in our analyses, indicating that FRAP generally does not modify the rate at which body composition changes over time. We did observe significant interaction between FRAP score and time on hand grip strength (p for interaction 0.003), suggesting that FRAP modifies the rate at which hand grip strength changes over time. We found significant interaction between FRAP score and sex only on FFMI (p for interaction 0.043) and between FRAP score and age only on AGR (p for interaction 0.046). Considering these findings, we additionally stratified all our analyses by sex and median age at baseline (Supplemental Tables 4.1.4 – 4.1.9). We observed that FRAP score was more strongly associated with FFMI in women (0.189, 95% CI 0.135; 0.243) compared to men (0.070, 95% CI 0.070; 0.133) after adjustment for all covariates, but these sex differences were generally not reflected in the other outcome parameters. Similarly, while FRAP score was more strongly associated with AGR in younger participants (−0.007, 95% CI −0.012; −0.001) compared to older participants (−0.0004, 95% CI −0.006; 0.006) after adjustment, this pattern was not reflected in the other outcome parameters.

DISCUSSION

In this prospective cohort study, higher total dietary antioxidant capacity (TDAC) was associated with higher fat-free mass index (FFMI), higher body mass index (BMI), lower body fat percentage (BF%) and lower android-to-gynoid fat ratio (AGR) across follow-up. We found no association between TDAC and the presence of sarcopenia, probable sarcopenia or hand grip strength. The observed associations were independent of degree of adherence to dietary guidelines. Overall, this combination of findings from our study indicates a positive association between TDAC and fat-free mass in particular. TDAC was positively associated with FFMI but was not associated with FMI. Hence, the decrease in body fat percentage we observe with higher TDAC is likely mainly due to higher fat-free mass rather than lower fat mass.

Several previous studies have examined the association between individual compounds with antioxidative properties and indicators of body composition. For example, a cross-sectional study of 3,182 participants found that serum levels of β-carotene and vitamin C, but not vitamin E, zinc or selenium, were lower in participants with

higher BMI.[37] Another cross-sectional study on a similar scale found that serum levels of magnesium, a cofactor for a number of antioxidant enzymes, were associated with lower BMI and waist circumference.[38] Several studies have also been performed that examined TDAC in relation to anthropometric measures. A systematic review reported that TDAC was examined in relation to waist circumference in several studies, two of which found a significant (inverse) association.[7,15,39] One of these two studies investigated TDAC in relation to abdominal obesity (defined as a waist circumference ≥ 95 cm) measured 3 years after baseline among 1,983 young adults, and reported lower occurrence of abdominal obesity across quartiles of TDAC after multivariable adjustment.[7] The other study reported lower waist circumference with higher trolox-equivalent antioxidant capacity (TEAC) among 266 young adults in a cross-sectional analysis adjusted only for energy intake and sex.[39] Another cross-sectional study found an association between measures of TDAC and obesity as measured by BMI, but not between TDAC and waist circumference.[40] Differences between studies with regards to the observed associations could be accounted for by differences in sample size, as a number of previous studies had considerably fewer participants available than ours and other larger studies.[7,8,39] Furthermore, a number of previous studies also did not adjust their analyses for cardiometabolic risk factors[39], or had a population that was demographically and ethnically different from ours.[7,8,40] Previous studies also differed with regards to the measure of TDAC that was investigated.[39–41] Notably, no studies thus far have investigated TDAC in relation to more detailed measures of obesity derived from DXA data. This is important considering that BMI alone fails to fully capture inter-individual differences in fat and lean mass.[42] Furthermore, when used as a measurement of adiposity, waist circumference may underestimate the association between adiposity and cardiometabolic risk factors when compared to DXA-derived measurements of adiposity.[43] These limitations emphasize the importance of studying more comprehensive measures of body composition over simple anthropometrics.

The positive association between TDAC and fat-free mass we observed in our study could be mediated by the reduction in oxidative stress levels that is associated with antioxidant consumption.[44] One of the major sources of oxidative stress is the presence of excess reactive oxygen species (ROS), which are chemically reactive molecules naturally produced in response to cellular stress and inflammatory processes.[45] While ROS have certain physiological functions at low concentrations, excess ROS production in response to stressors has adverse effects on cellular functioning.[45] High levels of ROS may specifically affect skeletal muscle mass and strength through a number of pathways.[46] For example, oxidative stress induces activation of proteolytic compounds and mediates the release of pro-inflammatory cytokines, which may lead to protein degradation and atrophy or loss of muscle fibers.[47] Previous studies have also

demonstrated that aging is associated with higher levels of ROS in skeletal muscle.[48,49] These adverse effects of ROS on muscle tissue, potentially exacerbated by increasing levels of ROS with aging, may in part be responsible for the commonly observed loss of muscle mass in the elderly.[47,50] Given that antioxidants have the ability to lower oxidative stress levels, a high consumption of antioxidants might reduce the extent to which these deleterious processes take place.[45] Increased consumption of dietary antioxidants may also help counteract the age-related deficiencies in the endogenous antioxidant defense system that have been reported in the elderly.[51] In spite of our observation that higher TDAC was associated with higher FFMI, we did not observe an association between TDAC and hand grip strength. This indicates that the increased muscle mass that is associated with higher TDAC is not also paired with increased muscle strength (Table 4.1.3). This discrepancy between findings for muscle mass and muscle strength could be explained by the fact that despite the correlation between these parameters, muscle strength may also be determined by neural factors in addition to muscle mass alone.[52] Furthermore, in a previous study, it was demonstrated that muscle mass accounted for only 13% of the variation in muscle strength among older adults.[53] We observed no association between TDAC and probability of probable sarcopenia or sarcopenia after adjustment for covariates. Sarcopenia is a complex and heterogeneous condition that can be defined according to different combinations of criteria within the EWGSOP2 definition.[23] Possibly, other factors than TDAC play a more prominent role in the pathogenesis of sarcopenia. We also had limited statistical power in this analysis due to the relatively low number of sarcopenia cases (n = 104) available. In addition, although we found that the association between TDAC and FFMI appeared to be somewhat stronger in women compared to men, previous literature has not provided consistent evidence of sex differences with relation to this association or the associations between individual antioxidants and anthropometrics.[37,54] However, in the case of our study, these sex differences could also be explained by differences in statistical power between the groups considering that we had more women (n = 2,581) than men (n = 2,014) available for analysis. The association between TDAC and android-to-gynoid fat ratio, and the variation of the strength of this association with age, has not been previously reported in the literature. Further research is needed in order to elucidate these findings.

The strengths of our study include its prospective design with repeated assessment of body composition over a period of, on average, more than six years. In addition, we had a large population available for analysis. We investigated a comprehensive measure of TDAC, which takes into account the potential synergistic effects of all antioxidants that are contained in the diet, rather than focusing on single antioxidative compounds. In addition, we analyzed advanced measures of body composition in

our study as opposed to only anthropometrics, enabling us to study the association between TDAC and body composition in greater detail than was previously possible. Furthermore, we were also able to adjust for a large number of covariates related to lifestyle, cardiometabolic status and dietary habits. Although it is possible that high TDAC could reflect an overall healthy diet because healthy foods are generally rich in antioxidants, we were able to demonstrate that our results persisted after adjustment for adherence to guidelines for a healthy diet. Several limitations should be taken into account when interpreting our findings. First, we estimated TDAC based on a Norwegian database listing the antioxidant content of different types of food.[22] It is possible that differences with regards to country of origin, growth conditions and processing of food have led to some error in the estimation of TDAC, although we did attempt to mitigate this by determining the closest Dutch food equivalent for products with multiple listings in the database. Second, we had no information available on the cooking methods used by participants. It has been demonstrated that cooking methods may also affect the antioxidant content of food.[55] Third, we had no data available on the use of food supplements in our study, so these could not be taken into account in our estimation of the TDAC. Fourth, the FFQ we used in order to assess dietary habits may inherently provide some measurement error, although our FFQ were both validated and shown to be adequate in ranking according to nutrient intake.[19,20] Fifth, we had a relatively limited number of repeated measurements available per participant, which may in turn limit the accuracy of the estimated longitudinal body composition profiles.

In conclusion, higher total dietary antioxidant capacity was associated with higher fat-free mass index in this longitudinal population-based cohort study of over 4500 middle-aged and elderly participants. Our findings indicate that increased consumption of antioxidants may have favorable effects on body composition and may play a role in preserving lean mass over time.

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Supplemental Table 4.1.1. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and fat mass index, fat-free mas index, android-to-gynoid fat ratio, body mass index and body fat percentage; excluding participants with selected comorbidities at baseline.

	Fat mass index (kg/m^2)	p-value	**Fat free mass index (kg/m^2)**	p-value	**Android-to-gynoid fat ratio**	p-value	**Body mass index (kg/m^2)**	p-value	**Body fat %**	p-value
Model 1[1]	-0.100 (-0.185; -0.015)	0.022	0.107 (0.038; 0.177)	0.003	-0.021 (-0.052; 0.010)	0.182	0.058 (-0.055; 0.170)	0.314	0.458 (-0.649; -0.266)	< 0.001
Model 2[2]	-0.049 (-0.131; 0.033)	0.243	0.123 (0.054; 0.192)	0.001	-0.026 (-0.056; 0.004)	0.093	0.117 (0.009; 0.226)	0.035	-0.350 (-0.536; -0.163)	< 0.001
Model 3[3]	-0.028 (-0.111; 0.054)	0.500	0.126 (0.056; 0.195)	< 0.001	-0.026 (-0.056; 0.004)	0.087	0.141 (0.032; 0.250)	0.011	-0.303 (-0.490; -0.116)	0.001

Total number of participants with one or more prevalent comorbidities was n = 1,268 (n = 366 cases of cancer other than non-melanoma skin cancer, n = 531 cases of type 2 diabetes, n = 113 cases of heart failure, n = 309 cases of coronary heart disease and n = 153 cases of a history of stroke), leaving n= 3,327 participants free of these comorbidities at baseline. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP score. [1]Model 1: adjusted for time interval, age, sex and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.2. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and hand grip strength; excluding participants with selected comorbidities at baseline.

	Probable sarcopenia (n cases = 166)	p-value	**Sarcopenia (n cases = 51)**	p-value
Model 1[1]	0.93 (0.75; 1.14)	0.477	0.95 (0.67; 1.35)	0.767
Model 2[2]	0.95 (0.76; 1.18)	0.627	0.94 (0.65; 1.35)	0.736
Model 3[3]	0.96 (0.77; 1.20)	0.720	0.92 (0.64; 1.34)	0.673

Total number of participants with one or more prevalent comorbidities was n = 1,123 (n = 323 cases of cancer other than non-melanoma skin cancer, n = 458 cases of type 2 diabetes, n = 102 cases of heart failure, n = 276 cases of coronary heart disease and n = 132 cases of a history of stroke), leaving n = 3,070 participants free of these comorbidities at baseline. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP score. [1]Model 1: adjusted for time interval, age, sex and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.3. Associations between Ferric Reducing Ability of Plasma (FRAP) score and (probable) sarcopenia; excluding participants with selected comorbidities at baseline.

	Hand grip strength (kg)	p-value
Model 1[1]	0.207 (-0.161; 0.574)	0.270
Model 2[2]	0.180 (-0.190; 0.550)	0.341
Model 3[3]	0.182 (-0.188; 0.553)	0.335

Sample size for analysis of n = 3,070. Total number of participants with one or more prevalent comorbidities was n = 782 (n = 227 cases of cancer other than non-melanoma skin cancer, n = 301 cases of type 2 diabetes, n = 89 cases of heart failure, n = 198 cases of coronary heart disease and n = 95 cases of a history of stroke), leaving n = 1,219 participants free of these comorbidities at baseline. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP score. [a]Model 1: adjusted for time interval, age, sex and Rotterdam Study cohort. [b]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [c]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.4. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and fat mass index, fat-free mas index, android-to-gynoid fat ratio, body mass index and body fat percentage; stratified by sex.

		Model 1[1]	p-value	Model 2[2]	p-value	Model 3[3]	p-value
Fat mass index (kg/m^2) *P for interaction 0.348*	Men	-0.116 (-0.209; -0.024)	0.014	-0.095 (-0.185; -0.006)	0.037	-0.079 (-0.169; 0.010)	0.082
	Women	-0.054 (-0.163; 0.056)	0.338	0.011 (-0.095; 0.118)	0.835	0.037 (-0.070; 0.144)	0.502
Fat-free mass index (kg/m^2) *P for interaction 0.043*	Men	0.063 (0.001; 0.125)	0.047	0.070 (0.007; 0.132)	0.029	0.070 (0.007; 0.133)	0.029
	Women	0.180 (0.126; 0.235)	< 0.001	0.183 (0.129; 0.237)	< 0.001	0.189 (0.135; 0.243)	< 0.001
Android-to-gynoid fat ratio *P for interaction 0.701*	Men	-0.030 (-0.071; 0.012)	0.161	-0.029 (-0.069; 0.012)	0.166	-0.028 (-0.068; 0.013)	0.181
	Women	-0.026 (-0.059; 0.006)	0.109	-0.026 (-0.057; 0.005)	0.103	-0.026 (-0.057; 0.005)	0.100
Body Mass Index (kg/m^2) *P for interaction 0.117*	Men	-0.058 (-0.185; 0.069)	0.370	-0.028 (-0.152; 0.095)	0.653	-0.012 (-0.136; 0.112)	0.850
	Women	0.126 (-0.018; 0.269)	0.087	0.194 (0.055; 0.332)	0.006	0.223 (0.083; 0.363)	0.002
Body Fat % *P for interaction 0.914*	Men	-0.354 (-0.572; -0.119)	0.003	-0.301 (-0.522; -0.080)	0.008	-0.261 (-0.482; -0.040)	0.021
	Women	-0.377 (-0.607; -0.146)	0.001	-0.234 (-0.460; -0.008)	0.043	-0.181 (-0.409; 0.046)	0.119

Sample size of n = 2,014 men and n = 2,581 women. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP score. [1]Model 1: adjusted for time interval, age and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score. Among women, we observed no differences in the strength of the association between FRAP score and fat-free mass index according to menopausal status (among a subset of n = 2,077 women with data on menopausal status, of whom n = 1,778 post-menopausal; p for interaction 0.365) or age (p for interaction 0.894).

Supplemental Table 4.1.5. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and hand grip strength; stratified by sex.

		Model 1[1]	p-value	Model 2[2]	p-value	Model 3[3]	p-value
Hand grip strength (kg) *P for interaction 0.082*	Men	-0.229 (-0.654; 0.195)	0.290	-0.286 (-0.716; 0.144)	0.192	-0.289 (-0.723; -0.145)	0.192
	Women	0.184 (-0.076; 0.445)	0.166	0.090 (-0.174; 0.353)	0.504	0.062 (-0.205; 0.329)	0.649

Sample size of n = 1,835 men and n = 2,358 women. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for time interval, age and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.6. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and (probable) sarcopenia; stratified by sex.

		Model 1[1]	p-value	Model 2[2]	p-value	Model 3[3]	p-value
Probable sarcopenia (n cases = 314) *P for interaction 0.546*	Men (n cases = 137)	0.98 (0.78; 1.24)	0.884	0.98 (0.77; 1.24)	0.872	0.99 (0.78; 1.25)	0.924
	Women (n cases = 177)	0.88 (0.71; 1.09)	0.233	0.93 (0.74; 1.15)	0.492	0.93 (0.74; 1.16)	0.506
Sarcopenia (n cases = 104) *P for interaction 0.931*	Men (n cases = 37)	0.87 (0.57; 1.33)	0.534	0.90 (0.58; 1.39)	0.630	0.90 (0.58; 1.39)	0.637
	Women (n cases = 67)	0.71 (0.52; 0.98)	**0.040**	0.74 (0.53; 1.03)	0.076	0.75 (0.54; 1.06)	0.101

Sample size of n = 909 men and n = 1,092 women. Results are presented as odds ratios (OR) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for age and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.7. Longitudinal associations between Ferric Reducing Ability of Plasma (FRAP) score and fat mass index, fat-free mas index, android-to-gynoid fat ratio, body mass index and body fat percentage; stratified by age.

		Model 1[1]	p-value	Model 2[2]	p-value	Model 3[3]	p-value
Fat mass index (kg/m^2) *P for interaction 0.889*	Age <= 62.3 years	-0.095 (-0.199; 0.009)	0.072	-0.029 (-0.131; 0.072)	0.570	-0.009 (-0.110; 0.093)	0.867
	Age > 62.3 years	-0.068 (-0.170; 0.034)	0.192	-0.036 (-0.135; 0.063)	0.475	-0.018 (-0.118; 0.081)	0.720
Fat-free mass index (kg/m^2) *P for interaction 0.691*	Age <= 62.3 years	0.107 (0.051; 0.163)	< 0.001	0.116 (0.059; 0.172)	< 0.001	0.117 (0.060; 0.174)	< 0.001
	Age > 62.3 years	0.133 (0.072; 0.193)	< 0.001	0.135 (0.075; 0.194)	< 0.001	0.142 (0.081; 0.202)	< 0.001
Android-to-gynoid fat ratio *P for interaction 0.046*	Age <= 62.3 years	-0.008 (-0.014; -0.002)	0.009	-0.008 (-0.013; -0.002)	0.009	-0.007 (-0.012; -0.001)	0.023
	Age > 62.3 years	-0.002 (-0.008; 0.004)	0.524	-0.001 (-0.007; 0.004)	0.625	-0.0004 (-0.006; 0.006)	0.884
Body Mass Index (kg/m^2) *P for interaction 0.794*	Age <= 62.3 years	0.010 (-0.126; 0.146)	0.880	0.087 (-0.045; 0.220)	0.196	0.107 (-0.025; 0.240)	0.113
	Age > 62.3 years	0.065 (-0.074; 0.204)	0.360	0.098 (-0.036; 0.233)	0.150	0.122 (-0.013; 0.258)	0.076
Body Fat % *P for interaction 0.942*	Age <= 62.3 years	-0.356 (-0.588; -0.124)	0.003	-0.220 (-0.449; 0.008)	0.059	-0.169 (-0.398; 0.060)	0.148
	Age > 62.3 years	-0.361 (-0.587; -0.135)	0.002	-0.285 (-0.505; -0.066)	0.011	-0.251 (-0.472; -0.029)	0.027

Sample size of n = 2,298 participants aged <= 62.3 (sample median) years and n = 2,297 participants aged > 62.3 years. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for time interval, age, sex and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.8. Associations between Ferric Reducing Ability of Plasma (FRAP) score and hand grip strength, stratified by age.

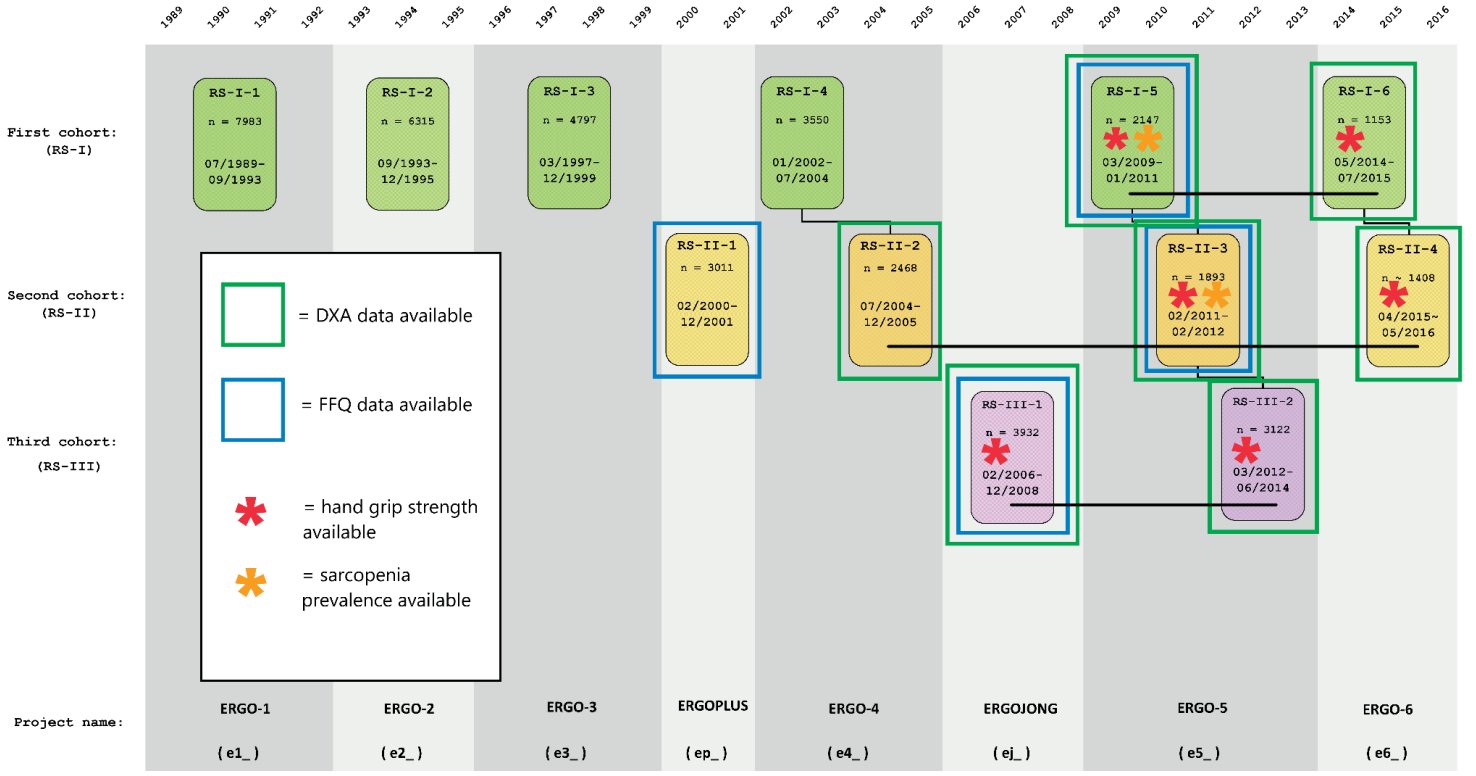
		Model 1[1]	p-value	Model 2[2]	p-value	Model 3[3]	p-value
Hand grip strength (kg)	Age <= 65.9 years	-0.167 (-0.601; 0.266)	0.449	-0.252 (-0.690; 0.186)	0.260	-0.247 (-0.687; 0.193)	0.271
P for interaction 0.546	Age > 65.9 years	0.675 (0.234; 1.116)	0.003	0.626 (0.185; 1.068)	0.005	0.584 (0.140; 1.029)	0.010

Sample size of n = 2,097 participants aged <= 65.9 (sample median) years and n = 2,096 participants aged > 65.9 years. Results are presented as regression coefficient (β) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for time interval, age, sex and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.

Supplemental Table 4.1.9. Associations between Ferric Reducing Ability of Plasma (FRAP) score and (probable) sarcopenia, stratified by age.

		Model 1[1]	p-value	Model 2[2]	p-value	Model 3[3]	p-value
Probable sarcopenia (n cases = 314) *P for interaction 0.362*	Age <= 75.7 (n cases = 78)	1.00 (0.76; 1.30)	0.981	1.03 (0.79; 1.35)	0.814	1.03 (0.78; 1.35)	0.836
	Age > 75.7 (n cases = 236)	0.90 (0.74; 1.09)	0.289	0.93 (0.76; 1.13)	0.446	0.94 (0.77; 1.15)	0.544
Sarcopenia (n cases = 104) *P for interaction 0.811*	Age <= 75.7 (n cases = 34)	0.70 (0.46; 1.06)	0.090	0.69 (0.45; 1.07)	0.096	0.72 (0.47; 1.11)	0.141
	Age > 75.7 (n cases = 70)	0.80 (0.58; 1.11)	0.191	0.87 (0.62; 1.22)	0.414	0.87 (0.61; 1.22)	0.414

Sample size of n = 1,001 participants aged <= 75.7 (sample median) years and n = 1,000 participants aged > 75.7 years. Results are presented as odds ratios (OR) with corresponding 95% CI per 1 standard deviation increment in FRAP. [1]Model 1: adjusted for sex, age and Rotterdam Study cohort. [2]Model 2: additionally adjusted for hypertension status, presence of dyslipidemia, daily alcohol consumption, daily physical activity, smoking status, highest attained level of education and serum glucose. [3]Model 3: additionally adjusted for adherence to dietary guideline score.



Supplementary Figure 4.1.1. Overview of Rotterdam Study cohorts and measurement points.

Chapter 5

General Discussion

DISCUSSION

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

Main findings

Dietary determinants of type 2 diabetes

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

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

Markers of inflammation and risk of type 2 diabetes

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

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

Determinants of body composition

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

Methodological considerations

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

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

Temporality and causality in observational study design

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

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

Determinants of internal validity

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

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

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

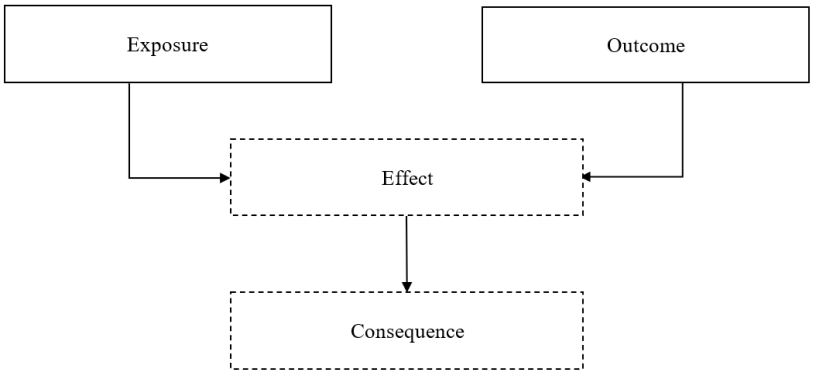


Figure 5.1.1. An exposure and outcome which share a common effect will be conditionally associated within levels of this common effect or a consequence of this common effect. Arrows indicate causal effects. Dashed lines indicate possible conditioning (adapted from Hernán et al., A Structural Approach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

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

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

Measurement of diet

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

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

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

Measurement of body composition

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

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

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

On generalization

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

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

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

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

Implications and future directions

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

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

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

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

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

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

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

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

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

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

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Chapter 6

Appendices

Summary
Samenvatting
Dankwoord
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SUMMARY

Type 2 diabetes remains a growing public health concern. This emphasizes the need for a better understanding of its determinants and how these are related to each other. One of the primary determinants of type 2 diabetes is composition of the diet. The biological pathways through which diet exerts is effects on health are numerous and complex. For instance, diet may affect body composition, in other words the absolute and relative quantities as well as the distribution of fat mass and fat-free mass, and thereby modulate disease risk. Previous research has also put forward the notion that composition of the diet may induce or mitigate a state of chronic low-grade inflammation. This inflammation is in itself closely associated with the pathophysiology of adiposity and, through multiple pathways related or unrelated to obesity, with risk of type 2 diabetes. Considering the high prevalence of type 2 diabetes and the severity of its complications, further insight into how these factors affect type 2 diabetes risk is of great importance.

In chapter 1, I offer a general introduction to the topics at hand as well a brief overview of the studies that form the basis of this thesis. In chapter 2, studies on dietary determinants of type 2 diabetes are described. Specifically, in chapter 2.1, I present our research on total dietary antioxidant capacity in relation to insulin resistance over time, risk of prediabetes and risk of type 2 diabetes. In this study, I found that higher dietary antioxidant consumption is associated with lower insulin resistance and lower risk of developing type 2 diabetes, underlining the presumed beneficial effects of antioxidant consumption. In chapter 2.2, I report how a relatively more plant-based diet, compared to a more animal-based diet, is related to these same endpoints. In this study, a plant-based diet index was constructed where a higher score indicates a higher consumption of plant-based products and a lower consumption of animal-based products. I report that a more plant-based diet is associated with lower insulin resistance and lower risk of prediabetes and type 2 diabetes. These findings strengthen dietary guidelines that recommend preferential intake of plant-based foods, and indicate that plant-based diets may exert health-promoting effects even if they are not strictly vegan or vegetarian.

The studies in chapter 3 are centered around markers of inflammation and their relation to risk of type 2 diabetes and cardiovascular disease. In chapter 3.1, I report that a higher serum level of uric acid, a marker associated with inflammatory processes, is associated with higher risk of prediabetes, specifically among women, but not with risk of type 2 diabetes. This underlines the potential role of uric acid as a determinant of the preliminary stages of type 2 diabetes. In chapter 3.2, I demonstrate that uric acid

may also serve a role as potential risk marker for cardiovascular disease. Specifically, I found that higher serum uric acid is associated with higher risk of fatal and non-fatal cardiovascular events. These associations were also especially pronounced among women, mainly among those with established type 2 diabetes. Further research is warranted to investigate whether serum uric acid is causally associated with these outcomes, and whether serum uric acid could inform clinical management decisions in this regard. Finally, in the research described in chapter 3.3, I confirm previous studies that have found a protective effect of coffee consumption on risk of type 2 diabetes. I also report, for the first time, that this association appears to be mediated by changes in serum levels of C-reactive protein, a marker of inflammation. This indicates that the beneficial effects of coffee consumption on risk of type 2 diabetes may occur in part due to mitigation of systemic inflammation by coffee consumption.

In the studies contained in chapter 4, I aimed to investigate determinants of body composition. In chapter 4.1, I report that dietary consumption of antioxidants is associated with favorable changes in body composition over time, providing further evidence for the putative beneficial health effects of antioxidant consumption. In chapter 4.2, I describe our research on dietary advanced glycation end-products, compounds with inflammatory potential that are formed when food is processed with high temperatures under low-moisture circumstances, in relation to body composition. Higher consumption of one of these compounds, carboxyethyl-lysine, was associated with detrimental changes in body composition over time. This places emphasis on the influence that cooking methods may have on the health effects of the diet, and further corroborates the notion raised by previous literature that inflammation may have adverse effects on body composition.

Finally, in chapter 5, I provide a general overview of the findings presented in this thesis paired with a discussion on methodological considerations and the potential implications of these findings. Overall, I found that several components of the diet may affect risk of obesity and type 2 diabetes through modulating inflammation. Among these are consumption of dietary antioxidants and coffee consumption, which may mitigate inflammation, as well as advanced glycation end-products, which may promote inflammation. The findings from this thesis also strengthen the notion that a more plant-based diet may have substantial beneficial health effects. In addition, I provide further evidence for the role of uric acid, a marker of inflammation, as a risk marker for type 2 diabetes and cardiovascular events in both early and late stages of disease. Future research may lead to the adaptation of these findings into dietary recommendations and clinical practice guidelines.

SAMENVATTING

Type 2 diabetes is een nog altijd in frequentie toenemend probleem voor de volksgezondheid. Dit benadrukt de noodzaak om de determinanten van deze ziekte beter te leren begrijpen, alsmede hoe deze determinanten met elkaar in verband staan. De samenstelling van het voedingspatroon is één van de belangrijkste determinanten van type 2 diabetes. Echter zijn de biologische mechanismen waarlangs voedingscomponenten hun gezondheidseffecten uitoefenen gecompliceerd en talrijk. Voeding heeft bijvoorbeeld invloed op lichaamssamenstelling, met andere woorden de verhouding en verdeling van vetmassa en vetvrije massa in het menselijk lichaam, en kan via deze weg het risico op ziekten vergroten of verkleinen. Eerder onderzoek heeft ook aangetoond dat de samenstelling van het voedingspatroon een bepaalde mate van chronische laaggradige ontsteking in het lichaam kan bewerkstelligen of deze ontsteking juist kan verminderen. Dergelijke chronische onsteking is op zichzelf weer geassocieerd met het ontstaan van overgewicht en, via mechanismen niet noodzakelijkerwijs direct gerelateerd aan overgewicht, met een hoger risico op type 2 diabetes. Gezien de hoge prevalentie van type 2 diabetes en de ernst van de potentiële complicaties van deze aandoening is beter inzicht in hoe deze factoren het risico op type 2 diabetes beïnvloeden van groot belang.

Hoofdstuk 1 van dit proefschrift bevat een algemene inleiding van het onderwerp in kwestie en een overzicht van de studies die samen dit proefschrift vormen. In hoofdstuk 2 beschrijf ik de studies waarin wij voedingscomponenten hebben onderzocht in relatie tot type 2 diabetes. In het bijzonder bevat hoofdstuk 2.1 mijn onderzoek naar het verband tussen de consumptie van antioxidanten en insulineresistentie, risico op prediabetes en risico op type 2 diabetes. Aan de hand van de resultaten van deze studie concludeer ik dat een hogere consumptie van antioxidanten geassocieerd is met lagere insulineresistentie en een lager risico op type 2 diabetes. Verder beschrijf ik in hoofdstuk 2.2 hoe wij de invloed van een voedingspatroon rijk aan plantaardige producten en arm aan dierlijke producten hebben onderzocht in relatie tot de eerder genoemde eindpunten. In dit onderzoek concludeer ik dat een meer plantaardig dieet verband houdt met lagere insulineresistentie en een lager risico op zowel prediabetes als type 2 diabetes. Deze bevindingen bieden verdere ondersteuning aan voedingsrichtlijnen die de consumptie van plantaardige producten aanbevelen. Tevens benadrukken deze resultaten dat een hogere consumptie van plantaardige producten gunstige effecten heeft op de gezondheid, ook indien het voedingspatroon als geheel niet volledig vegetarisch of veganistisch is.

De studies in hoofdstuk 3 zijn gericht op biologische markers van ontsteking en hun verhouding tot risico op type 2 diabetes en cardiovasculaire aandoeningen. In hoofdstuk 3.1 beschrijf ik dat een hoog serum urinezuur, een marker geasocieerd met onstekingsprocessen, in verband staat met een hoger risico op prediabetes, met name bij vrouwen. In deze studie werd echter geen verband gevonden tussen serum urinezuur en risico op type 2 diabetes binnen personen met reeds bestaande prediabetes. Deze resultaten suggereren dat serum urinezuur een bruikbare marker zou kunnen zijn voor de vroege stadia in de ontwikkeling van type 2 diabetes. Daarnaast heb ik in de studie beschreven in hoofdstuk 3.2 aangetoond dat hoog serum urinezuur geasocieerd is met zowel cardiovasculaire ziekte als cardiovasculaire sterfte, in het bijzonder bij vrouwen en bij personen met type 2 diabetes. Verder onderzoek is noodzakelijk om vast te stellen of het hier een causaal verband betreft en om te bepalen of serum urinezuur met dit oogmerk een rol zou kunnen hebben in de klinische praktijk. Daarnaast bevestig ik in het onderzoek beschreven in hoofdstuk 3.3 eerdere studies die hebben aangetoond dat de consumptie van koffie mogelijk een beschermend effect heeft op het ontstaan van type 2 diabetes. Tevens is dit de eerste studie die aantoont dat deze associatie mogelijk gemediëerd wordt door koffie-geïnduceerde veranderingen in serum C-reactief proteïne, een biologische marker van ontsteking. Dit betekent dat het beschermende effect van koffieconsumptie op het risico van type 2 diabetes mogelijk tot stand komt door ontstekingsremmende effecten van koffie.

In de studies beschreven in hoofdstuk 4 heb ik mij gericht op determinanten van lichaamssamenstelling. In het onderzoek in hoofdstuk 4.1 concludeer ik dat consumptie van antioxidanten gunstige effecten heeft op herhaadelijk gemeten lichaamssamenstelling, hetgeen de vermeende gezondheidsbevorderende effecten van antioxidanten verder ondersteunt. Hoofdstuk 4.2 bevat mijn onderzoek naar het verband tussen geavanceerde glycatie-eindproducten, ofwel eiwitten en lipiden die ontsteking kunnen bewerkstelligen en gevormd worden wanneer voedsel op hoge temperatuur onder droge omstandigheden wordt bereid, en lichaamssamenstelling. In deze studie constateer ik dat hogere consumptie van één van deze eindproducten, carboxy-ethyl-lysine, geassocieerd is met ongunstige verandering van lichaamssamenstelling door de tijd heen. Deze bevinding benadrukt de potentiële invloed van bereidingsmethoden op de gezondheidseffecten van voedsel en onderstreept de hypothese dat ontsteking veranderingen in lichaamssamenstelling kan bewerkstelligen.

Tot slot bied ik in hoofdstuk 5 een overzicht van de bevindingen van dit proefschrift. In dit hoofdstuk behandel ik ook methodologische overwegingen bij het uitgevoerde onderzoek en beschrijf ik enkele implicaties van de gerapporteerde bevindingen. Over het geheel genomen suggereren de resultaten van dit proefschrift dat meerdere

voedingscomponenten het risico op overgewicht en type 2 diabetes kunnen beïnvloeden door effecten op ontsteking. Het betreft onder andere antioxidanten en koffie, die ontsteking kunnen verminderen, maar ook geavanceerde glycatie-eindproducten, die ontsteking juist kunnen bevorderen. Deze bevindingen benadrukken ook het gegeven dat een voedingspatroon gebaseerd op plantaardige producten aanzienlijke gezondheidsbevorderende effecten zou kunnen hebben. Daarnaast bieden deze resultaten verder inzicht in de mogelijke rol van serum urinezuur als een biologische marker voor type 2 diabetes en cardiovasculaire ziekten. Tezamen met toekomstig onderzoek zouden deze bevindingen vertaald kunnen worden naar voedingsrichtlijnen en de klinische praktijk.

PHD PORTFOLIO

Name PhD student	Niels van der Schaft
Department	Epidemiology, Erasmus University Medical Center Rotterdam
Research School	Netherlands Institute for Health Sciences (NIHES)
PhD period	October 2015 – October 2020
Promotor	prof. dr. M.A. Ikram
Co-promotor	dr. ir. T. Voortman

	Year	ECTS
Training		
Master of Science in Health Sciences, NIHES, Rotterdam, the Netherlands		
Core curriculum		
Study design	2015	4.3
Biostatistical Methods I: Basic Principles	2015	5.7
Biostatistical Methods II: Classical Regression Models	2015	4.3
Specialization		
Principles of Research in Medicine and Epidemiology	2015	0.7
Introduction to Public Health	2015	0.7
Primary and Secondary Prevention Research	2015	0.7
Fundamentals of Medical Decision Making	2015	0.7
Clinical Epidemiology	2015	5.7
Methodologic Topics in Epidemiologic Research	2015	1.4
Methods of Clinical Research	2015	0.7
Clinical Practice-relevant Therapeutic Trials	2015	0.7
Pharmaco-epidemiology and Drug Safety	2016	1.9
Advanced Topics in Clinical Trials	2016	1.9
Advanced Analysis of Prognosis Studies	2016	0.9
Principles of Epidemiologic Data-analysis	2016	0.7
Elective courses		
Health Economics	2015	0.7
Repeated Measurements in Clinical Studies	2016	1.4
Causal Inference	2016	0.7
Logistic Regression	2016	1.4
Joint Models for Longitudinal and Survival Data	2016	0.7
Genome Wide Association Studies	2016	0.7
Courses at other institutions		
Society and Health (Harvard University)	2016	1.4
Study Design in Clinical Epidemiology (Harvard University)	2016	1.4
Nutrition & Physical Activity (University of Cambridge)	2016	1.4

	Year	ECTS
General academic courses		
Scientific Writing	2016	2.0
Attended seminars		
Seminars at the department of Epidemiology	2015 – 2020	2.0
2020 meetings	2015 – 2020	2.0
Nutrition & Lifestyle meetings	2015 – 2020	2.0
Conferences		
Annual Dutch Diabetes Research Meeting (oral)	2018	
European/International Conference on Obesity 2020 (poster)	2020	
Teaching activities		
MSc thesis supervision Oguzhan Akgündüz	2020	4.0
Other		
Peer review of manuscripts for scientific journals	2017-2020	0.5

1 ECTS (European Credit Transfer System) is the equivalent of 28 working hours.

PROPOSITIONS

1. Diet may affect metabolic health through modulation of the inflammatory response (this thesis).

2. Composition of the diet may affect body composition in ways not adequately captured by traditional anthropometrics, emphasizing the importance of using advanced body composition measurement techniques (this thesis).

3. Serum uric acid has potential as a stage-specific risk marker for type 2 diabetes and cardiovascular disease (this thesis).

4. Consumption of antioxidants in the context of a healthy diet has favorable effects on body composition and risk of type 2 diabetes (this thesis).

5. Better adherence to a more plant-based diet may provide significant benefits to population health (this thesis).

6. The close relationship between nutrition and health deserves a more prominent role in clinical practice.

7. Preventive medicine is a key area of responsibility for any physician.

8. The mathematical sciences wield their particular language made of digits and signs, no less subtle than any other. (Jorge Luis Borges, *Palabrería para versos,* 1926)

9. Science demands that facts not be subordinated to opinions, but that opinion be subordinated to facts. (Bertolt Brecht, unpublished version of *Leben des Galilei*, 1939)

10. The history of science is crosshatched with lines of additive and corrective thought. This is how we try to arrive at truth. Truth accumulates. It can be borrowed and paid back. (Don DeLillo, *Ratner's Star,* 1976)

11. La idea es un jaque a la verdad. (José Ortega y Gasset, *La Rebelión de las Masas*, 1929)

If I tell you that the city toward which my journey tends is discontinuous in space and time, now scattered, now more condensed, you must not believe the search for it can stop.

- Italo Calvino, *Le Città Invisibili,* 1972.