Dietary fatty acids and cardiovascular disease risk in observational studies

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Vetzuren uit de voeding en het risico op hart- en vaatziekten
in observationele studies
(met een samenvatting in het Nederlands)

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Chapter 1.

General introduction

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General introduction

Dietary fat.

Dietary fat comprises all lipids that are present in the foods we consume. Fat enhances the texture, taste, and aroma of food, which is why products rich in fat are generally perceived as very palatable. Dietary fat has several important functions. For instance, fat is an important source of energy for our body. Also, it carries fat-soluble vitamins and aids their absorption in the intestines. Fat provides important building blocks for cell membranes, and some types of fat are precursors for compounds that contain hormone-like or inflammatory properties [1, 2]. However, besides these valuable roles, certain classes of fat, including saturated fat and *trans* fatty acids (**Textbox 1**), may also contribute to the development of cardiovascular disease (CVD) [2]. This chapter provides a summary of these different fatty acid classes and their relation with CVD risk with the main emphasis on the saturated fatty acids.

Textbox 1. Classification of dietary fat.

Dietary fats are commonly classified based on the type of fatty acids that it contains. Ninety-eight percent of dietary fat is made up of triglycerides, each containing one glycerol molecule and three fatty acid molecules. A fatty acid contains a chain of hydrocarbon atoms. This chain can differ in length, and in the position and the number of double bonds. Based on the number of double bonds, fatty acids can be classified into saturated fatty acids (no double bonds), monounsaturated fatty acids (MUFA; one double bond) and polyunsaturated fatty acids (PUFA; more than one double bond) (**Figure 1**). The double bond(s) in the majority of the PUFA and MUFA are created so that the hydrogen atoms of two attached carbons are on the same side of the chain. This is called the *cis*-configuration. Fatty acids with this configuration therefore are called *cis*-PUFA or *cis*-MUFA. In a small group of unsaturated fatty acids, called the *trans* fatty acids, the hydrogen atoms are on the opposite side of the chain, which is called the *trans*-configuration. Within each of the abovementioned fatty acid classes, distinction can be made between the individual fatty acids. This is done based on the carbon-chain length, and on the position of the double bonds. PUFA with a double bond situated at the third or the sixth carbon atom from the methyl-end of the molecule are called *n*-3 PUFA or *n*-6 PUFA, respectively. [1, 3]

Dietary saturated fat and coronary heart disease: the debate.

The classic diet heart hypothesis[4, 5] is the notion that dietary saturated fat plays an essential role in the development of atherosclerosis and thereby of coronary heart disease (CHD) in humans . This hypothesis originates from the early 1950s, and was based on the combined results of human feeding trials that reported increased serum cholesterol levels in men after

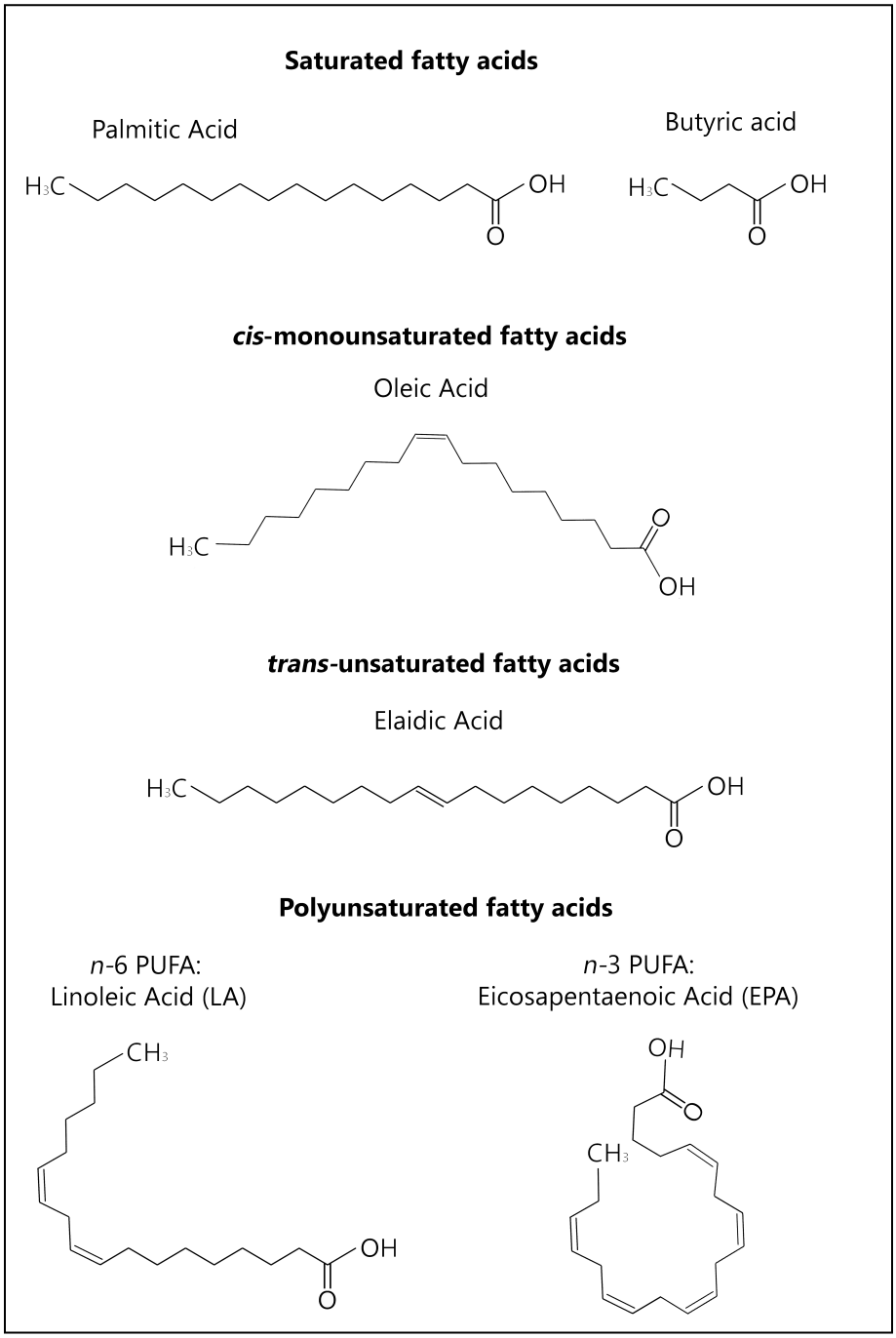

Figure 1. Example of different types of fatty acids, with their molecule structure which shows the carbon chain-lengths and number of single and double bonds.

a diet high in saturated fat [5-8], and of observational studies that showed a high risk of CHD in subjects with high serum cholesterol levels [9, 10]. In 1961, the American Heart Association (AHA) published the first official dietary guideline that included the advice to reduce the intake of saturated fat and to increase the intake of polyunsaturated fat [11]. Although, the dietary recommendation to limit saturated fat intake was already criticized for lack of sufficient evidence [12], over time, many other health organisations and governing bodies followed and issued a similar advice in their dietary guidelines [4, 13-18]. The recommendation to lower saturated fat intake remained virtually unchanged in international guidelines over the last 50 years [2, 19-22]. However, the controversy remained present [23-31].

That dietary SFA increases serum cholesterol levels is shown in plenty controlled trials [32], and appears to be irrefutable. It is the link between SFA and clinically manifest CHD that is controversial because there is no consistent scientific evidence that undeniably supports it. The first studies that examined the relationship between SFA and CHD outcomes were ecological studies [33] and migration studies [34], that reported higher saturated fat intakes in countries with higher CHD incidence. However, because of their geographical design, the findings are at most suggestive, not conclusive. Besides, the associations were not controlled for any potential confounding, and therefore many other factors may have explained the differences in CHD incidence between countries. The Seven Countries Study was also criticized for having excluded several countries which, if they were included in the analyses, would have nullified the observed correlation [35]. Around 2009, the debate intensified when the results of observational cohort studies, in which the associations were statistically corrected for potential confounders, did not confirm the association between SFA and CHD [36]. Later meta-analyses of observational studies did not observe a significant association between SFA and CHD incidence either [37-39].

This leads to the question whether dietary SFA is indeed not related to CHD risk, regardless of its cholesterol raising effects, or whether the null-findings in cohort studies are the result of either methodological issues such as residual confounding and misclassification, or of other reasons that are yet unknown. There are, for instance, several factors that the above-mentioned meta-analysed cohort studies failed to consider, which may play a role in the association between SFA and CHD. Three of these factors are discussed below.

1. The role of the substituting macronutrient.

The association between SFA and CHD may depend on the macronutrient that replaces SFA when its intake is lowered [40]. Randomized controlled trials (RCTs) show that PUFA, MUFA and carbohydrates each affect the blood lipid profile to a different extent when they replace SFA. Replacement of SFA with *cis*-PUFA results in the greatest beneficial change in the blood lipid profile, followed by *cis*-MUFA. The effects of replacement with carbohydrates are the least beneficial [32]. In line with those findings are the results from controlled trials on SFA and hard CHD outcomes, that showed that the replacement of SFA with PUFA was related to a lower risk of incident CHD [41, 42] , whereas the substitution of SFA with carbohydrates was not [41]. However, the included trials have the limitations that about half

of them were secondary prevention trials, which limits the generalisability of the results. Also, the majority of the trials was rather short-term. In half of the trials the follow-up was shorter than 4.5 years. Therefore, for long-term effects and primary prevention purposes the findings from observational cohort studies on this topic are still important.
In 2009, a pooled analysis of 11 observational cohort studies showed that the substitution of SFA with PUFA was related to a lower risk of CHD, whereas substitution with carbohydrates and MUFA were related to a higher risk of incident CHD and unrelated to CHD mortality [43]. Regardless, new meta-analyses of a total of 22 observational studies that were conducted after that time did not take into account the substituting macronutrient [37, 38].

2. The role of the individual saturated fatty acids.

Another factor that perhaps should be considered is the SFA type, i.e., the carbon-chain length of the SFA (**Textbox 2 and Table 1**). Controlled trials showed that the effect on serum cholesterol concentrations varies across the even-chained SFA with 12 to 18 carbons [32]. Replacement of carbohydrates with lauric acid, myristic acid or palmitic acid resulted in increased levels of serum total cholesterol, HDL-cholesterol and LDL-cholesterol. However, the ratio of total- to HDL-cholesterol decreased when carbohydrates were replaced with lauric acid, and replacement with stearic acid appeared to be neutral and was not associated with a change in the cholesterol concentrations. There is no evidence on the effects of different SFA on hard CHD outcomes from trials, and there are two previous prospective cohort studies that aimed to disentangle the individual SFA and their association with CHD incidence [44, 45]. In those studies the intake of the sum of long even-chained SFA (12 through 18 carbons) was related to a higher CHD risk, whereas SFA with chain lengths up to 10 carbons were not.

Textbox 2.

The classification of dietary saturated fat differs across the literature, but is generally defined as follows: short-chain (3 to 7 carbons), medium-chain (8 to 13 carbons), long-chain (14 to 20 carbons) and very long-chain (20 or more carbons) [46]. Most SFA in the diet contain an even number of carbons, and the most abundant SFA are the long-chain SFA palmitic acid, and stearic acid, followed by myristic acid and lauric acid. SFA with 10 carbons or less, and the odd-chain SFA pentadecylic acid and margaric acid together generally make up less than 10% of the total SFA intake. SFA with chains over 20 are primarily produced in the human body, and sparsely present in the human diet.[47]

3. The role of the food source.

Finally, the food source of saturated fat may play a role [48]. SFA is predominantly consumed through animal products, including dairy and meat. It is hypothesized that the effect of SFA may depend on its food source, for instance because of the different content of individual SFA, or because of (interactions with) other nutrients in these food sources. Studies on the

intake of SFA from specific food sources are scarce. One previous cohort study made a distinction between different food sources and observed that the intake of SFA from dairy sources was inversely associated with CHD, whereas the association between SFA from meat and incident CHD was potentially adverse [49]. Despite the fact that the three above-mentioned aspects and their supporting evidence are not new, still many observational studies did not take them into account in their analyses [37, 39]. Moreover, no studies have been conducted that consider all three aspects in a single study population, which makes it difficult to combine the evidence into one solid answer to the question whether SFA and CHD are related.

Table 1. Common names of saturated fatty acids

Common name	Short name*
Butyric acid	4 : 0
Caproic acid	6 : 0
Caprylic acid	8 : 0
Capric acid	10 : 0
Lauric acid	12 : 0
Myristic acid	14 : 0
Pentadecylic acid	15 : 0
Palmitic acid	16 : 0
Margaric acid	17 : 0
Stearic acid	18 : 0
Arachidic acid	20 : 0
Behenic acid	22 : 0
Lignoceric acid	24 : 0

* Refers to the number of carbon atoms and the number of double bonds

Dietary unsaturated fatty acids and CVD.

The consumption of (*cis*-)poly unsaturated fatty acids (PUFA) is associated with a lower risk of CVD, particularly CHD. As opposed to saturated fat, the evidence for the relation between PUFA and CVD is considered to be consistent and convincing. In several observational cohort studies [43, 50, 51], and randomized controlled trials (RCTs) [41, 42] a higher intake of PUFA was related to a lower CHD risk when substituted for SFA [41-43, 50], for carbohydrates [50], and without a defined substitute [51]. Evidence from trials showed that the very long chain *n*-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which make up only ~1% of the total PUFA intake [52], have beneficial effects on cardiovascular risk factors, such as blood pressure levels [53] and plasma triglyceride levels [54]. The main food sources of these very long chain *n*-3 PUFA are fish and seafood [55]. Fish consumption and supplementation of EPA and DHA reduced the risk of cardiovascular events in early trials [56-59] conducted before 2000. Contrary to these earlier trials, the more recent trials [60-64]

as well as observational studies [65-68] observed no clear association between EPA and DHA and CVD events, whereas fish consumption was associated with reduced risks of CVD in observational studies [69, 70]. These findings initiated another minor debate [71], and suggests that perhaps the benefits of EPA and DHA may be driven by fish consumption as such, or an interplay with other nutrients fish [72].
The association between consumption of *cis*-MUFA and CVD risk is not as clear as for PUFA[73]. Trials have shown that compared with SFA, *cis*-MUFA has a favourable effect on the blood lipid profile [32]. However, in observational studies no clear association has been observed between the substitution of MUFA for SFA and risk of CVD [41, 43].
On the adverse effects of *trans*-(unsaturated) fatty acids on CVD risk on the other hand, the existing evidence is very clear and consistent. *Trans* fatty acids are naturally present in small amounts in meat and dairy. *Trans* fatty acids are also formed by partial hydrogenation of vegetable oils and fish oil [74]. This is an industrial process, used to harden these oils, which was widely used in the food industry since its introduction in the early 1900s. In 1990, a trial showed that *trans* fatty acids raised blood levels of LDL-cholesterol and lowered levels of HDL-cholesterol [75]. These effects were confirmed in the trials that followed [76], and in observational studies *trans* fat consumption was observed to be associated with a higher CHD risk [77]. Therefore, the *trans* fat content of food products has been reduced since approximately 1994 [78]. In the Netherlands, for instance, the *trans* fat content of spreads and cooking fats reduced dramatically between 1994 and 1998 [78, 79]. The advised upper intake limit for *trans* fat of 1en% per day in the dietary guidelines followed in 2000 and thereafter [80, 81]. Yet, a recent study showed that the average *trans* fat intake in several countries was still too high in 2010 [82].

The overall quality of the fatty acid consumption.

Even though there is still room for discussion on the exact effects of the different classes of dietary fat on CVD, it is clear that there are differences between, and even within, the classes. Since fatty acids are never consumed on their own, but are all simultaneously present in a diet, perhaps we should look for an alternative classification, which considers the quality of total dietary fat. Recently, a different classification of fatty acids was introduced: the lipophilic index [83]. Rather than on the structure of the fatty acids, this classification is based on their melting points. It is proposed that the lipophilic index may better explain metabolic processes that cause CHD.

Objective and outline of this thesis

The objective of this thesis was to examine the association between dietary fatty acids and CVD risk in observational studies, with the main focus on the SFA.
In chapter 2 we examined the relative validity and reproducibility of the food frequency questionnaire, which was used in the EPIC-NL cohort, against twelve 24-hour recalls for its ability to rank individuals based on their intake of individual fatty acids. Chapter

3 includes four observational studies in prospective cohorts. In chapter 3.1 and 3.2 we examined whether baseline consumption of SFA was associated with incident CHD during follow up in the two Dutch cohorts EPIC-NL and the Rotterdam Study, and whether this association depended on the substituting macronutrient, the type of SFA, and the food source of SFA. In chapter 3.3 we investigated whether baseline consumption of individual SFA differing in carbon chain length was related to myocardial infarction in a cohort from the United Kingdom (EPIC-Norfolk), and in a cohort from Denmark (Diet, Cancer and Disease cohort). In chapter 4 we investigated whether the baseline intake of total fish and types of fish, which are rich in *n-3* PUFA, was related to CVD during follow up in the EPIC-NL cohort. In chapter 5 we examined whether the fluidity of the baseline dietary fatty acid profile, calculated as the lipophilic index and lipophilic load, were cross-sectionally related to concentrations of biochemical cardiovascular risk factors, and whether they were related to CHD and stroke incidence during follow up. Chapter 6 covers a study in which we investigated whether substitution of SFA for other macronutrients occurred during follow up, and whether this was similarly related to serum cholesterol changes as modelled substitution in baseline data only. This study was performed among a subpopulation from the PROSPECT-EPIC cohort. In chapter 7, we discuss the main findings of this thesis in broader context, followed by their practical implications and suggestions for future research. Finally, chapter 8 and 9 include summaries of this thesis in English and in Dutch, respectively.

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Chapter 2.

Reproducibility and relative validity of an FFQ to estimate the intake of fatty acids

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Abstract

We investigated the validity and reproducibility of the FFQ used in the Dutch European Investigation of Cancer and Nutrition cohort, in order to rank subjects according to intakes of fatty acid classes and individual fatty acids. In total, 121 men and women (23-72 years) filled out three FFQ at 6-month intervals between 1991 and 1992. As a reference method, they filled out twelve monthly 24-h dietary recalls (24HDR) during the same year. Intra-class correlation coefficients for the FFQ showed moderate to good reproducibility across all fatty acids (classes and individual) in men (0.56 through 0.81) and women (0.57 through 0.83). In men, Spearman's correlation coefficients (r_s) for the FFQ compared to the 24HDR indicated moderate to good relative validity (r_s=0.45 through 0.71) for all fatty acids, except arachidonic acid and marine PUFA (r_s<0.40). In women, relative validity was moderate to good for MUFA and *trans*-fatty acids (TFA) and the majority of SFA (r_s=0.40 through 0.66), was fair for the short-chain SFA and lauric acid (r_s=0.30 to 0.33) and was fair to moderate for PUFAs (r_s=0.22 to 0.47). Bland-Altman plots showed good agreement between the FFQ and 24HDR, and proportional bias for fatty acids with very low intakes. In conclusion, the FFQ showed good reproducibility for subject ranking based on intakes of fatty acids (classes and individual). The relative validity measures indicated that the FFQ is an adequate tool to rank subjects according to intakes of high-abundant fatty acids, but less for low-abundant fatty acids.

Introduction

The food frequency questionnaire (FFQ) is a frequently used tool to measure dietary intakes in epidemiological studies on diet and disease. A self-administered semi-quantitative FFQ was used to measure the habitual consumption of foods and nutrients in the Dutch cohorts of the European Prospective Investigation into Cancer and nutrition (EPIC-NL) [1].

In 1991, before the start of the EPIC-NL study, the FFQ was validated against twelve 24 hour dietary recalls (24HDR) to study its ability to rank subjects according to several foods [2] and nutrients [3], including total fat. However, up to today, this FFQ has not been validated for classes of fatty acids and individual fatty acids, although over time it has become evident that effects of dietary fats on (cardiovascular) health may differ across classes [4], and potentially even across individual fatty acids within these classes [5, 6]. For the purpose of studying disease risks in relation to individual fatty acids in the EPIC-NL cohort, it is essential to assess the ability of its FFQ to capture their intake.

Several other FFQ were validated against 24HDR or food records for their ability to rank subjects according to several, but not all, individual fatty acids. The majority was focused on individual polyunsaturated fatty acids (PUFA) [7-16] and oleic acid (18:1n-9) [7-9, 11-16], and the validity varied from fair (correlation coefficients (*r*) between 0.20 and 0.40) up to good (*r* between 0.60 and 0.80). Concerning individual saturated fatty acids (SFA), studies focused on validating the medium- and long- chained SFA only [7, 9, 11, 12, 15, 16], of which only two [12, 15] reported on the validity of pentadecylic (15:0) and margaric (17:0) acid [15], or capric (10:0) and lauric (12:0) acid [12]. All reported moderate to good relative validity [7, 11, 12, 15, 16], except for one, which observed fair to moderate validity [9]. The relative validity for *trans*-fatty acid (TFA) intake was studied less often than the other fatty acid classes [8, 11, 17], and ranged from poor [11] to good [17].

Other validity studies were done in different, non-Dutch, populations with different dietary patterns. Since the validity of an FFQ is specific to the study population and FFQ, we cannot translate the validity of other FFQ to the EPIC-NL FFQ. Therefore, in the present study, the reproducibility and relative validity of the FFQ, used in the EPIC-NL study, for measuring fatty acid classes and individual SFA, including short- and medium-chain SFA, TFA, monounsaturated fatty acids (MUFA) and PUFA were investigated.

Methods

Study population and data collection

Description of the study population as well as the collection and processing of the data have been described in detail elsewhere [2]. In short, the validation study was carried out before the actual enrolment of the EPIC-NL cohort members, and started in 1991. A total of 960 healthy Dutch men and women from two ongoing projects in four towns were invited

for the study by postal mail. These subjects were representative of the EPIC-NL cohort members. Of the 240 (25%) subjects who responded positively, 134 subjects were selected, equally distributed across the four towns, between both sexes, and in 20-year age groups. A total of sixty-three men and fifty-eight women, aged 23-72 years old, completed the study. The results presented in this article apply to those 121 subjects. Data were collected over a period of 13 months, starting in October 1991. To assess the reproducibility, the FFQ was administered three times; in months 1, 7 and 13. During the same period, twelve 24HDR were administered once every month in order to assess relative validity. The questionnaire was self-administered and contained questions on the habitual consumption frequency of seventy-nine main food items during the preceding year. Frequencies could be indicated in times per day, per week, per month or per year. For twenty-one foods, the questionnaire contained photographs of different portion sizes. For other foods, natural or household units were used to indicate portion size. The questionnaire contained additional questions about preparation methods and additions, and provided blank spaces for specification of brand names of margarines and cooking fats. Of the twelve 24HDR, six were administered face-to-face and six by telephone without previous warning. For most subjects, the recall days included one Saturday and one Sunday, and all other weekdays were on average recalled twice. The recalls were performed by trained nutritionists and dietitians, and most subjects were interviewed by the same interviewer throughout the study period.

Data processing and data analyses

For each FFQ and 24HDR assessment, dietary intakes were calculated for each individual subject. The Dutch food composition table 1998 (digital update) was used to calculate the intake of individual fatty acids in grams per day. To correct for under-representation of weekend days, the weighted average of 24HDR was calculated with a weight of one for weekdays and two for weekend days. The nutrient residual method was used to adjust fatty acid intakes for total energy intake [18]. As the majority of fatty acids were not normally distributed (data not shown), intakes were expressed in medians with interquartile ranges. To compare the median intakes of the first FFQ (FFQ1) with FFQ2, FFQ3, and the 24HDR, the Wilcoxon's signed-rank test was used. Intra-class correlation coefficients (ICC) were calculated with a two-way mixed model to obtain the reproducibility of the FFQ. To investigate the relative validity between FFQ1 and the weighted average of the twelve 24HDR, Spearman's rank correlation coefficients (r_s) were calculated. In addition, weighted kappa (κ_w) coefficients were calculated to assess the degree of agreement in fatty acid intake quintiles according to the FFQ1 versus the 24HDR. The ICC, r_s, and κ_w were interpreted according to the following classification: poor (≤0.20), fair (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80) or excellent (>0.80). All above-mentioned analyses were performed for both crude and energy-adjusted intakes. To assess absolute agreement between FFQ1 and the 24HDR, we constructed Bland-Altman plots for energy-adjusted fatty acid intakes only. In addition, we investigated whether potential bias was proportional to the levels of energy-adjusted fatty acid intake using linear regression analyses.

Linear regression analysis showed that the relationship between fatty acid intakes as measured by the FFQ and as measured by the 24HDR differed significantly for men and women. Therefore, all analyses were stratified for sex. All the analyses were performed in SPSS version 20.0 (IBM, Armonk, NY, USA) or SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

A detailed description of the baseline characteristics of the study population can be found elsewhere [2]. In short, the mean (± standard deviation) age of men and women was 42.6 (± 11.1) years and 49.0 (± 14.6) years, respectively. The average body mass index was 25.5 (± 2.9) kg/m^2 in men and 24.9 (± 3.5) kg/m^2 in women. Furthermore, 28% of both men and women attained higher vocational education or attended university.

The crude fatty acid intakes as measured by the FFQ and the 24HDR are shown in **Tables 1 and 2** for men and women, respectively. Energy-adjusted intakes are presented in **Supplemental Tables 1 and 2**. In both men and women, FFQ1 overestimated intakes of 16:0, TFA, and total MUFA as well as individual MUFA and PUFA, as compared with the weighted average of the 24HDR. Similarly, median intakes measured with FFQ1 were significantly higher than those measured with FFQ3, except for PUFA.

Table 3 presents the ICC of the three repeated FFQ. ICC for crude fatty acids ranged from 0.56 to 0.75 in men, and from 0.57 to 0.82 in women, indicating moderate to good reproducibility. The results were comparable for energy-adjusted fatty acids. The r_s for the fatty acids as measured by FFQ1 and the weighted average of the 24HDR are shown in **Table 4**. In men, the relative validity was moderate to good for crude intakes of total and individual SFA and MUFA, TFA, linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3), with r_s between 0.53 and 0.67. For energy-adjusted intakes of these fatty acids the coefficients were slightly different but still fell within the same range, except for stearic acid (18:0) (r_s = 0.47) and ALA (r_s = 0.45), which were lower. Relative validity was lower for the low-abundant PUFA, including arachidonic acid (AA; 20:4n-6) (r_s =0.42), and the marine *n*-3 PUFA eicosapentanoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (r_s < 0.40). Energy adjustment did not materially change these coefficients.

In women, the r_s between FFQ1 and the 24HDR showed moderate to good relative validity for all SFA (r_s from 0.51 to 0.62), except for caprylic acid (8:0) (r_s = 0.35) and lauric acid (r_s = 0.33), for which validity was fair. Energy adjustment lowered most correlations (r_s from 0.30 to 0.50), except for palmitic acid (16:0) (r_s = 0.62) and capric acid (r_s = 0.66). For MUFA and TFA, the r_s were, respectively, 0.63 and 0.56 for crude intakes and 0.58 and 0.49 for energy-adjusted intakes. For individual PUFA, the r_s varied from 0.33 to 0.44 for *n*-6 PUFA and from 0.28 to 0.36 for *n*-3 PUFA. The correlation coefficients for energy-adjusted PUFA intakes were higher for AA and EPA, but lower for total PUFA, ALA and DHA.

Table 1. Fatty acid intakes (g/d) in medians (IQR) for the three measurements of FFQ and the weighted average 24HDR in 63 men

			FFQ1		FFQ2		FFQ3		24HDR	
			Median	IQR	Median	IQR	Median	IQR	Median	IQR
SFA	Total		42.6	32.7 - 54.5	40.8*	31.3 - 49.8	39.2**	29.4 - 47.5	42.3*	31.2 - 49.2
	Butyric acid	4:0	0.54	0.33 - 0.89	0.43***	0.27 - 0.70	0.46**	0.31 - 0.68	0.56	0.37 - 0.76
	Caproic acid	6:0	0.40	0.24 - 0.62	0.31***	0.19 - 0.50	0.33**	0.22 - 0.49	0.39	0.27 - 0.56
	Caprylic acid	8:0	0.30	0.20 - 0.40	0.23***	0.15 - 0.38	0.23**	0.16 - 0.35	0.28	0.2 - 0.37
	Capric acid	10:0	0.58	0.35 - 0.75	0.47***	0.31 - 0.66	0.46**	0.32 - 0.72	0.50**	0.35 - 0.65
	Lauric acid	12:0	2.05	1.32 - 2.71	1.68**	1.12 - 2.44	1.57**	1.03 - 2.14	1.69	1.26 - 2.36
	Myristic acid	14:0	4.4	3.1 - 5.5	3.9**	2.5 - 5.1	3.9*	2.5 - 4.7	4.0	2.8 - 5.1
	Pentadecylic acid	15:0	0.56	0.41 - 0.73	0.48***	0.33 - 0.65	0.50**	0.34 - 0.64	0.54	0.38 - 0.71
	Palmitic acid	16:0	20.1	15.7 - 26.1	19.0**	14.5 - 22.9	18.9***	14.4 - 21.5	19.5**	14.1 - 22.8
	Margaric acid	17:0	0.44	0.31 - 0.53	0.40***	0.28 - 0.46	0.39**	0.28 - 0.48	0.39	0.31 - 0.5
	Stearic acid	18:0	9.4	7.5 - 12.3	9.1*	7.1 - 11.2	8.6**	6.8 - 10.6	9.4	7.2 - 11.4
MUFA	Total		40.5	31.9 - 51.7	38.6	30.6 - 49.7	36.8*	29.4 - 45.2	37.2**	29.0 - 45.4
	Oleic acid	18:1n-9	19.9	14.6 - 26.7	19.5	13.7 - 25.2	18.8**	14.2 - 24.2	20.0	15.4 - 26.7
TFA	Total		4.2	3.1 - 6.1	4.0*	3.0 - 5.1	3.8**	2.7 - 5.2	3.8*	2.7 - 5.2
PUFA	Total		23.1	17.6 - 31.0	23.2	18.8 - 29.9	22.2	16.7 - 28.2	18.3***	14.4 - 23.7
n-6	Total		16.9	12.4 - 22.3	17.7	12.9 - 21.3	15.9	12.7 - 20.5	12.7***	9.1 - 15.3
	Linoleic acid	18:2n-6	16.7	12.4 - 22.2	17.6	12.8 - 20.9	15.8	12.6 - 20.3	12.5***	8.9 - 15.1
	Arachidonic Acid	20:4n-6	0.02	0.02 - 0.04	0.02	0.02 - 0.04	0.02*	0.02 - 0.03	0.03**	0.02 - 0.05
n-3	Total		1.58	1.24 - 2.11	1.56	1.17 - 2.01	1.52*	1.08 - 1.93	1.47*	1.00 - 1.94
	Alpha-linolenic acid	18:3n-3	1.44	1.09 - 1.87	1.44	1.02 - 1.86	1.36*	0.94 - 1.72	1.24**	0.88 - 1.67
	Eicosapentanoic acid	20:5n-3	0.03	0.01 - 0.05	0.02	0.01 - 0.05	0.03	0.01 - 0.05	0.02	0.00 - 0.12
	Docosahexanoic acid	22:6n-3	0.07	0.04 - 0.13	0.06*	0.04 - 0.12	0.06	0.04 - 0.12	0.05	0.02 - 0.17

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; TFA, trans fatty acid; PUFA, polyunsaturated fatty acid

Table 5 presents the κ_w between FFQ1 and the 24HDR**.** In men, the agreement between FFQ1 and the 24HDR was fair for crude intake of lauric acid and moderate for the other individual SFA and total SFA (κ_w from 0.40 to 0.48). After energy adjustment, the agreement was fair for the short-chain SFA, caprylic acid, lauric acid and margaric acid (κ_w from 0.34 to 0.38), and moderate for all other SFA (κ_w from 0.44 to 0.52). Moderate agreement was observed for crude intakes of MUFA and TFA, and all PUFA, except AA (κ_w = 0.31) and marine *n*-3 PUFA (median κ_w = 0.21), which were considered fair. In general, the κ_w were slightly lower for energy-adjusted intakes of MUFA, TFA and PUFA. In women, κ_w between FFQ1 and the 24HDR were 0.47, 0.41, 0.50 and 0.43 for crude intakes of, respectively, total SFA, capric acid, palmitic acid and stearic acid (18:0). For the other SFA, κ_w coefficients were lower, ranging from 0.19 to 0.39. For crude intakes of MUFA and TFA, κ_w coefficients were 0.43 and 0.34, respectively. κ_w coefficients for PUFA ranged from 0.17 (EPA) to 0.28 (total PUFA). Energy adjustment in general lowered the κ_w coefficients for all fatty acids.

Bland-Altman plots showed systematic, non-proportional overestimation by FFQ1 as compared with the weighted average of the 24HDR of intakes of palmitic acid and TFA in both men and women (**Supplemental Figures 1 to 44)**. In men, proportional bias was observed for butyric acid (4:0), caproic acid (6:0) and pentadecylic acid, indicating underestimation at lower intake levels and overestimation at higher intake levels. In addition, for PUFA and LA, the overestimation was positively proportional to the levels of intake. For AA and n-3 PUFA, the proportional bias was negative, demonstrating underestimation by the FFQ at higher levels of intake. In women, a slight overestimation was observed for most SFA, which was positively proportional for capric acid only. Intake of total PUFA was systematically overestimated, showing no proportional bias, whereas the overestimation of LA increased with increased levels of intake, and intakes of AA, EPA and DHA showed negatively proportional bias.

Discussion

The reproducibility of the FFQ, used in EPIC-NL, was moderate to good for all fatty acid classes and individual fatty acids in both men and women. In men, the relative validity of the FFQ was moderate to good for all fatty acids, but fair for the low-abundant long-chain PUFA. In women, moderate to good relative validity was observed for SFA that are highly abundant in the Dutch diet, as well as for TFA and MUFA. The relative validity of low-abundant SFA and PUFA was fair to moderate, with the lowest validity observed for the marine *n*-3 fatty acids. Compared with the weighted average of the 24HDR, the FFQ generally overestimated fatty acid intake, and showed proportional bias for low-abundant fatty acids, particularly the short-chain SFA and the PUFA.

Strengths of this study include the size of the study population and the equal distribution of subject characteristics such as age and sex. Furthermore, we used data from twelve repeated

24HDR and the FFQ was administered three times at 6-month intervals. A limitation of the study is that the reference method we used, the 24HDR, has correlated errors with the FFQ, such as the reliance on memory, socially desirable answering and use of the same food composition database for calculations of nutrient intakes. Such correlated errors can lead to artificially high correlations between the two methods [19]. A reference method that has no correlated errors to the FFQ is the biomarker. Fatty acid levels measured in, for instance, erythrocytes, plasma, or adipose tissue can be used as biomarkers for dietary fatty acid intake, but only for the (largely) exogenously derived ones such as EPA, DHA, TFA, pentadecylic acid and margaric acid. Fatty acid biomarkers do not reflect dietary intakes of fatty acids that are largely endogenously derived, such as SFAs, and MUFAs [20]. For the present study population, no biomarkers were available. A previous study in a subsample of the total EPIC cohort (which apart from EPIC-NL includes cohorts from nine other countries [21]) compared mean plasma phospholipid fatty acid profiles with mean intakes of food groups as measured by the country-specific FFQ, including the EPIC-NL FFQ [22]. In that study, exogenously derived fatty acids significantly correlated with those foods that are important contributors to their intake. To illustrate, plasma phospholipid measures of the sum of pentadecylic acid and margaric acid were correlated with dairy product intake as measured by the FFQ. Also, 18:1*n-9t* correlated with intakes of dairy foods and margarine, and DHA correlated with fatty fish intake. This indirectly suggests that the EPIC FFQ are well capable of measuring the intakes of these fatty acids. However, we should be careful with directly applying this to the EPIC-NL FFQ as the previous findings are based on combined study populations from different European countries with each having their own FFQ, and it does not compare estimates on the individual fatty acid level. Our study showed that the reproducibility of the FFQ for fatty acid intake assessment in general was good, with ICC ranging from 0.56 to 0.83. These ICC are of the same magnitude as those presented in other studies that assessed the reproducibility of an FFQ for classes of fatty acids [23-26] and a limited number of individual PUFA [25, 26]. One study reported lower ICC ranging from 0.28 for total PUFA to 0.61 for DHA [27]

We observed an overestimation of intake of the majority of fatty acids assessed by the first FFQ as compared with the third FFQ, which is in line with a previous reproducibility study on dietary fatty acid measurements [27]. The first FFQ also overestimated the fatty acid intakes as compared with the 24HDR, which was also observed in several previous validation studies [8-10, 13], although not in all [15]. Overestimation is very common for questionnaires that cover more than 100 food items and pertain to a long time period [13], such as the FFQ used in our study.

In general, the relative validity for subject ranking in our study was lower among women than among men. This is in line with the lower validity among women in a previous validation study of this FFQ for food groups that largely contribute to the fatty acid intake, including cheese, nuts and seeds, and biscuits and pastries [2]. Previously, it was shown that under-reporting more often applies to foods that are rich in fats [28], and some studies

[29-32], although not all [33-35], showed that under reporters are more often women, which may explain the lower validity we observed.

Energy adjustment is often used in validation studies to cancel out correlated errors between the two measurement tools [19]. In the present study, energy adjustment of fatty acid intake did not improve the validity, and in many cases even lowered the validity. This is in contrast to what is expected based on a study that reported improvement of the validity of three different FFQ after energy adjustment [36]. It is unclear why energy adjustment caused lower relative validity in our study.

In general, the relative validity of the FFQ in the present study was moderate to good for intakes of individual SFA. Results from previous validation studies on SFA with chain lengths of ten carbons and over that used 24HDR [9, 15] or (weighed) food records [7, 11, 12, 16] as their reference method were similar to ours. The ability to rank subjects according to intake of SFA that are less abundant in the diet, including short-chain SFA and odd-chain SFA, was less among women. To our knowledge, no previous studies validated an FFQ against 24HDR or diet records for shorter-chain SFAs. It is conceivable that because of the small between-subject and withinssubject variation in intake of these SFA, overestimation by the FFQ as compared with the 24HDR will easily lead to changes in subject ranking, and thus to lower validity.

For measurement of individual PUFA, and in particular the marine *n*-3 PUFA, which are less abundant in the Dutch diet, the relative validity was low in our study, and considered fair. Previous validation studies showed varying results for the measurement of EPA and DHA. Some studies observed fair validity ($r < 0.40$) [10, 13, 15], similar to our study, whereas other studies report moderate ($0.40 \leq r < 0.60$) [7, 8, 12, 16] to good validity ($r \geq 0.60$) [14].

The lower validity in our study may be caused by the type of reference method used. Studies that showed the lowest validity all used 24HDR [8, 10, 13, 15], whereas the reference method in the majority of studies that showed higher validity were food records [7, 12, 14, 16]. Other validation studies used erythrocytes [37-40], adipose tissue [41] or plasma [11, 38, 39] as their reference method. Such biomarkers are considered to be a better reference for n-3 PUFA than 24HDR and food records, because of their uncorrelated errors to the FFQ. The observed validity in these biomarker studies ranged from fair [40-42] to excellent [39]. In general, the validity was higher for FFQ that were specifically developed to measure n-3 PUFA intake [37-39], than for FFQ that were, similar to the EPIC-NL FFQ, developed with the aim to measure the total diet [40-42]. This illustrates another potential explanation for the lower validity in our study. In the EPIC-NL FFQ, intakes of fish products, the main food sources of EPA and DHA, were not asked separately but aggregated into three items, which could have led to an underestimation of intake [43]. Correspondingly, a previous validation study of the FFQ used in the present study [2] showed similar fair validity for intake of fish ($r = 0.32$ in men, $r = 0.37$ in women).

In contrast to the underestimation of EPA and DHA, LA intake was overestimated by the FFQ as compared with the 24HDR in our study population. This overestimation increased with higher intake levels, and may be caused by the additional and detailed questions about added fats and margarines in the questionnaire, which are an important source of LA in the

population.
The validity of an FFQ is specific to the FFQ and to the study population it is administered to. In general, validation studies show obvious differences in validity across FFQ and also across all types of fatty acids. There is no indication that one particular fatty acid is commonly better captured by FFQ as compared with another fatty acid. This implies that we cannot generalise the validity of one FFQ to another, but each FFQ needs to be validated separately for its ability to measure fatty acids.

To conclude, the FFQ used in EPIC-NL showed moderate to good reproducibility for the assessment of intakes of specific classes and individual fatty acids. Furthermore, for the fatty acids that are highly abundant in the Dutch diet, this FFQ is an adequate tool to rank people according to their intakes. Relative validity was less for intakes of low-abundant fatty acids including short-chain SFA, AA and marine *n*-3 PUFA.

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Conflicts of interest

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Supplemental Materials

The following supplemental materials are available online through the website of the British Journal of Nutrition *.

Supplemental table 1.	Energy adjusted fatty acid intakes (g/d) in medians with interquartile ranges (IQR) for the three measurements of the food frequency questionnaire (FFQ) and the weighted average 24 hour dietary recalls (24HDRs) in 63 men
Supplemental table 2.	Energy adjusted fatty acid intakes (g/d) in medians with interquartile ranges (IQR) for the three measurements of the food frequency questionnaire (FFQ) and the weighted average 24 hour dietary recalls (24HDRs) in 58 women.
Supplemental figures 1 through 44.	Bland-Altman plots of energy adjusted intakes of fatty acids for men (in blue) and women (in red) separately.

* URL: https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/reproducibility-and-relative-validity-of-a-ffq-to-estimate-the-intake-of-fatty-acids/27852BCD5F649541BE42A38D7B5D535A#fndtn-supplementary-materials.

Chapter 3.

Saturated fatty acids and CHD in observational cohort studies

Chapter 3.1.

The EPIC-NL cohort

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Abstract

Background: The association between saturated fatty acids (SFA) intake and coronary heart disease (CHD) risk is debated.

3.1

Objective: We sought to investigate whether dietary SFAs were associated with CHD risk and whether associations depended on (1) the substituting macronutrient; (2) the carbon chain length of SFAs; and (3) the SFA food source.

Design: Baseline (1993-1997) SFA intake was measured with a food frequency questionnaire among 35,597 participants from the EPIC-NL cohort. CHD risks were estimated with multivariable Cox regression for the substitution of SFA with other macronutrients, and for higher intakes of total SFA, individual SFAs, and SFA from different food sources.

Results: During 12 years of follow-up 1,807 CHD events occurred. Total SFA intake was associated with a lower CHD risk (Hazard Ratio (HR) per 5en% = 0.83, 95% CI: 0.74, 0.93). Substituting SFA with animal protein, *cis*-monounsaturated fat, polyunsaturated fat (PUFA) or carbohydrates was significantly associated with higher CHD risks (HRs per 5 % of energy between 1.27 and 1.37). Slightly lower CHD risks were observed for higher intakes of the sum of butyric (4:0) through capric (10:0) acid (HR per standard deviation (SD) = 0.93, 95% CI: 0.89, 0.99), myristic acid (14:0) (HR_{SD}: 0.90, 95% CI: 0.83, 0.97), the sum of pentadecylic (15:0) and margaric (17:0) acid (HR_{SD}: 0.91, 95% CI: 0.83, 0.99) and for SFA from dairy sources, including butter (HR_{SD}: 0.94, 95% CI: 0.90, 0.99), cheese (HR_{SD}: 0.91, 95% CI: 0.86, 0.97), and milk and milk products (HR_{SD}: 0.92, 95% CI: 0.86, 0.97).

Conclusions: In this Dutch population, higher SFA intake was not associated with higher CHD risks. The lower CHD risk observed did not depend on the substituting macronutrient but appeared to be driven mainly by the sum of butyric through capric acid, sum of pentadecylic and margaric acid, myristic acid, and SFA from dairy sources. Residual confounding by cholesterol-lowering therapy and *trans*-fat or limited variation in SFA and PUFA intake may explain our findings. Analyses need to be repeated in populations with larger differences in SFA intake and different SFA food sources.

Introduction

Limiting the intake of dietary saturated fatty acids (SFAs) is an important component of recommendations for the prevention of coronary heart disease (CHD). High SFA intake is associated with higher blood low-density lipoprotein (LDL)-cholesterol levels [1], an established risk factor for CHD [2]. However, the association between SFA and CHD is now heavily debated [3-5], in part because evidence on this link appears to originate mainly from results of early ecologic studies [6], secondary prevention studies and short-term biomarker studies [7-9], whereas a direct link between SFA and CHD in prospective cohort studies is lacking. A meta-analysis that included 16 cohort studies showed no association between SFA intake and CHD risk, with a relative risk (RR) of 1.07 (95% CI: 0.96, 1.19) in the highest compared with the lowest quintile of intake [10]. An update of this meta-analysis, including 4 additional prospective cohort studies [11] as well as a meta-analysis of a selection of 12 cohort studies [12], observed similar null associations with RRs of 1.03 (95% CI: 0.98, 1.07) [11] and 1.06 (95% CI: 0.95, 1.17) [12]. However, the association between SFA and CHD may depend on several factors that were not taken into account in all 3 meta-analyses.

First, the association may depend on the macronutrients that replace SFA in the diet. A pooled analysis of 11 cohort studies showed that the association between SFA and CHD differed when SFA was replaced by polyunsaturated fat (PUFA) as opposed to carbohydrates or monounsaturated fat (MUFA) [13].

Second, specific types of SFA that differ in carbon chain length may also differ in their effects on blood lipids and thereby on CHD risk. SFA consists predominantly of the long-chain fatty acids stearic acid (18:0), palmitic acid (16:0), myristic acid (14:0) and lauric acid (12:0). A meta-analysis of 60 controlled trials showed that compared with carbohydrates these different types of SFAs vary in their effect on blood lipid levels [1]. The Nurses' Health Study (NHS) is the only prospective cohort study to our knowledge that specifically addressed the relation between dietary SFAs differing in carbon chain length and CHD [14]. This cohort study observed a moderately increased CHD risk for the sum of longer-chain SFAs (lauric acid through stearic acid), whereas for short- to medium-chain SFAs [butyric (4:0) through capric (10:0) acid] no associations with CHD were observed.

Finally, different food sources of SFA may modulate the effect of SFA on CHD risk. The major food sources of SFA are of animal origin, including meat and dairy products. In addition to the difference in specific SFAs in these products, other nutrients in these foods (and the way they interact with SFAs) could affect the risk of CHD. Accordingly, in the Multi-Ethnic Study of Atherosclerosis (MESA) each 5 g/d intake of dairy SFA was associated with a 16% lower risk of CHD, whereas each 5 g/d intake of meat SFA was related to a 29% higher risk of CHD [15].

In this study we examined the association between SFA intake and incident CHD risk and whether associations differed based on *1*) the type of macronutrient that replaces SFA, *2*)

the type of SFA (differing in carbon chain length) and *3*) the food source of SFA.

Methods

Study population

The EPIC-NL (European Prospective Investigation into Cancer and Nutrition–Netherlands) cohort consists of the Prospect-EPIC and MORGEN (Monitoring Project on Risk Factors for Chronic diseases) cohorts. Both cohorts were set up simultaneously between 1993 and 1997 and recruited a total of 40,011 participants. The design and rationale of EPIC-NL are described in detail elsewhere [16]. In brief, the Prospect-EPIC study included 17,357 women aged 49 - 70 years who lived in or near Utrecht and who participated in a nationwide breast cancer screening program. The MORGEN cohort consisted of 22,654 men and women aged 20-65 y selected from random samples of the Dutch population in 3 Dutch towns (Doetinchem, Amsterdam and Maastricht). All participants signed informed consent before inclusion. Both studies complied with the Declaration of Helsinki. Prospect-EPIC was approved by the institutional review board of the University Medical Center Utrecht, and MORGEN was approved by the medical ethics committee of the Netherlands Organization for Applied Scientific Research (TNO). At baseline, a general questionnaire and a food frequency questionnaire (FFQ) were administered, and a physical examination was performed that included blood pressure measurements, anthropometry and blood sampling [16].
For this study, we excluded subjects who withheld permission for linkage with vital status and death registries (n = 2,717); subjects with missing questionnaires (n = 172); subjects with an implausible energy intake based on the ratio of reported energy intake to estimated basal metabolic rate, i.e., the top or bottom 0.5% of the ratio (n = 342); and prevalent cases of cardiovascular disease at baseline (n = 1,183), leaving a total of 35,597 subjects for analysis.

Intake of foods, saturated fat and other nutrients

Food intake was assessed by a self-administered FFQ that measured the mean consumption frequency of 79 main food categories during the year before study enrollment [17]. This FFQ allowed for the estimation of the habitual intake of 178 food items. Portion sizes were estimated with use of photographs of several food items. Based on frequencies and portion sizes, the mean daily intake (g/d) was calculated for each subject individually. The intakes of all macronutrients and micronutrients were then calculated based on an updated version of the computerized Dutch food composition table 1996 [18]. Intakes of SFAs differing in chain length were calculated based on the Dutch food composition table 1998 (digital update; available on request from the National Institute for Public Health and the Environment). Before the start of the study, the FFQ was validated against twelve 24-h recalls among 121 men and women [19]. Pearson correlation coefficients showed good relative validity for intakes of fat (men: 0.63; women: 0.61), carbohydrates (men: 0.76; women: 0.74) and protein (men: 0.76; women: 0.71) [19]. Spearman rank correlation

coefficients showed reasonable to good validity for intakes of total SFA and the individual SFAs included in this study (butyric acid through stearic acid), ranging from 0.47 to 0.71 in men and from 0.30 to 0.66 in women [20]. Furthermore, the FFQ showed good reproducibility for the measurement of both total and individual SFAs, with intraclass correlation coefficients ranging from 0.58 to 0.73 in men and from 0.66 to 0.83 in women.

Because of very low intakes of butyric, caproic (6:0), caprylic (8:0) and capric acids, these SFAs were summed and evaluated as short- to medium-chain SFAs in the present study. For the same reason, intakes of pentadecylic (15:0) and margaric (17:0) acids were summed and evaluated as such. Based on the food groups that are predefined in the Dutch food composition table 1996 (NEVO)[18], we identified the following 7 mutually exclusive food groups that together contributed ~82% of the mean total SFA intake in the study population: cheese, meat, 'milk and milk products', fats, butter, cakes, and snacks. We separated the fats group into 2 subgroups based on the SFA content: 'hard and solid fats' (including margarines and fats in wrappers and solid frying fats, all of which contained ≥20 g SFAs/100 g of product) and 'soft and liquid fats' (including soft margarines, vegetable oils, liquid fats and frying oils, all of which contained <20 g SFAs/100 g of product). The remaining food groups, which each contributed <2.5 % to the total SFA intake, were aggregated and labeled as 'other sources'. Total SFA was defined as the sum of individual fatty acids with only single bonds between the carbon atoms in the fatty acid chain. SFA intake from each food group was calculated by summing the amount of total SFA present in all foods included in that group. Total carbohydrates comprised all types of carbohydrates except dietary fibre. *Cis*-MUFA included fatty acids with one double carbon bond with a *cis*-configuration (**Supplemental Figure 1)**. Total PUFA included fatty acids with multiple double bonds and with *cis*- and/or *trans* configurations (S**upplemental Figure 2**). *Trans*-fat was the sum of all *trans*-MUFAs and *trans*-PUFAs**.** Protein intake was divided in animal- and vegetable-derived protein based on whether the food source was of animal or vegetable origin. Alcohol consumption was categorized as follows: 0, 0.1-6.0, 6.1-12.0, 12.1-24.0 and >24 g/day for women and 0, 0.1-6.0, 6.1-12.0, 12.1-24.0, 24.1-60.0 and >60 g/day for men. The international table compiled by Foster-Powell et al. [21] was used to obtain the glycemic index (GI) of foods. Intake variables of total SFA, SFAs differing in carbon chain lengths, and SFA from specific food groups, and other macronutrient intake variables were expressed as percentages of total energy intake (en%). Other nutrients were adjusted for total energy intake through use of the residual method [22].

Other baseline assessments

Information on demographic characteristics, presence of chronic diseases and cardiovascular disease risk factors was obtained with the general questionnaire at baseline. Smoking status was categorized as never, former or current. Education was defined in 3 categories: low (primary education up to completing intermediate vocational education), intermediate (up to higher secondary education) or high (higher vocational education and university). On the basis of information about the duration and types of physical activity, which were

assessed through a validated questionnaire, the Cambridge Physical Activity Index (CPAI) was calculated [23], and participants were divided into 4 categories for physical activity level (inactive, moderately inactive, moderately active and active).

During the physical examination at baseline, body weight, height and waist circumference were measured. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Mean systolic and diastolic blood pressure were obtained by calculating the mean of 2 sequential measurements that were performed in the supine position with a cuff on the left arm through use of either a Boso Oscillomat (Bosch & Son, Jungingen, Germany) (Prospect-EPIC) or a random zero sphygmomanometer (MORGEN). Hypertension was considered present when at least one of the following criteria were met: systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, self-reported use of antihypertensive medication, or self-report of physician-diagnosed hypertension. Total cholesterol concentrations were measured using enzymatic methods, and HDL-cholesterol and LDL-cholesterol were measured with use of a standard homogeneous assay with an enzymatic endpoint.

Ascertainment of CHD

Morbidity data were obtained from the Dutch Center for Health Care Information, which holds a standardized computerized registry of hospital discharge diagnoses. Admission files from general and university hospitals in the Netherlands have been stored continuously since 1990. The records contain data on sex, date of birth, dates of admission and discharge, at least 1 principal diagnosis and up to nine optional additional diagnoses. All events were coded by qualified medical administrative personnel in the hospitals according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9). The National Medical Registry checked the data and collected them in the hospital discharge diagnosis database, which is linked to the cohort based on information of birthdate, sex, postal code and general practitioner with a validated probabilistic method [24]. Information on vital status was obtained through linkage with municipal registries, and causes of death were obtained through linkage with Statistics Netherlands (CBS). We identified all first-ever CHD events (ICD-9; 410–414, 427.5, 798.1, 798.2, 798.9). Follow-up was complete until 1 January 2008.

Data analysis

Baseline characteristics of the study population were calculated across quintiles of total SFA intake in percentage of energy and presented as means with SDs for normally distributed variables, medians with IQRs for variables that were not normally distributed, or percentages for categorical variables. Pearson correlations between intakes of total SFA, SFA from food sources, and SFAs differing in carbon chain length were calculated. Person-years were calculated as the time between the date of study entry and the date of first-ever CHD event, date of death, loss to follow-up or end of follow-up (1 January 2008),

whichever came first.
We used Cox proportional hazard regression models to calculate Hazard Ratios (HR) with 95% CIs for the association between SFA intake and risk of CHD incidence (fatal and nonfatal). Total SFA intake was evaluated per 5% of energy and entered as a continuous variable into the Cox regression models. In addition to a crude model (model 1), 3 models were constructed to adjust for potential confounding. As potential confounders, we considered known risk factors for CHD and covariables that were associated with SFA intake and CHD risk in our population. Model 2 was adjusted for age. Model 3 was additionally adjusted for sex, total energy intake, BMI, waist circumference, education level, physical activity index, smoking status and alcohol intake (in categories). Model 4 was additionally adjusted for intakes of *trans*-fat, animal protein, and vegetable protein (all in en%), and for energy-adjusted intakes of vitamin C, fibre, and dietary cholesterol. The HRs for SFA intakes after adjustment model 1, 2 and 3 can be interpreted as the CHD risk for an increased intake of energy from total SFA (or SFA type) at the expense of intakes of energy from all other types of fats, carbohydrates and protein. Because of additional adjustment for *trans*-fat, animal protein, vegetable protein (and the sum of other SFAs), the HRs after adjustment for model 4 can be interpreted as the CHD risk for an increased intake of energy from total SFA (or SFA type) at the expense of intakes of energy from PUFA, *cis*-MUFA and carbohydrates.

To estimate the risk of CHD when energy intake from SFA was substituted by an equal amount of energy from each of the other macronutrients, all 4 Cox models were converted into substitution models. These models included intakes of PUFA, *cis*-MUFA, *trans*-fat, total carbohydrates, animal protein and vegetable protein (all expressed per 5 en%), as well as total energy intake from all macronutrients except energy from alcohol consumption. By excluding SFA intake from the models, the HR for each macronutrient can be interpreted as the difference in CHD risk for each additional intake of 5 en% from that particular macronutrient at the expense of 5 en% from SFA [22]. To distinguish between the quality of carbohydrates, subjects were ranked based on their GI intake. The analyses in which SFA was substituted with total carbohydrates were then stratified for tertiles of this GI distribution [25]. In this way, the substitution of SFA with carbohydrates in GI tertiles 1, 2 and 3 represented the substitution of SFA with carbohydrates in subjects with a low-, medium- and high-GI diet, respectively. Intakes of SFAs differing in carbon chain length or SFA from different food sources were separately evaluated by entering them into the Cox models as continuous variables per 1 SD of intake. The SDs for the sum of butyric through capric acid, lauric acid, myristic acid, palmitic acid, the sum of pentadecylic and margaric acid, and stearic acid were 0.27 en%, 0.24 en%, 0.44 en%, 1.19 en%, 0.11 en%, and 0.66 en%, respectively. The SDs for SFA from butter, cheese, milk and milk products, meat, cakes, snacks, hard and solid fats, soft and liquid fats, and other sources were 1.42 en%, 1.95 en%, 1.45 en%, 1.44 en%, 0.83 en%, 0.40 en%. 1.25 en%, 0.50 en%, and 1.06 en%, respectively. The 4 previously mentioned Cox models were used, with additional adjustment in model 4 for the sum of all other consumed SFAs. To identify whether nonlinear associations existed,

Table 1. Baseline characteristics[1,2] across quintiles of the saturated fat intake (in en%) in 35597 subjects of the EPIC-NL cohort

	Quintiles of total saturated fat intake (in en%)				
	Q1	Q2	Q3	Q4	Q5
Median intake (IQR)	11.7 (2.3 - 12.8)	13.6 (12.8 - 14.2)	14.9 (14.2 - 15.5)	16.2 (15.5 - 17.1)	18.4 (17.1 - 28.7)
Subjects (n)	7119	7120	7119	7120	7119
Age (years)	48.1 (± 12.5)	47.8 (± 12.5)	48.5 (± 12.0)	49.7 (± 11.5)	52.3 (± 10.2)
Male (%)	28	29	26	23	19
High education level (%)	24	23	21	18	15
BMI (kg/m^2)	25.3 (± 3.8)	25.5 (± 3.8)	25.6 (± 4.0)	25.8 (± 4.0)	26.1 (± 4.3)
Waist circumference (cm)	84.5 (± 11.2)	85 (± 11.3)	85.1 (± 11.3)	85.4 (± 11.4)	85.7 (± 11.8)
High physical activity (%)	43	44	43	42	39
Current smokers (%)	29	29	28	30	34
Hypertension (%)	37	36	36	37	39
Systolic blood pressure (mmHg)	126.4 (± 19.2)	125.6 (± 18.4)	125.7 (± 18.7)	126.6 (± 19.0)	127.5 (± 19.4)
Diastolic blood pressure (mmHg)	77.9 (± 10.8)	77.7 (± 10.5)	77.7 (± 10.7)	78.2 (± 10.6)	78.1 (± 10.6)
Cholesterol (mmol/L)	5.6 (± 1.2)	5.6 (± 1.1)	5.6 (± 1.1)	5.7 (± 1.1)	5.9 (± 1.1)
HDL (mmol/L)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)
Ratio total: HDL	4.2 (± 1.5)	4.3 (± 1.5)	4.2 (± 1.5)	4.3 (± 1.5)	4.4 (± 1.5)
Energy intake (kcal)	1910 (± 596)	2043 (± 602)	2097 (± 602)	2099 (± 597)	2106 (± 606)
Saturated fat (g/d)	24.2 (± 8.3)	30.8 (± 9.2)	34.7 (± 10.0)	37.9 (± 10.9)	44 (± 13.2)
Sum of butyric to capric acid (en%)	0.4 (± 0.2)	0.6 (± 0.2)	0.6 (± 0.2)	0.7 (± 0.2)	0.9 (± 0.3)
Lauric acid (en%)	0.4 (± 0.2)	0.5 (± 0.2)	0.6 (± 0.2)	0.7 (± 0.2)	0.8 (± 0.2)
Myristic acid (en%)	1 (± 0.2)	1.2 (± 0.2)	1.4 (± 0.2)	1.6 (± 0.3)	2 (± 0.4)
Palmitic acid (en%)	5.1 (± 0.6)	6 (± 0.4)	6.5 (± 0.4)	7.1 (± 0.4)	8 (± 0.7)

Pentadecylic & margaric acid (en%)	0.3 (± 0.1)	0.3 (± 0.1)	0.3 (± 0.1)	0.4 (± 0.1)	0.5 (± 0.1)
Stearic acid (en%)	2.5 (± 0.4)	2.9 (± 0.3)	3.2 (± 0.3)	3.5 (± 0.3)	4 (± 0.5)
% from butter[3]	3.7 (2.0 - 6.1)	4.1 (2.5 - 6.7)	4.4 (2.7 - 7.6)	4.9 (3.1 - 9.7)	6.6 (3.5 - 17.2)
% from cheese[3]	13.5 (6.7 - 21.1)	14.6 (8.5 - 22.3)	15.6 (9.5 - 23.4)	16.5 (10.2 - 24.5)	18.6 (11.2 - 27.8)
% from milk and milk products[3]	15.7 (9.0 - 23.4)	15.8 (9.9 - 22.5)	15.9 (10.1 - 22.6)	15.7 (10.3 - 21.9)	14.1 (8.8 - 20.8)
% from meat[3]	16.9 (10.5 - 24.2)	17.3 (11.5 - 23.6)	17.3 (11.4 - 23.3)	16.9 (11.3 - 22.5)	15.7 (10.3 - 21.6)
% from cakes[3]	5.1 (2.3 - 9.3)	5.6 (2.9 - 9.6)	5.8 (3.0 - 9.6)	5.7 (3.0 - 9.5)	5 (2.4 - 8.5)
% from snacks[3]	2.3 (0.8 - 5.0)	2.4 (0.9 - 4.5)	2.2 (0.9 - 4.2)	1.9 (0.8 - 3.6)	1.3 (0.5 - 2.6)
% from hard. solid fats[3]	4.5 (1.4 - 8.8)	5.9 (2.5 - 10.6)	7.1 (3.2 - 11.9)	8.2 (3.9 – 14.0)	9.8 (4.6 - 16.7)
% from soft. liquid fats[3]	5.3 (2.8 - 8.5)	4.7 (2.4 - 7.4)	4.3 (2.2 - 6.9)	3.6 (1.6 – 6.0)	2.3 (0.7 - 4.5)
% from other sources[3]	21.8 (16.9 - 27.7)	19.3 (15.0 - 24.5)	17.2 (13.5 - 22.2)	15.5 (11.9 - 19.8)	12.6 (9.4 - 16.7)
Polyunsaturated fatty acids (en%)	6.7 (± 1.9)	6.9 (± 1.8)	7 (± 1.7)	6.9 (± 1.7)	6.7 (± 1.7)
Cis-monounsaturated fatty acids (en%)	8 (± 1.7)	9 (± 1.6)	9.6 (± 1.6)	10.2 (± 1.6)	11.1 (± 1.8)
Trans-fat (en%)	1 (± 0.4)	1.2 (± 0.4)	1.3 (± 0.4)	1.4 (± 0.5)	1.6 (± 0.5)
Animal protein (en%)	9.2 (± 2.8)	9.6 (± 2.5)	9.9 (± 2.4)	10.2 (± 2.3)	10.8 (± 2.4)
Vegetable protein (en%)	6 (± 1.2)	5.8 (± 1.0)	5.6 (± 0.9)	5.4 (± 0.8)	5 (± 0.8)
Carbohydrates (en%)	49.5 (± 7.0)	47 (± 5.5)	45.4 (± 5.0)	43.6 (± 4.8)	40.5 (± 5.0)
Glycemic Index[4]	54.2 (51.4 - 56.9)	54.8 (52.4 - 57.2)	54.9 (52.6 - 57.2)	55 (52.7 - 57.2)	55.1 (52.7 - 57.3)
Alcohol (g/d)	8.6 (1.1 – 24.0)	6.4 (1.0 - 18.5)	5.2 (0.9 - 14.9)	4.2 (0.7 - 12.2)	2.9 (0.3 - 10.3)
Cholesterol (mg/d)[4]	182.1 (± 54.1)	203.7 (± 52.2)	216.3 (± 49.0)	230.4 (± 52.4)	255.9 (± 58.5)
Fibre (g/d)[4]	24.9 (± 5.7)	24.1 (± 4.7)	23.4 (± 4.4)	22.8 (± 4.2)	21.7 (± 4.2)
Vitamin C (mg/d)[4]	128.5 (± 56.0)	114.8 (± 44.0)	108.4 (± 40.8)	102.8 (± 38.7)	94 (± 36.1)

[1] Baseline characteristics are expressed as means with standard deviations or as percentages, unless stated otherwise.

[2] Expressed in medians with interquartile ranges. [3] Adjusted for total energy intake.

quadratic terms of the SFA intake variables were included into the fourth model. *P* values for quadratic terms were between 0.1 and 0.9 for all SFA intake variables except for SFA from milk. However, construction of restricted cubic splines showed no significant non-linear association between SFA from milk and CHD ($P = 0.06$) (**Supplemental Figure 3**). The proportional hazards assumption was tested by calculating Schoenfeld residuals and visual inspection of log-log plots, which showed no significant deviations. We performed a series of sensitivity analyses. We checked for possible effect modification by sex by adding a product term of sex with SFA to the final models. To check whether blood cholesterol or blood pressure were possible intermediates, we adjusted the fourth model for either the baseline total cholesterol: HDL cholesterol ratio or systolic blood pressure. To minimize the possibility of reverse causation, we repeated the analyses in the population after excluding the first 2 y of follow-up. Because baseline dietary data could be unrelated to events occurring after a very long follow-up time, we repeated our analyses for the first 5 y of follow-up only by censoring everyone in the study population who in the first 5 y did not experience an event and was not lost to follow-up. Furthermore, we performed separate analyses for nonfatal CHD events ($n = 1,649$) only, because previous published studies have suggested that associations may differ for CHD mortality compared with nonfatal CHD [13]. Because of the low number of CHD deaths in our population (n=158), we did not perform a separate analysis for CHD mortality only. We repeated the analyses with age as the underlying time axis and additional stratification by birth year in 5-y intervals to adjust for calendar effects [26]. Finally, we checked whether differences in associations were observed between the substitution of SFA with n-3 PUFAs versus n-6 PUFAs, as suggested previously [27]. All statistical analyses were executed in SAS 9.2 (SAS Institute, Cary, North Carolina, USA), and *P* values <0.05 (2-sided) were considered statistically significant.

Results

Baseline characteristics

The baseline characteristics of the total study population are presented in **Table 1.** Compared with subjects with the lowest intake, subjects with a high intake of SFA were more likely to be older women who smoked and who had a higher BMI and waist circumference, higher blood pressure, higher total cholesterol: HDL cholesterol ratio, and less education and physical activity. Subjects with high SFA intake also reported higher intakes of *cis*-MUFA, *trans*-fat, cholesterol, animal protein, and calcium and lower intakes of carbohydrates, vegetable protein, fibre, vitamin C, and alcohol.

The mean baseline intake of total SFA in the population was 15.0 en% ± 2.7 en%. Over 97% of the population exceeded the upper intake limit of 10 en% per day as recommended by the Health Council of the Netherlands [28]. Most SFA intake was represented by the long-chain SFAs palmitic acid (51.2%) and stearic acid (25.5%) (**Figure 1**). The main food sources of SFA were cheese (17.4%), milk and milk products (16.6%), meat (17.5%), hard and solid fats (8.6%), and butter (7.3%) (**Figure 2**). Pearson correlation coefficients of intakes of all individual SFAs ranged between 0.30 and 0.63, except for palmitic and stearic acids,

which were highly correlated (r = 0.92), because of shared food sources (**Table 2**). The main food sources of palmitic acid and stearic acid were meat and cheese. Milk and milk products and cheese were the top 2 contributors of the sum of butyric through capric acid, lauric acid, myristic acid, and the sum of pentadecylic and margaric acids (**Supplemental Figure 4**). The percentages of *cis*-MUFA and PUFA provided by the predefined SFA food groups can be found in **Supplemental Figures 5 and 6**.

Total SFA intake and CHD risk

Over a median follow-up time of 12.2 y 1,807 incident CHD cases were documented; 158 (8.7 %) of these were fatal. After multivariable adjustment for lifestyle and dietary factors (model 4), a higher intake of energy from SFA was significantly associated with a 17% lower CHD risk (HR per 5 en%: 0.83, 95%: CI 0.74, 0.93) (**Table 3**). **Table 4** presents the HRs for the association between a higher intake of energy from carbohydrates, *cis*-MUFA, PUFA, or protein at the expense of an equal amount of energy from SFA and incident CHD. After full adjustment (model 4), the substitution of SFA with total carbohydrates ($HR_{5en\%}$: 1.23, 95% CI: 1.09, 1.40), *cis*-MUFA ($HR_{5en\%}$: 1.30, 95% CI: 1.02, 1.65), PUFA ($HR_{5en\%}$: 1.35, 95% CI: 1.14, 1.61) or animal protein ($HR_{5en\%}$: 1.37, 95% CI: 1.14, 1.65) was significantly associated with higher CHD risks. We observed differences in CHD risk when SFA was substituted with carbohydrates differing in GI values. The higher CHD risk for substitution of SFA with high-GI carbohydrates was statistically significant ($HR_{GI>56}$: 1.27, 95% CI: 1.03, 1.56), whereas the CHD risk for substitution with low-GI carbohydrates was not statistically significant ($HR_{GI<53}$: 1.14, 95% CI :0.91, 1.43). No significant association with CHD risk was observed for the substitution of SFA with vegetable protein ($HR_{5en\%}$: 0.81, 95% CI: 0.57, 1.17).

Intake of SFA differing in carbon chain length and risk of CHD

Table 3 shows the HRs for the associations between intakes of SFAs differing in carbon chain length and risk of CHD. After adjustment for lifestyle and dietary factors (model 4), slightly but significantly lower CHD risks were observed for each additional SD of intake of energy from short-to medium-chain SFAs (HR: 0.93, 95% CI: 0.89, 0.99), myristic acid (HR: 0.90, 95% CI: 0.83, 0.97), and the sum of pentadecylic and margaric acids (HR: 0.91, 95% CI: 0.83, 0.99). No significant associations were observed for intakes of lauric (HR: 0.97, 95% CI: 0.91, 1.02), palmitic (HR: 1.00 95% CI: 0.91, 1.10) or stearic (HR: 1.05, 95% CI: 0.97, 1.14) acid.

Intake of SFA from food sources and risk of CHD

After adjustment for lifestyle and dietary factors (model 4), slightly but significantly lower CHD risks were found for each additional SD of intake of SFA from butter (HR: 0.94, 95% CI: 0.90, 0.99), SFA from cheese (HR: 0.91, 95% CI: 0.86, 0.97), and SFA from milk (HR: 0.92, 95% CI: 0.86, 0.97) (**Table 5**). No significant associations were observed for intakes of SFA from other food sources.

Table 2. Pearson correlation coefficients[1] between intakes (in en%) of total saturated fatty acids (SFA), SFA from its main food sources, and individual SFA

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
(1)	Total SFA	1														
(2)	Butyric - capric acid (4:0 - 10:0)	0.59	1													
(3)	Lauric acid (12:0)	0.55	0.73	1												
(4)	Myristic acid (14:0)	0.82	0.84	0.72	1											
(5)	Palmitic acid (16:0)	0.94	0.40	0.35	0.68	1										
(6)	Pentadecylic & margaric acid (15:0 & 17:0)	0.75	0.92	0.59	0.92	0.63	1									
(7)	Stearic acid (18:0)	0.88	0.30	0.34	0.55	0.92	0.50	1								
(8)	SFA from butter	0.44	0.16	0.22	0.48	0.41	0.28	0.32	1							
(9)	SFA from cheese	0.42	0.74	0.37	0.57	0.29	0.76	0.23	0.012	1						
(10)	SFA from milk	0.24	0.49	0.48	0.48	0.13	0.40	0.03	-0.04	-0.10	1					
(11)	SFA from meat	0.26	-0.27	-0.20	-0.08	0.45	-0.03	0.51	-0.03	-0.18	-0.16	1				
(12)	SFA from cakes	0.17	0.21	0.41	0.18	0.08	0.12	0.09	-0.03	0.01	0.02	-0.15	1			
(13)	SFA from snacks	-0.03	-0.25	-0.21	-0.23	0.08	-0.23	0.09	-0.10	-0.16	-0.18	0.04	-0.10	1		
(14)	SFA from hard, solid fats	0.41	-0.09	0.02	0.15	0.41	0.04	0.37	0.03	-0.10	0.004	0.18	-0.04	-0.03	1	
(15)	SFA from soft, liquid fats	-0.11	-0.11	-0.08	-0.17	-0.15	-0.15	-0.13	-0.08	-0.03	-0.10	-0.04	-0.07	-0.08	-0.20	1
(16)	SFA from other sources	-0.01	-0.29	-0.20	-0.28	0.04	-0.31	0.18	-0.07	-0.19	-0.23	-0.17	-0.03	0.32	-0.09	-0.06

[1] All *P* values were <0.0001 unless stated otherwise.

[2] *P* value < 0.05, 3 P value = 0.2, 4 P value = 0.7

Table 3. Multivariable HR with 95% CI [1] for the associations between the intake of total and individual saturated fatty acids (SFA) with CHD incidence

	Median intake (en%)	HR expressed per	HR (95% CI) Model 1[2]	HR (95% CI) Model 2[3]	HR (95% CI) Model 3[4]	HR (95% CI) Model 4[5]
Total SFA	14.9	5.00 en%	1.14 (1.05, 1.24)	1.02 (0.94, 1.10)	0.94 (0.86, 1.02)	0.83 (0.74, 0.93)
Butyric - capric acid (4:0 - 10:0)	0.62	0.27 en%	0.99 (0.94, 1.03)	0.85 (0.81, 0.90)	0.95 (0.90, 1.00)	0.93 (0.89, 0.99) [6]
Lauric acid (12:0)	0.61	0.24 en%	1.04 (1.00, 1.09)	0.88 (0.84, 0.93)	0.96 (0.91, 1.00)	0.97 (0.91, 1.02) [6]
Myristic acid (14:0)	1.44	0.44 en%	1.05 (1.01, 1.10)	0.92 (0.87, 0.96)	0.95 (0.90, 0.99)	0.90 (0.83, 0.97) [6]
Palmitic acid (16:0)	6.50	1.19 en%	1.06 (1.02, 1.11)	1.05 (1.01, 1.10)	0.98 (0.94, 1.03)	1.00 (0.91, 1.10) [6]
Pentadecylic (15:0) & margaric (17:0) acid	0.35	0.11 en%	1.03 (0.99, 1.08)	0.91 (0.87, 0.95)	0.96 (0.91, 1.01)	0.91 (0.83, 0.99) [6]
Stearic acid (18:0)	3.20	0.66 en%	1.08 (1.03, 1.13)	1.08 (1.03, 1.12)	1.00 (0.95, 1.04)	1.05 (0.97, 1.14) [6]

[1] Obtained from Cox proportional hazards regression models

[2] Crude model

[3] Adjustment for age

[4] Additional adjustment for sex, total energy, BMI, waist circumference, education level, physical activity level, smoking status, and alcohol intake (categories)

[5] Additional adjustment for trans-fat, vegetable protein, animal protein (all in en%) and energy adjusted intakes of cholesterol, fibre, and vitamin c

[6] Additional adjustment for the sum of other SFA.

Table 4. Multivariable HR with 95% CI[1] for the association between the consumption of 5% energy from different macronutrients at the expense of 5% energy from total saturated fat (SFA), while keeping total energy intake constant, and incident coronary heart disease risk.

	HR (95% CI) Model 1[2]	HR (95% CI) Model 2[3]	HR (95% CI) Model 3[4]	HR (95% CI) Model 4[5]
Carbohydrates for SFA	0.84 (0.75, 0.95)	1.31 (1.16, 1.48)	1.19 (1.05, 1.34)	1.23 (1.09, 1.40)
Carbohydrates with low GI for SFA[6]	0.90 (0.73, 1.11)	1.26 (1.02, 1.56)	1.14 (0.92, 1.41)	1.14 (0.91, 1.43)
Carbohydrates with medium GI for SFA[6]	0.89 (0.71, 1.11)	1.50 (1.18, 1.89)	1.31 (1.03, 1.67)	1.35 (1.05, 1.73)
Carbohydrates with high GI for SFA[6]	0.84 (0.70, 1.01)	1.43 (1.17, 1.75)	1.23 (1.01, 1.51)	1.27 (1.03, 1.56)
Cis-MUFA for SFA	0.71 (0.56, 0.90)	1.57 (1.24, 1.99)	1.27 (1.00, 1.61)	1.30 (1.02, 1.65)
PUFA for SFA	1.09 (0.91, 1.29)	1.52 (1.28, 1.81)	1.31 (1.10, 1.55)	1.35 (1.14, 1.61)
Protein for SFA	0.86 (0.73, 1.02)	1.41 (1.19, 1.67)	1.25 (1.05, 1.48)	1.29 (1.08, 1.54)
Animal protein for SFA	0.98 (0.82, 1.16)	1.57 (1.32, 1.87)	1.35 (1.13, 1.62)	1.37 (1.14, 1.65)
Vegetable protein for SFA	0.47 (0.35, 0.62)	0.83 (0.63, 1.11)	0.88 (0.67, 1.16)	0.81 (0.57, 1.17)

[1] Obtained from Cox proportional hazards regression models

[2] Includes intakes of total carbohydrates, *cis*-MUFA, PUFA, trans-fat, animal protein and vegetable protein (all expressed per 5 en%), as well as total energy (excluding energy from alcohol intake)

[3] Additional adjustment for age

[4] Additional adjustment for sex, BMI, waist circumference, education level, physical activity level, smoking status, and alcohol intake (categories).

[5] Additional adjustment for energy adjusted intakes of cholesterol, fibre, and vitamin c

[6] Number of cases for low GI: 591; medium GI: 524; high GI: 692

Table 5. Multivariable HR with 95% CI [1] for the associations between the intake of saturated fat (SFA) from its main food sources with CHD incidence.

	Median intake (en%)	HR expressed per	HR (95% CI) Model 1[2]	HR (95% CI) Model 2[3]	HR (95% CI) Model 3[4]	HR (95% CI) Model 4[5]
SFA from butter	0.62	1.42 en%	1.04 (1.00, 1.09)	0.99 (0.94, 1.03)	0.97 (0.92, 1.01)	0.94 (0.90, 0.99)
SFA from cheese	2.15	1.95 en%	0.96 (0.92, 1.01)	0.89 (0.85, 0.94)	0.96 (0.92, 1.01)	0.91 (0.86, 0.97)
SFA from milk and milk products	2.14	1.45 en%	1.04 (0.99, 1.09)	0.96 (0.92, 1.01)	0.99 (0.95, 1.04)	0.92 (0.86, 0.97)
SFA from meat	2.33	1.44 en%	1.19 (1.14, 1.24)	1.20 (1.15, 1.25)	1.07 (1.02, 1.12)	1.00 (0.95, 1.06)
SFA from cakes	0.75	0.83 en%	0.99 (0.95, 1.04)	0.86 (0.82, 0.91)	0.97 (0.93, 1.02)	0.96 (0.91, 1.02)
SFA from snacks	0.28	0.40 en%	0.80 (0.76, 0.84)	1.10 (1.05, 1.16)	1.03 (0.98, 1.09)	1.03 (0.97, 1.10)
SFA from hard and solid fats	0.95	1.25 en%	1.12 (1.07, 1.17)	1.08 (1.03, 1.12)	0.99 (0.95, 1.03)	0.97 (0.91, 1.02)
SFA from soft and liquid fats	0.54	0.50 en%	1.07 (1.02, 1.12)	1.04 (1.00, 1.09)	1.01 (0.97, 1.06)	0.99 (0.95, 1.04)
SFA from other sources	2.35	1.06 en%	0.79 (0.75, 0.84)	0.99 (0.94, 1.05)	0.96 (0.91, 1.01)	0.94 (0.88, 1.01)

[1] Obtained from Cox proportional hazards regression models

[2] Crude model

[3] Adjustment for age

[4] Additional adjustment for sex, total energy, BMI, waist circumference, education level, physical activity level, smoking status, and alcohol intake (categories)

[5] Additional adjustment for the sum of all other SFA, trans-fat, animal protein, vegetable protein, and energy adjusted intakes of vitamin c, fibre and cholesterol.

Sensitivity analyses

We observed no significant effect modification by sex (*P* values all between 0.2 and 0.9), except for SFA from cheese (*P* = 0.03). Stratification for sex in the model for SFA from cheese showed that the lowered risk was stronger in women (HR: 0.89, 95% CI: 0.83, 0.96) than in men (HR 0.97, 95% CI: 0.88, 1.07). Our results did not materially change after including the baseline total cholesterol: HDL cholesterol ratio or systolic blood pressure in the models (**Supplemental Tables 1 and 2**), excluding the first 2 y of follow-up (**Supplemental Table 3**), or analyzing the first 5 y of follow-up only (**Supplemental Table 4**), or analyzing nonfatal CHD events only (data not shown). The results for the analysis with age as the underlying time axis did not differ from the analysis with follow-up time as time axis (e.g., HR per 5en% of total SFA intake: 0.83, 95% CI: 0.74, 0.93). In addition, distinguishing between *n*-3 PUFAs (mean intake: 1.2 ±0.5 g/d) and *n*-6 PUFAs (mean intake: 10.7 ±4.9 g/d) as a replacement for SFA did not yield different results (data not shown).

Discussion

In this prospective cohort study in 35,597 Dutch men and women, a higher intake of total SFA was associated with a lower risk of incident CHD. This association did not depend on the substituting macronutrient but rather on the chain length and food source of SFAs, with slightly lower CHD risks for higher intakes of the sum of butyric through capric acid, myristic acid, the sum of pentadecylic and margaric acids, and SFA from dairy sources (milk and milk products, cheese, and butter).

Strengths of this study include the prospective study design, long follow-up period, large number of CHD events, and robustness of findings in sensitivity analyses. Although we adjusted for a wide range of potential confounders, we cannot exclude that residual confounding partly explains our findings. For instance, our study lacks information on the initiation of cholesterol lowering therapy during follow-up. It is conceivable that individuals with high SFA intake have high cholesterol concentrations [(1)] and will become eligible for cholesterol-lowering therapy during follow-up. In ~15% of the EPIC-NL cohort that is examined every 5 y, it was indeed observed that cholesterol-lowering therapy increased from <2% at baseline to >10% at 10 y follow-up [(29)]. Cholesterol-lowering therapy is a confounder, and would reduce CHD risk substantially [(30)], which may at least partially explain the observed reduced CHD risk associated with SFA intake. Another limitation is that SFA intake was measured with use of an FFQ, a tool that relies on self-reporting. However, a validation study [(20)] showed reasonable to good reproducibility and relative validity for SFA intake .

Three recent meta-analyses, including the study results of a total of 22 observational cohorts, observed no association between SFA intake and CHD incidence [(10-12)]. We also did not observe an increased CHD risk with higher total SFA intake in this cohort found instead a reduced risk. Although this differs from the meta-analyses, it has been reported previously. In the MESA cohort, an even lower CHD risk was observed ($HR_{5en\%}$: 0.73, 95% CI:

0.56, 0.96) [15]. Neither the MESA cohort study nor the meta-analyses [10-12], considered the macronutrients that substituted SFA, which may affect the association between SFA and CHD [31]. Our results for the substitution of SFA with *cis*-MUFA [13], total carbohydrates [13], and carbohydrates differing in GI [25] are essentially in line with most previous cohort studies [13, 25], although a recent updated analysis in the NHS and Health Professionals Follow Up Study showed lower CHD risks for the replacement of SFA with MUFA and with carbohydrates from whole grains [32]. A meta-analysis of trials showed no significant association between replacing SFA with MUFA, carbohydrates or protein and CHD events; however, these results were based on a limited number of studies and events with high heterogeneity [33].To our knowledge, no previous cohort studies have investigated the association between substitution of SFA with animal protein and CHD risk. The inverse association between the substitution of SFA with PUFA and CHD risk in our study conflicts with a consistent body of evidence from previous trials that investigated the effects on blood lipids [1] or CHD outcomes [33, 34], as well as evidence from cohort studies [13, 32, 35]. All these previous studies showed inverse associations between the substitution of SFA with PUFA and CHD risk, but one study did not show these associations [36]. We are not certain what causes the discrepancy between our results and those from the other studies. Perhaps our analyses were limited by the small SFA intake range (IQR: 13.2-16.6 en%) at a high mean level of intake (15.0 en%). In populations with SFA intakes covering a wider range, the association may be different than in our study. To illustrate this point, the range of SFA intake in the pooled cohort study [13] was wider (with 80% central ranges between 6 en% and 26.9 en%). Furthermore, because the range of PUFA intake was small (IQR: 5.6-7.9 en%), this may have limited the possibility to model the substitution of these 2 macronutrients. Another explanation for our findings may be that certain PUFA food sources consumed in our study population also contained *trans*-fat at that time. For instance, the most important PUFA source, margarines (17%) (**Supplemental Figure 7**), also provided 9% of the *trans*-fat intake in our population (**Supplemental Figure 8**). Residual confounding caused by underestimating *trans*-fat intakes may be present in the observed associations between the substitution of SFA with PUFA or MUFA, because of the gradual but drastic reduction of the amount of *trans*-fats in margarines and spreads between 1994 and 1997 [37]. Altogether, the lower CHD risk for higher SFA at the expense of PUFA intake needs to be interpreted with caution.

When we distinguished between chain lengths of SFA, we observed differences in associations with CHD risk. In our study, higher intakes of the short-to medium-chain SFA (sum of butyric through capric acid), myristic acid, and the sum of pentadecylic and margaric acids, which are all mainly derived from dairy sources, were associated with a slightly reduced CHD risk. Intakes of lauric acid (which is also largely derived from dairy, but also from coconut oil) however, as well as the long-chain SFAs palmitic acid and stearic acid, were not associated with CHD risk. In contrast to our findings, a meta-analysis of 60 controlled trials showed that compared to carbohydrates the serum LDL-raising effects of the even-chained SFAs with 12-18 carbons decreased with increasing chain length [1]. To our knowledge, the associations between SFAs differing in carbon chain length and CHD risk were previously

investigated only in the NHS [14] that found no associations with short- to medium-chain SFAs (butyric through capric acid) and moderately increased IHD risk for long-chain SFAs (lauric through stearic acids). This suggests that short- to medium-chain SFA appear to be more beneficial for cardiovascular disease risk than the long-chain SFAs, which is in line with our findings.

The results we observed for SFAs differing in carbon chain length and CHD risk, correspond in part with our results for SFA from food sources. Our results suggest that the inverse association between total SFA and CHD was mainly driven by SFA from dairy sources. To our knowledge, the associations between SFA from food sources and CHD risk were previously investigated in the MESA study [15] only. Our findings for SFA from dairy are in line with the results from MESA, which reported a 29% lower CHD risk per 5 en% (HR per 5en%: 0.71, 95% CI: 0.52, 0.98). The null association between SFA from other sources and CHD in our study is also in line with the results from MESA. On the other hand, MESA observed a nonsignificant increased CHD risk for higher intake of SFA from meat (HR per 5 en%: 1.57, 95% CI: 0.98, 2.51) [15], whereas in our study this association was essentially null. It is unclear whether the association between SFA from dairy and CHD in our study is attributable to the type of SFA or to interactions of SFA with other components in dairy such as calcium, magnesium or potassium, or whether it is caused by residual or unmeasured confounding from specific nutrients in dairy.

Whether the risk differences observed in our study are attributable to the SFA type or its food source or to unmeasured confounding, remains unclear for now and warrants investigation.
To conclude, in this Dutch population with a relatively high SFA intake from dairy sources and modest range in SFA and PUFA intake, we observed a lower CHD risk with a higher intake of SFA that did not depend on the type of substituting macronutrient. The association seems mainly driven by short- to medium-chain SFAs, myristic acid, the sum of pentadecylic and margaric acids, and SFA from dairy sources including butter, cheese and milk and milk products. We cannot exclude confounding by unmeasured initiation of cholesterol-lowering therapy during follow-up. The fact that we did not observe a lower CHD risk for substitution of SFA with PUFA may have been caused by residual confounding by *trans*-fat or by the small range in PUFA intake in this cohort. Further investigation is necessary in other populations with similar as well as different dietary patterns before definitive conclusions can be drawn.

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Supplemental Materials

The following supplemental materials are available online through the website of the American Journal of Clinical Nutrition *.

Supplemental figure 1	Percentages of individual cis-monounsaturated fatty acids to the total cis-MUFA intake.
Supplemental figure 2.	Percentages of n-3 and n-6 polyunsaturated fatty acids intake tot the total PUFA intake.
Supplemental figure 3.	Restricted cubic splines of multivariable HRs of incident ischemic heart disease risk according to the intake of SFA from milk and milk products.
Supplemental figure 4.	Contributors to the intake of saturated fatty acids, differing in carbon chain length.
Supplemental figure 5.	Average intake of cis-monounsaturated fatty acids from the main food sources of total saturated fatty acids.
Supplemetntal figure 6.	Average intake of polyunsaturated fatty acids from the main food sources of total saturated fatty acids.
Supplemental figure 7.	Main contributors to the total intake of polyunsaturated fat
Supplemental figure 8.	Main contributors to the total intake of trans fat
Supplemental table 1.	HRs for the association between total saturated fat, individual SFAs and SFAs from specific food sources and ischemic heart disease, with and without adjustment for total cholesterol: HDL ratio or systolic blood pressure, in two subpopulations

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Supplemental table 2.	HRs for the association between the substitution of different macronutrients for total saturated fat, and incident ischemic heart disease risk, with and without adjustment for total cholesterol: HDL ratio or systolic blood pressure, in two subpopulations
Supplemental table 3.	HRs for the association between the substitution of different macronutrients for total saturated fat, and incident ischemic heart disease risk after exclusion of the first two years of follow-up
Supplemental table 4.	HRs for the association between the substitution of different macronutrients for total saturated fat, and incident ischemic heart disease risk during the first 5 follow-up years only.

* URL: http://ajcn.nutrition.org/content/103/2/356/suppl/DCSupplemental

3.1

Chapter 3.2

The Rotterdam Study

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Abstract

Objective: We assessed whether the association between dietary saturated fatty acids (SFA) and incident coronary heart disease (CHD) depends on the food source, the carbon chain length of SFA, and the substituting macronutrient.

Approach and results: From the Rotterdam Study, 4722 men and women (≥55 years) were included. Baseline (1990-1993) SFA intake was assessed using a validated food frequency questionnaire. CHD (nonfatal myocardial infarction and fatal CHD) was ascertained by medical records. Using multivariable Cox regression analysis, we calculated CHD risks for higher intakes of total SFA, SFA from specific food sources, SFA differing in carbon chain length, and substituting other macronutrients instead of SFA.

During a median follow-up of 16.3 years, 659 CHD events occurred. Total SFA intake was not associated with CHD risk (Hazard Ratio (HR) per 5 en%: 1.13, 95% CI: 0.94, 1.22), and neither was SFA from specific food sources. A higher CHD risk was observed for palmitic acid (16:0) intake (HR_{SD}: 1.26, 95% CI: 1.05, 1.15) but not for SFA with other chain lengths. Except for a higher CHD risk for substitution of SFA with animal protein ($HR_{5en\%}$: 1.24, 95% CI: 1.01, 1.51), substitution with other macronutrients was not associated with CHD.

Conclusion: In this Dutch population, we observed that a higher intake of palmitic acid, which accounts for ~50% of the total SFA intake, was associated with a higher CHD risk, as was substitution of total SFA with animal protein. Nevertheless, we found no association between total SFA intake and CHD risk, which did not differ by food source.

Introduction

The classic hypothesis that dietary saturated fatty acids (SFA) increase low-density lipoprotein (LDL) cholesterol levels and thereby the risk of coronary heart disease (CHD) remains controversial [1]. Three recent meta-analyses, including a total of 22 prospective cohort studies, did not confirm an association between SFA intake and CHD risk [2-4]. However, these studies focused solely on total SFA intake, neglecting other factors that should be considered: the food source of SFA, the differences in carbon chain lengths of SFA, and the substituting macronutrients. To illustrate, the MESA (Multi-Ethnic Study of Atherosclerosis) study observed a significantly lower CHD risk for people with a higher intake of SFA from milk but not from other sources [5]. Furthermore, in the NHS (Nurses' Health Study), associations with CHD differed for intakes of short- to medium-, even-chained SFAs (butyric acid (4:0) to capric acid (10:0)) as compared with longer even-chained SFAs (lauric acid (12:0) to stearic acid (18:0)) [6], showing a significantly higher CHD risk for the latter only. Regarding the substituting macronutrient, cohort studies [7-9] and trials [10, 11] generally showed that the isocaloric substitution of SFA with polyunsaturated fatty acids (PUFA) may have beneficial effects on CHD risk [7-11], whereas the substitution of SFA with carbohydrates appears to have no [9] or an adverse association [8] with CHD. For the substitution of SFA with monounsaturated fatty acids (MUFA), the results are inconclusive up to now, showing both protective [9] and adverse [8] associations with CHD risk.

Despite the recommendation in nutrition guidelines to lower dietary SFA, the intake is still high in many countries, including the Netherlands [12]. According to the food consumption surveys [13, 14] ~ 90% of the Dutch population exceeds the recommended upper intake limit of 10 en% of SFA per day, with dairy products providing ~ 30% of the total SFA intake [13, 14]. In contrast to what could be expected, a higher SFA intake was recently associated with a significantly lower CHD risk in a Dutch cohort study, mainly driven by SFA from dairy products and SFA subtypes that are primarily derived from dairy [15]. Thus, the association between SFA and CHD risk may depend on type and source of SFA. Nevertheless, the evidence on the association between intake of individual SFAs and SFA from specific food sources is limited. Elucidating the role of different sources of SFA is important for shaping future dietary guidelines, because many recent dietary guidelines are food-based. Therefore, we aimed to investigate the association between intake of total SFA, SFA from specific food sources, and SFAs differing in carbon chain lengths and CHD. Furthermore, we investigated the association between SFA and CHD, taking into account substitution with other macronutrients. For these purposes, we used data from the Rotterdam Study, a Dutch cohort consisting of middle-aged and elderly men and women.

Materials and methods

Study population

This study was embedded in the Rotterdam Study I (RS-I). Details on the objectives and design have been described previously [16]. In brief, starting in 1990 all men and women 55 years and older living in the Ommoord district of Rotterdam, the Netherlands, were invited to participate in the study. A total of 7983 subjects (78%) agreed to participate and were included. Between 1990 and 1993, baseline data were collected. First, a trained research assistant interviewed the subjects at home. Next, all subjects were invited for a physical examination and a dietary assessment at the research center. All subjects gave written informed consent. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports.

Dietary intake assessment

Baseline dietary intake of 170 food items was assessed by a trained dietician using a validated, semi-quantitative food frequency questionnaire (FFQ). The questionnaire was adapted for the use in the elderly and validated against multiple food records in a sample of the Rotterdam Study (n = 80)[17]. After adjustment for age, sex and total energy intake, Pearson correlation coefficients for total fat, MUFA, PUFA, SFA, linoleic acid, and cholesterol ranged from 0.39 (for SFA) to 0.52 (for linoleic acid and PUFA).

Dietary intake of total and individual SFAs, as well as other nutrients, was calculated with use of the Dutch food composition table of 1998 (digital update, available on request from the National Institute for Public Health and the Environment (RIVM)).

Intakes of SFA and other macronutrients were converted to kilocalories, by multiplying their intake in grams by 9 kilocalories (kcal) for fats and 4 kcal for carbohydrates and protein. These values were expressed as a percentage of the total intake of kcal consumed (en%). Other dietary intake variables were adjusted for total energy intake by means of the nutrient residual method[18].

For the present analysis, intakes of butyric acid, caproic acid (6:0), caprylic acid (8:0), and capric acid, as well as intakes of pentadylic acid (15:0) and margaric acid (17:0) were summed, because of their low intakes. For the calculation of SFA intake by food source, items were clustered into the following mutually exclusive food groups: butter, cheese, milk, meat, cakes and cookies, hard and solid fats, soft and liquid fats and a rest group defined as other sources (**Supplemental Table S1)**. These groups were based on the predefined groups in the Dutch food composition table 1996 (NEVO) [19].

Outcome assessment

In the present study, incident CHD included the following underlying outcomes: fatal and nonfatal myocardial infarction (MI) or definite coronary mortality [20], and follow-up until January 2011 was used. Information on the definition and collection of cardiac outcomes in the Rotterdam Study has been described in detail elsewhere [20]. In brief, information on vital status and the date of death were collected through digital linkage with municipality records and digital files from GPs. Based on information from medical records, a study physician independently determined the cause of death, which was subsequently validated by a medical specialist, whose judgment was considered decisive. Classification of fatal CHD was performed according to the definitions from widely endorsed international guidelines [20, 21]. Information on incident CHD was collected by automated digital linkage of the study database to digital files from GPs in the study area and coded using the ICPC coding system. Additional information was obtained from hospitals if the automated follow-up system or the medical records contained insufficient information. Within the Rotterdam Study, a validation study for evaluating the clinical follow-up event registration of incident MI was performed [20]. This validation study (n = 100) showed that the clinical follow-up system of the Rotterdam Study had a 98% case finding of hospitalized MIs.

Assessment of other variables

Most covariates were assessed at baseline by home interview (1990 - 1993). Body weight, height, waist circumference, and blood pressure were assessed at the study center. Body mass index (BMI) was calculated by dividing body weight (in kg) by the squared value of body height (in m). Blood pressure was measured twice after a five-minute rest, using a random-zero sphygmomanometer at the right upper arm, while participants were in sitting position. The mean of these two measurements was used for analysis. Hypertension was defined as systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg or blood pressure lowering medication with indication hypertension [22]. Smoking was defined as current, former or never. Highest education and net household income were added to the model as proxy for socioeconomic status (SES). Education was coded as low (primary, primary plus higher not completed and lower vocational education), middle (lower secondary, intermediate vocational and general secondary education) or high (higher vocational education & university). Household income was coded low (<1900), middle (1900-3500) or high (>3500) in Dutch Guilders (equivalent to ≈ 1588 euro) per month.

Serum total and HDL cholesterol were determined by an automatic enzymatic procedure in non-fasting blood samples (Monotest Cholesterol kit , Boehringer Mannheim Systems) [23]. The use of serum lipid reducing agents and antihypertensive drugs was registered during the home interview by trained research assistants [24]. Physical activity was assessed at the 3rd visit (between 1997 and 1999), using the Zutphen Study Physical Activity Questionnaire. Total time spend on physical activity was calculated by the sum of minutes per week for each type of activity [25-27].

Population for analysis

Diet was not assessed in subjects who were institutionalized and therefore could not visit the research center for a dietary interview (n = 1462). Dietary data were available for 5435 (68%) of the 7983 subjects. Dietary data was missing for those included during the pilot phase of the study (between 1989 and 1990, institutionalized subjects, and subjects with dietary data deemed unreliable (i.e. when subjects had difficulties with recall of their food intake, or when dementia was suspected). Of the 5435 subjects with complete dietary data we excluded 39 subjects who signed no informed consent for collection of follow-up data, or who were lost to follow up, as well as 674 subjects with prevalent CVD. A total of 4722 subjects were left for the present analysis.

Data analysis

Study population characteristics

We calculated Pearson's correlation coefficients between total SFA and SFA from food sources or types of SFA (all in en%). Baseline characteristics of the study population were calculated across quintiles of the total SFA intake distribution (in en%), and expressed as means with standard deviations (SD), medians with interquartile ranges (IQR), or percentages. We used the multiple imputation procedure in SPSS to deal with missing data on covariates (**Supplemental Table S2)**. Ten imputation datasets were constructed.

Total SFA and CHD incidence

We calculated Hazard Ratios (HRs) with 95% confidence intervals (CI) for the association between total SFA intake per 5 en% and incident CHD, by using Cox' proportional hazards regression models. The first model was adjusted for age. The second model was additionally adjusted for sex, total energy intake, BMI, waist circumference, income level (categories), education level (categories), physical activity, smoking status (categories), and alcohol intake (categories). The third and final model was additionally adjusted for intakes of trans fat, animal protein, vegetable protein (all in en%), and energy adjusted intakes of fiber, vitamin C, and cholesterol. Potential confounders included known risk factors from existing literature, and all co-variables that were associated with SFA intake as well as CHD risk in our study population.

SFA from specific food sources or differing in carbon chain length

The associations between intakes of SFA from specific food sources or SFA differing in carbon chain length and CHD risk were calculated separately, and expressed per standard deviation (SD) of intake. We adjusted the HRs for potential confounders by using the same three Cox models as used for the evaluation of total SFA intake, along with additional adjustment for the sum of all other consumed SFA in the third model. A high number of subjects reported not to consume butter (67,2%). Therefore we calculated HRs per SD of SFA

intake from butter in consumers only (n = 1551).

Substitution of SFA with other macronutrients and CHD incidence

To estimate the CHD risk with an increased intake of energy from PUFA, *cis*-MUFA, vegetable protein, animal protein or carbohydrates, at the expense of an equal amount of energy from SFA, the Cox' models were converted into substitution models [18]. This was done as follows. All macronutrients, except alcohol, were included into all Cox models separately (per 5 en%) as well as summed to represent total energy intake. By excluding SFA intake, the HR for each macronutrient represented the CHD risk difference for a 5 en% higher intake of that particular macronutrient and a concomitant lower intake of SFA. The HRs were adjusted for the same confounders as listed above. To identify whether the association between substitution of SFA with carbohydrates and CHD depended on the quality of the carbohydrates, we stratified the models for tertiles of the GI intake distribution, as was done previously [15, 28]. In addition, we built a Cox model in which energy from SFA was substituted with energy from carbohydrates from whole grains and carbohydrates from refined starch and sugars (**Supplemental Table S3**).

Additional analyses

As sensitivity analysis we excluded the first two years of follow up to limit the possibility of reverse causation. The present associations are based on baseline dietary measurements. The relationship between these baseline measures and CHD events may have reduced after longer follow up time. Therefore, we repeated the analyses in the first eight years of follow up, by censoring all subjects who, during that time period, were not lost to follow-up and did not suffer from a CHD event. We additionally adjusted model 3 for total: HDL cholesterol ratio and systolic blood pressure, to see whether these factors were possible intermediates in the association between SFA and CHD. We tested whether associations were non-linear, by adding quadratic terms of SFA intake variables to the final third model. In addition, we calculated HRs across quartiles of the SFA intake distributions, using the lowest quartile as the reference.

All analyses were done using SAS version 9.3 (SAS Institute, Cary, North Carolina, 191 USA) or SPSS version 23.0 (IBM, Armonk, NY, USA). Two-sided p-values < 0.05 were considered statistically significant. Cox proportional hazards assumptions were investigated by visual inspection of log-log plots, showing no deviations.

Results

Population characteristics

Subjects in the higher quintiles of SFA intake (in en%) were older, more often women and smoker, less educated, and less physically active (**Table 1**). Furthermore, these participants had higher intakes of PUFA, *cis*-MUFA, *trans*-fat, and cholesterol and lower intakes of

Table 1. Baseline characteristics* of 4722 subjects of the Rotterdam Study across quintiles of total saturated fat intake (in en%).

	Q1	Q2	Q3	Q4	Q5
	11.2 (± 1.4)	13.9 (± 0.5)	15.6 (± 0.5)	17.5 (± 0.6)	20.8 (± 1.9)
Subjects (n)	944	945	944	945	944
Age (years)	65.2 (± 7.2)	66.3 (± 7.3)	66.2 (± 7.4)	67.2 (± 7.8)	68.4 (± 8.0)
Male (%)	38	40	41	38	33
High education level (%)	10	10	9	8	7
BMI (kg/m2)	26.3 (± 3.6)	26.3 (± 3.6)	26 (± 3.6)	26.3 (± 3.7)	26.4 (± 3.8)
Waist circumference (cm)	89.2 (± 11.0)	90.2 (± 10.7)	89 (± 11.0)	89.8 (± 11.1)	90.3 (± 11.3)
Physical activity (minutes/week)†	2680 (± 1118)	2625 (± 1116)	2654 (± 1082)	2533 (± 1144)	2423 (± 1161)
Current smokers (%)	22	20	24	24	27
Hypertension (%)	55	54	53	50	55
Systolic blood pressure (mmHg)	138.5 (± 21.0)	138.7 (± 22.0)	138.5 (± 22.7)	137.4 (± 20.7)	139.5 (± 22.0)
Diastolic blood pressure (mmHg)	74.5 (± 11.0)	74.4 (± 10.8)	74.4 (± 11.2)	72.8 (± 11.0)	73.4 (± 11.4)
Total cholesterol (mmol/L)	6.7 (± 1.2)	6.7 (± 1.1)	6.6 (± 1.2)	6.7 (± 1.2)	6.8 (± 1.2)
HDL cholesterol (mmol/L)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)
Energy intake (kcal)	1834 (± 470)	1960 (± 488)	2001 (± 490)	2024 (± 493)	2081 (± 552)
Saturated fat (g/d)	23.0 (± 6.9)	30.3 (± 7.7)	34.8 (± 8.7)	39.4 (± 9.8)	48.2 (± 13.5)
SFA by chain length (en%):					
Butyric (4:0) - capric (10:0) acid	0.5 (± 0.2)	0.7 (± 0.2)	0.8 (± 0.2)	0.8 (± 0.2)	1.0 (± 0.3)
Lauric acid (12:0)	0.6 (± 0.3)	0.7 (± 0.3)	0.8 (± 0.3)	0.9 (± 0.4)	1.0 (± 0.4)
Myristic acid (14:0)	1.1 (± 0.2)	1.4 (± 0.2)	1.6 (± 0.3)	1.9 (± 0.3)	2.4 (± 0.5)
Palmitic acid (16:0)	5.2 (± 0.7)	6.2 (± 0.4)	6.9 (± 0.5)	7.6 (± 0.5)	8.9 (± 0.9)
Pentacecylic (15:0) & margaric (17:0) acid	0.3 (± 0.1)	0.4 (± 0.1)	0.4 (± 0.1)	0.5 (± 0.1)	0.5 (± 0.1)

Stearic acid (18:0)	2.7 (± 0.5)	3.2 (± 0.4)	3.5 (± 0.4)	3.8 (± 0.5)	4.3 (± 0.5)
SFA by food source (% of total SFA)‡:					
butter	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 - 0.4)	0.0 (0.0 - 15.7)	17.7 (0.0 - 28.6)
cheese	18.6 (11.2 - 26.9)	20.0 (13.1 - 27.4)	20.1 (13.6 - 28.1)	18.6 (12.7 - 26.0)	16.6 (11.2 - 22.9)
milk	11.6 (6.3 - 18.0)	11.5 (6.7 - 17.2)	11.3 (6.5 - 17.5)	11.6 (7.0 - 17.5)	10.9 (6.1 - 17.7)
meat	19.8 (13.6 -26.9)	17.7 (13.0 - 23.6)	17.4 (12.4 - 22.9)	16.0 (10.8 - 21.5)	13.3 (9.1 - 18.5)
cakes	7.9 (2.9 - 14.5)	9.7 (4.6 - 15.1)	8.6 (4.1 - 14.6)	8.4 (3.8 - 15.4)	7.4 (3.3 - 13.2)
hard, solid fats	5.9 (1.1 - 11.9)	8.9 (3.8 - 15.4)	10 (4.7 - 18.3)	12.1 (4.8 - 21.3)	12.3 (5.2 - 22.9)
soft, liquid fats	9.5 (5.3 - 12.8)	7.5 (4.1 - 10.2)	6.1 (1.9 - 8.6)	4.0 (0.3 - 6.5)	0.4 (0.0 - 3.2)
other sources	16.8 (12.5 - 22.1)	14.6 (11.0 - 19.0)	12.9 (9.6 - 17.9)	11.7 (8.4 - 15.9)	8.9 (6.2 - 12.9)
PUFA (en%)	7.5 (± 2.8)	7.5 (± 2.7)	7.3 (± 2.5)	6.9 (± 2.6)	5.9 (± 2.2)
cis-MUFA (en%)	7.8 (± 4.3)	8.3 (± 4.1)	8.8 (± 4.2)	8.7 (± 4.0)	8.6 (± 3.9)
Trans fat (en%)	1.8 (± 0.5)	2.2 (± 0.6)	2.5 (± 0.7)	2.8 (± 0.7)	3.5 (± 1.0)
Animal protein (en%)	11.2 (± 3.5)	10.9 (± 2.9)	11.0 (± 2.8)	11.0 (± 2.7)	10.9 (± 2.6)
Vegetable protein (en%)	6.2 (± 1.5)	5.8 (± 1.1)	5.6 (± 1.0)	5.4 (± 0.9)	5.0 (± 0.9)
Carbohydrates (en%)	47.7 (± 7.5)	45.2 (± 6.2)	43.3 (± 6.0)	41.8 (± 5.6)	39.0 (± 5.6)
Glycemic Index	58.4 (± 3.8)	58.8 (± 3.4)	58.9 (± 3.4)	58.9 (± 3.5)	59.0 (± 3.6)
Alcohol (g/d)	5.3 (0.2 - 20.9)	4.0 (0.3 - 17.3)	3.6 (0.3 - 14.4)	3.0 (0.2 - 13.4)	2.5 (0.1 - 10.9)
Cholesterol (mg/d)	185 (± 53)	211 (± 49)	229 (± 49)	246 (± 52)	281 (± 59)
Fibre (g/d)	28.4 (± 6.7)	26.9 (± 5.9)	25.8 (± 5.3)	24.6 (± 4.8)	23.2 (± 4.8)
Vitamin C (mg/d)	142 (± 59)	132 (± 53)	125 (± 52)	120 (± 46)	113 (± 45)

* Presented as means with standard deviations or in percentages, unless stated otherwise

† Physical activity was measured at the 3rd visit between 1997 and 1999, using the Zutphen Study Physical Activity Questionnaire including questions on walking, cycling, gardening, diverse sports, hobbies and on housekeeping.

‡ Presented as medians with interquartile ranges

vegetable protein, carbohydrates, fibre and vitamin C. With the exception of lauric acid (12:0) (r <0.41), the intakes of the individual SFAs were highly correlated (**Supplemental Table S4**). The highest correlations were observed for myristic acid (14:0) and the sum of pentadecylic (15:0) and margaric (17:0) acid (r = 0.88), for palmitic acid and stearic acid (r = 0.84), and for myristic and palmitic acid (r = 0.80). The top 5 food sources of total SFA intake were cheese (20.0%), meat (17.8%), milk (13.1%), solid fats (12.3%) and cakes and cookies (10.1%) (**Figure 1**). Approximately 74% of total SFA intake consisted of the long-chain SFAs palmitic acid and stearic acid (**Figure 2**). The top food source of palmitic and stearic acid was meat, and the top food sources of all other SFA were milk and cheese (**Supplemental Figure S1**).

SFA intake and CHD incidence

During a median follow up of 16.3 years (IQR: 10-18 years), 569 CHD events occurred. **Table 2** shows the HRs for the association between total SFA per 5 en% of intake and incident CHD risk. After adjustment for age, sex, and lifestyle and dietary risk factors (model 3), intake of total SFA was not significantly associated with CHD (HR per 5 en%: 1.13, 95% CI: 0.94, 1.36).

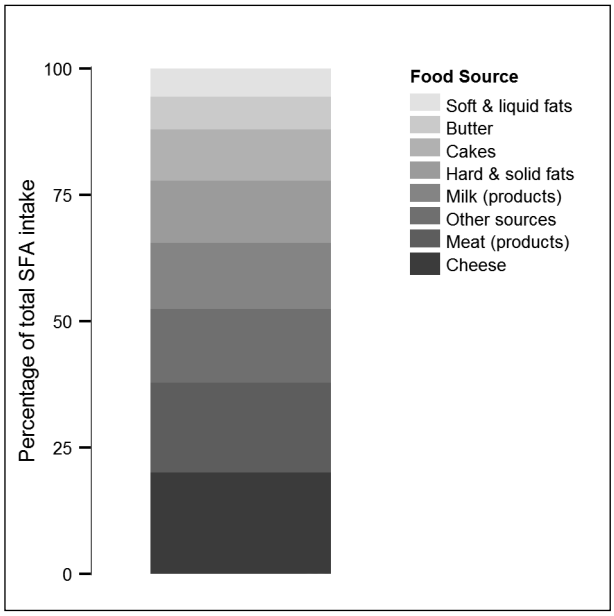

Figure 1. Contributions of food groups, in percentages, to the total saturated fat intake

Intake of SFA from specific food sources or differing in carbon chain length and CHD incidence

Table 3 shows no significant associations between SFA from specific food groups and risk of CHD after multivariable adjustment (model 3). The HRs ranged from 1.03 to 1.08 (p

>0.46), except for the HR for SFA from meat, which was 1.09 per SD of intake (95% CI: 0.99, 1.20, *P* value = 0.10). With respect to the individual SFAs differing in carbon chain length (**Table 2**), no statistically significant associations with CHD risk were observed (model 3), except for palmitic acid, which was associated with a higher CHD risk of 26% (95% CI: 1.05, 1.52) per SD of additional intake. For stearic acid we observed a nonsignificant HR of 1.11 (95% CI: 0.98 ,1.25) per SD.

Substitution of SFA intake with other macronutrients and CHD incidence

Table 4 presents the estimated HRs for 5% lower intake of energy from SFA and a concomitant higher intake of energy from other macronutrients. No statistically significant associations with incident CHD were observed for substitution of SFA with carbohydrates, *cis*-MUFA, and PUFA. The results for substitution of SFA with carbohydrates, while taking into account the GI of the diet, did not differ from the results for substitution with total carbohydrates. Neither did substitution with carbohydrates from whole grains ($HR_{5en\%}$: 0.78, 95% CI: 0.53, 1.15) or with carbohydrates from refined starch and sugars ($HR_{5en\%}$: 0.85, 95% CI: 0.60,1.20). Distinguishing between animal and vegetable protein showed that substitution of SFA with animal protein was significantly associated with an increased CHD risk ($HR_{5en\%}$: 1.24, 95% CI:1.01, 1.51), whereas substitution with vegetable protein was not ($HR_{5en\%}$: 0.88, 95% CI: 0.50, 1.53).

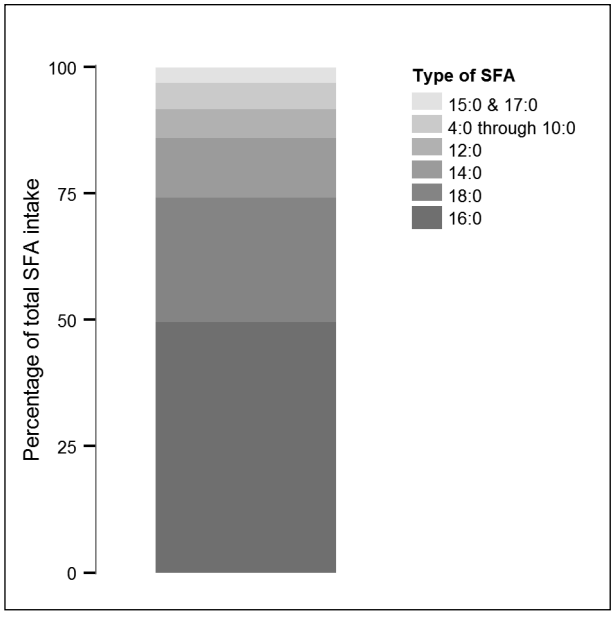

Figure 2. Contributions of individual saturated fatty acids, in percentages, to the total saturated fat intake

Additional analyses

Exclusion of the first 2 years of follow-up did not alter the results (data not shown). However, in repeat analysis within the first 8 years, during which 222 CHD events occurred, we observed a significantly higher CHD risk of 16% for SFA from meat (HR per SD = 1.16,

Table 2. Multivariable HR with 95% CI* for the associations of intakes of total saturated fatty acids (SFA; per 5en%) and SFA differing in carbon chain lengths (per SD) with incident CHD risk.

	HR expressed per	Median intake (en%)	HR (95% CI) Model 1†	HR (95% CI) Model 2‡	HR (95% CI) Model 3§
Total SFA	5.0 en%	15.7	1.10 (0.97, 1.23)	1.10 (0.97, 1.24)	1.13 (0.94, 1.36)
Sum butyric (4:0) through capric (10:0) acid	0.3 en%	0.7	1.00 (0.93, 1.09)	1.05 (0.97, 1.14)	0.99 (0.89, 1.09)‖
Lauric acid (12:0)	0.4 en%	0.7	0.98 (0.90, 1.07)	1.02 (0.94, 1.11)	1.01 (0.92, 1.10)‖
Myristic acid (14:0)	0.6 en%	1.6	1.04 (0.95, 1.12)	1.05 (0.97, 1.14)	0.96 (0.74, 1.24)‖
Palmitic acid (16:0)	1.4 en%	6.9	1.11 (1.02, 1.20)	1.09 (1.00, 1.18)	1.26 (1.05, 1.52)‖
Sum pentacecylic (15:0) and margaric (17:0) acid	0.1 en%	0.4	1.06 (0.98, 1.15)	1.07 (0.99, 1.16)	1.00 (0.87, 1.15)‖
Stearic acid (18:0)	0.7 en%	3.4	1.10 (1.02, 1.20)	1.09 (1.00, 1.18)	1.11 (0.98, 1.25)‖

* Obtained from Cox' proportional hazards regression models

† Adjustment for age.

‡ Additional adjustment for sex, total energy, BMI, waist circumference, education level (categories), income level, physical activity level, smoking status, and alcohol intake.

§ Additional adjustment for intakes of trans fat, vegetable protein, animal protein (all in en%) and energy adjusted intakes of cholesterol, fibre, vitamin c.

‖ Additional adjustment for the sum of all other SFA.

Table 3. Multivariable HR with 95% CI* for the associations between the intake of saturated fatty acids (SFA) from its main food sources per SD of intake with incident CHD risk.

	HR expressed per	Median intake (en%)	HR (95% CI) Model 1†	HR (95% CI) Model 2‡	HR (95% CI) Model 3§
SFA from butter‖	2.8 en%	3.6	0.98 (0.85, 1.14)	0.98 (0.85, 1.14)	1.08 (0.88, 1.31)
SFA from cheese	1.9 en%	2.9	1.01 (0.93, 1.09)	1.02 (0.94, 1.11)	1.03 (0.94, 1.13)
SFA from milk	1.5 en%	1.8	1.05 (0.97, 1.14)	1.06 (0.98, 1.15)	1.04 (0.94, 1.14)
SFA from meat	1.4 en%	2.6	1.15 (1.06, 1.24)	1.08 (0.99, 1.18)	1.09 (0.99, 1.20)
SFA from cakes	1.4 en%	1.3	0.92 (0.84, 1.00)	0.98 (0.90, 1.07)	1.02 (0.92, 1.13)
SFA from snacks	0.1 en%	0.0	0.95 (0.86, 1.05)	0.94 (0.85, 1.04)	0.95 (0.86, 1.05)
SFA from hard and solid fats	2.0 en%	1.4	1.06 (0.97, 1.14)	1.08 (0.92, 1.08)	1.05 (0.92, 1.19)
SFA from soft and liquid fats	0.6 en%	0.8	1.01 (0.93, 1.10)	1.01 (0.93, 1.10)	1.07 (0.97, 1.18)
SFA from other sources	1.0 en%	2.0	0.99 (0.91, 1.07)	1.00 (0.92, 1.08)	1.05 (0.96, 1.15)

* Obtained from Cox' proportional hazards regression models
† Adjustment for age.
‡ Additional adjustment for sex, total energy intake, BMI, waist circumference, education level, income level, physical activity, smoking status, and alcohol intake.
§ Additional adjustment for intakes of trans fat, animal protein, vegetable protein, energy adjusted intakes of vitamin c, fibre, and cholesterol, and for the sum of all other SFA.
‖ In butter consumers only (n=1551; 182 CHD cases)

Table 4. Multivariable HR with 95% CI * for the association between the consumption of 5% energy from different macronutrients at the expense of 5% energy from saturated fatty acids (SFA), while keeping total energy intake constant, and incident CHD risk.

5 en% decrease of	5 en% increase of	HR (95% CI). Model 1†	HR (95% CI). Model 2‡	HR (95% CI). Model 3§
SFA	Carbohydrates	0.92 (0.85, 1.00)	0.96 (0.86, 1.07)	0.90 (0.80, 1.02)
SFA	Low GI carbohydrates‖	0.97 (0.84, 1.12)	1.02 (0.84, 1.23)	0.96 (0.78, 1.19)
SFA	Medium GI carbohydrates‖	0.91 (0.79, 1.05)	0.92 (0.76, 1.11)	0.84 (0.68, 1.05)
SFA	High GI carbohydrates‖	0.96 (0.84, 1.08)	0.95 (0.80, 1.14)	0.94 (0.77, 1.14)
SFA	*cis*-MUFA	1.04 (0.94, 1.16)	1.08 (0.97, 1.20)	1.09 (0.98, 1.22)
SFA	PUFA	0.98 (0.81, 1.19)	0.99 (0.80, 1.23)	0.90 (0.71, 1.15)
SFA	Protein	1.16 (0.99, 1.37)	1.16 (0.96, 1.40)	1.22 (1.00, 1.49)
SFA	Animal protein	1.21 (1.02, 1.43)	1.21 (0.99, 1.47)	1.24 (1.01, 1.51)
SFA	Vegetable protein	0.87 (0.57, 1.32)	0.86 (0.56, 1.32)	0.88 (0.50, 1.53)

* Obtained from Cox' proportional hazards regression models

† Includes intakes of total carbohydrates, *cis*-MUFA, PUFA, trans-fat, animal protein and vegetable protein (all expressed per 5 en%), as well as total energy (excluding energy from alcohol intake), and adjustment for age.

‡ Additional adjustment for sex, BMI, waist circumference, education level, income level, physical activity level, smoking status and alcohol intake.

§ Additional adjustment for energy adjusted intakes of cholesterol, fibre, vitamin c.

‖ Number of cases per tertile of GI value distribution: low, 192; medium, 181; high, 196.

Abbreviations: GI, glycemic index; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

95% CI: 1.00, 1.34, $P = 0.045$) (**Supplemental Table S5**). Also, we observed a significantly lower risk of CHD for a higher intake of SFA from cakes and cookies ($HR_{SD} = 0.76$, 95% CI: 0.64, 0.90). Furthermore, CHD risks were higher and statistically significant for intakes of palmitic acid (HR_{SD}: 1.62, 95% CI: 1.23, 2.15) and stearic acid (HR_{SD}: 1.22, 95% CI: 1.01, 1.47) **(Supplemental Table S6)**. Similarly, significantly higher CHD risks were observed for substitution of SFA with *cis-M*UFA ($HR_{5en\%} = 1.36$, 95% CI: 1.14, 1.62), and with animal protein ($HR_{5en\%} = 1.54$, 95% CI: 1.14, 2.07) (**Supplemental Table S7)**. Additional adjustment for total cholesterol: HDL cholesterol ratio and systolic blood pressure did not change the HRs **(Supplemental Tables S8 through S10)**. Analyses including quadratic terms of SFA intake gave us no indication to suspect significant nonlinear associations between SFA and CHD (all $P > 0.3$). Moreover, analyses across quartiles of SFA intake confirmed our findings for continuously expressed SFA intake (data not shown).

Discussion

In this prospective cohort study, total SFA was not associated with incident CHD risk, and differentiation of SFA intake according to food sources had no conclusive effect on the association. Higher intake of palmitic acid, which contributes ~ 50% of the total SFA intake, was associated with a higher risk of CHD, whereas SFAs with other carbon chain lengths were not. Aside from the significantly higher CHD risk for substitution of SFA with animal protein, substitution with other macronutrients was not associated with CHD.

A recent study estimated that 9.5% of annually occurring CHD deaths in the Netherlands were attributable to a nonoptimal SFA intake (>10 en% per day) [29]. In this study, we observed no association between total dietary SFA and incident CHD, which is in line with the results of 3 recent meta-analyses [2-4]. However, when we separated SFA based on carbon chain length, we found a harmful association with palmitic acid, the predominant type of SFA in the diet. This is in line with the results from the NHS [6], but differs from those of the EPIC-NL (European Investigation into Cancer and Nutrition-Netherlands) cohort [15], in which palmitic acid was not associated with CHD, but a significant inverse association was observed for the short-to medium-chained SFAs [15]. Inverse associations were also observed before between circulating very long chain SFAs (with ≥20 carbons) and CHD risk[30]. However, we cannot directly compare these latter findings to the findings of our study, because very long-chain SFAs are primarily synthesized in vivo from very long-chain unsaturated fatty acids. Therefore these very long-chain SFAs may represent a different type of diet than a diet high in SFAs. We found no associations of SFA according to food source, including dairy products. In contrast, EPIC-NL [15] and the MESA study [5] found inverse associations for SFA from dairy products. Especially the discrepancies with the EPIC-NL cohort are noteworthy. Similar to the Rotterdam Study, the EPIC-NL cohort consists of Dutch men and women, who were recruited in the early 1990s. Other similarities between the 2 cohorts include the use of an FFQ to measure dietary intake and the use of the national nutrient database of 1998 for the calculation of SFA consumption. Furthermore, the intakes of total SFA and SFA subtypes were comparable between the 2 cohorts.

One may speculate what explains the difference in results between our study and EPIC-NL. They may, at least in part, be explained by residual confounding due to differences in socioeconomic factors, which are reflected by the higher age, lower level of education, and higher BMI in our study. Another explanation could be the difference between the FFQs. In both cohorts the top contributor to the mean palmitic acid intake was meat, providing ~ 22% of its intake. In both cohorts, the association between meat derived SFA and CHD was nonsignificant. Nevertheless, although in EPIC-NL the estimated CHD risk for a higher intake of SFA from meat was null, in our study we observed a nonsignificantly higher risk of 9%, which became stronger (16%) and statistically significant for a shorter follow-up time of 8 years. This discrepancy may be explained by the fact that our FFQ included more detail on meat intake (34 single items and an open field to fill in types of cold cuts) than the FFQ used in EPIC-NL (8 aggregated items). This may have led to less nondifferential misclassification based on meat intake in the present study as compared with EPIC-NL, which could explain why we were able to pick up both the association with palmitic acid and the suggestive association with meat-derived SFAs. Moreover, we observed a significantly higher CHD risk for the substitution of SFA with animal protein but not vegetable protein. Together with the results for palmitic acid and for meat-derived SFA, it could be argued that these 3 risk estimates all reflect harmful effects of meat or meat-derived nutrients.

Apart from the results of the EPIC-NL study [15] and of a meta-analysis of secondary prevention trials [31], the evidence from trials [10, 11, 32] and observational studies [7-9] up to now suggests that the substitution of SFA with PUFA could provide cardiovascular benefits. Although we observed no significant association between the substitution of SFA with PUFA and CHD, our effect estimate is in line with these findings. One could question whether we had limited power to detect statistical significance, since some of the previous studies [8, 9] included a 10-fold of the number of events in our study. Our effect estimate for the substitution of SFA with *cis*-MUFA is also in line with the results of a pooled analysis ($HR_{5en\%}$ = 1.19, 95% CI: 1.00, 1.42) [8] but in contrast to the significantly lower CHD risk observed for the substitution of SFA with MUFA in a recent analysis in NHS and HPFS ($HR_{5en\%}$: 0.85, 95% CI: 0.74, 0.97) [9]. Remarkably, we observed a statistically significant and much higher CHD risk for the substitution of SFA with *cis*-MUFA ($HR_{5en\%}$ = 1.36, 95 CI% 1.14, 1.62) in sensitivity analysis within the first 8 years of follow-up and thus including fewer events (n = 222). Possibly, our risk estimate for the substitution of SFA with *cis*-MUFA could be a reflection of the presence of residual confounding by *trans*-fat, which during the early 1990s was still a larger fraction of the total MUFA intake. The nonsignificant association with CHD for the substitution of SFA with carbohydrates in our study did not change when taking into account the carbohydrate quality. These findings fit the existing evidence up to now, which is inconclusive, both on the effects on CHD risk of substitution of SFA with total carbohydrates [8, 15, 28] and on the effects of substitution of SFA with carbohydrates differing in quality [9, 15, 28].

Even though we did not observe conclusive effects of substituting macronutrients, other

than animal protein, we cannot exclude the possibility that other substitution effects exist. The method used for substitution in observational studies like ours, may not be ideal to answer this study question. Although our study design was longitudinal, substitution of macronutrients was statistically modeled using dietary data that were derived at one time point. Thus, the replacement of macronutrients within subjects was not actually occurring, but rather a simultaneous comparison. Subjects with a relatively high intake of SFA, and a relatively low intake of another macronutrient, such as PUFA, were compared to subjects with a relatively high intake of PUFA and a relatively low intake of SFA. In our study, there was no other option, because we only had baseline dietary data available. However, up to now, in the limited number of studies that do have repeated dietary measures, the statistical modeling of substitution is done in a similar, simultaneous fashion [9]. The only difference lies in the fact that they repeat the substitution modelling for each time point, at which the dietary intake was measured. Furthermore, with IQRs of 13.5 to 18.0 en% for SFA and 4.9 to 8.8 en% for PUFA, the variation in the intake range of PUFA across the SFA intake distribution in our study may not have been large enough to model the intended substitution. An experimental study design would be ideal, but is often not feasible, to study substitution effects on clinically manifest disease. Therefore, to gain more insight on this matter, it is necessary to investigate to what extent simultaneous baseline measures of macronutrients in observational studies represent real life substitution and whether they provide suitable data to model the effects of substitution. Such an investigation would be achievable in a large cohort with long follow-up time and repeated dietary measures.

Given the high costs of cardiovascular disease in terms of health-care expenditure and quality of life, prevention strategies are paramount. Dietary factors are important modifiable risk factors that can be targeted for the prevention of cardiovascular diseases. Our study adds to the existing evidence, which together suggest that not total SFA intake, but the type and source may be important in terms of cardiovascular disease risk. However, to be able to facilitate dietary recommendations related to saturated fat intake and related foods, future studies should determine whether dietary interventions to reduce saturated fat from specific food sources or with particular chain lengths could indeed have a beneficial effect on cardiovascular health.

Strengths of our study include the prospective study design, the large size of the study population, and the long follow-up time. One of its limitations is the use of an FFQ, which relies on self-reporting and is therefore subject to measurement error. A validation study of the FFQ showed that compared to the dietary history method its relative validity for the measurement of total SFA was moderate, with a correlation coefficient of 0.39 [17]. In case of subject misclassification, this is likely unrelated to the outcome and thus nondifferential, which generally leads to bias towards the null [33]. This may explain why we did not observe an association between total SFA intake and CHD. Also, the observed associations in our study therefore may actually be stronger. Second, we used baseline dietary measurements only, under the assumption that 2 decades of follow-up is an appropriate exposure time

window for this study. Sensitivity analysis showed that during a shorter follow-up time of the first 8 years only, some of the observed associations became stronger. This could either indicate that the exposure time window was too long or may reflect changes in eating behavior or food composition. Dietary stability was not assessed in our study population, but results from the Dutch food consumption surveys showed changes in the diet of the general Dutch population between 1987 and 2010. In general, a shift was observed from high fat dairy to low fat dairy, and from unprocessed to processed meat[34]. Nevertheless, it was shown by others that the association between dietary fatty acids and cardiovascular disease risk are fairly similar when using a baseline measurements versus dietary assessment close the outcome [35]. The population we used for the present analysis was free of cardiovascular disease at baseline, aged ≥55 years, and non-institutionalized. Also, the major food sources of SFA intake were dairy (33%) and meat (18%). Therefore, the associations we observed may only be generalizable to a relatively healthy population of older age with a similar dietary pattern.

To conclude, the results of this study support the notion that in studies on the association between SFA and CHD, focusing on dietary SFA as a whole is too simplistic. Even though total SFA intake was not related to CHD risk in this prospective cohort study, and we observed no conclusive effect of SFA per food source, a higher intake of palmitic acid only was related to a higher CHD risk. Also, substitution of SFA with animal protein was associated with a higher risk of CHD. Translation of our findings to nutritional guidelines would be too premature, considering that the existing evidence up to now is limited and inconsistent. However, in future studies on SFA and CHD, it is of importance to consider the type of SFA and its substituting macronutrient. Furthermore, we need to investigate to what extent substitution modeling in observational data is feasible.

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Disclosures

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Supplemental Materials

The following supplemental materials are available online through the website of Atherosclerosis, Thrombosis and Vascular Biology *.

Supplemental figure 1	Contributions of different food sources (%) to the intake of individual saturated fatty acids in the Rotterdam Study.
Supplemental table 1	Food items included in each food group contributing to the total saturated fatty acids intake
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* URL: http://atvb.ahajournals.org/content/36/9/2011/tab-supplemental

Chapter 3.3.

EPIC-Norfolk and the Danish Diet, Cancer and Disease Cohort

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Abstract

Background: The effect of individual saturated fatty acids (SFAs) on serum cholesterol levels depends on their carbon-chain length. Whether the association with myocardial infarction (MI) also differs across individual SFAs is unclear.

Objective: To examine the association between the baseline consumption of individual SFAs, differing in chain lengths ranging from 4 through 18 carbons, and the risk of incident MI during follow-up.

Design: We used data from 22132 and 54603 participants from UK EPIC-Norfolk and EPIC-Denmark, respectively. Baseline intakes of SFAs and other nutrients were assessed through validated, country-specific food frequency questionnaires. Cox regression analysis was used to calculate the Hazard Ratios (HR) and 95% confidence intervals (CI) between intake categories of individual SFAs and incident MI risk, for each cohort separately.

Results: Over median follow-up times of 18.8 years in the UK and 13.6 years in Denmark, respectively, 1209 and 2318 MI events occurred. Mean (± SD) total SFA intake was 13.3 (± 3.5) en% in EPIC-Norfolk, and 12.5 (± 2.6) en% in EPIC-Denmark. After multivariable adjustment, the summed intakes of butyric (4:0) through capric acid (10:0) and the intake of myristic acid (14:0) were inversely associated with MI risk. HRs for the upper intake categories versus the lowest of butyric through capric acid intake, and of myristic acid were 0.77 (95% CI: 0.59, 1.01, P_{trend} = 0.04) and 0.73 (95% CI: 0.53, 1.02), P_{trend} = 0.06) respectively in the UK, and 0.74 (95% CI: 0.60, 0.91, P_{trend} = 0.02) and 0.81 (95% CI: 0.63, 1.06, P_{trend} = 0.05) in Denmark. The other individual SFAs were not associated with MI.

Conclusion: The results from the present study suggest that the association between SFA and MI risk depends on the carbon chain-length. Intervention studies are needed to investigate whether the observed differences in observational studies are caused by the SFAs as such or by residual confounding.

Introduction

Limiting the intake of dietary saturated fatty acids (SFAs) is an important component of the dietary recommendations for the prevention of coronary heart disease (CHD) [1-4] . A high intake of SFAs, compared with carbohydrates is associated with higher serum low-density lipoprotein (LDL) cholesterol concentrations [5], which is an established risk factor for CHD. However, the link between SFAs and CHD has been heavily debated for years now, because of inconsistent results from observational cohort studies [6-9].

One of the proposed explanations for the inconsistent findings in meta-analyses of these cohort studies is that the association between SFAs and CHD differs across types of SFAs. SFAs consist of chained hydrocarbons with a carboxyl-group on one end, and a methyl-group on the other. The length of the carbon chains differs across dietary SFAs, ranging from 4 to 24 carbons. The most abundant SFAs in the diet are the long-chain SFAs palmitic acid (16 carbons; 16:0), and stearic acid (18:0), which contribute approximately 75% of the total SFAs intake in the Western diet [10].

A recently updated meta-analysis of 52 controlled trials showed that the effect of dietary SFA on serum cholesterol levels in humans differed depending on the chain-length [5]. Compared with carbohydrates, the intake of lauric acid (12:0), myristic acid (14:0), and palmitic acid all increased serum total-cholesterol and LDL-cholesterol concentrations. However, they also increased HDL-cholesterol concentrations. Lauric acid decreased the ratio of total cholesterol to HDL-cholesterol, and stearic acid had a neutral effect on serum cholesterol [5]. Considering that an increased LDL-cholesterol, and particularly an increased ratio of total cholesterol to HDL-cholesterol [11], are predictors of CHD risk, not all SFAs may be equally harmful. Approaching SFAs as a whole in observational studies may therefore have obscured the association with CHD.

Four previous prospective cohort studies [12-15] indeed observed various associations with CHD when individual SFAs were separated in the analyses, but their findings are inconsistent. In the Nurses' Health Study (NHS) [12, 15] and the Health Professional Follow-up Study (HPFS) [15], SFAs with chain lengths ≥12 carbons were associated with a higher CHD risk. In the Rotterdam study, only palmitic acid was related to an increased risk [14]. In the EPIC-NL cohort, the SFAs with chain lengths ≤10 carbons and the odd-chain SFAs, pentadecylic acid (15:0) and margaric acid (17:0), were related to a lower CHD risk [13].

Addressing the relations of individual SFAs with CHD risk in other populations will yield more insight into if and how individual SFAs relate to CHD risk. Therefore, the objective of this study was to investigate the association between individual SFAs and MI risk in a UK and a Danish cohort.

Methods

Study population

For this study, we used data from EPIC-Norfolk (European Investigation into Cancer and Nutrition-Norfolk cohort) and from the Danish Diet, Cancer, and Health cohort (further referred to as EPIC-Denmark). Both cohorts are part of the international multicenter EPIC study [16]. Detailed descriptions of the design and rationale of both cohorts can be found elsewhere [17, 18]. In brief, the recruitment of both cohorts took place between 1993 and 1997. Participants of EPIC-Norfolk were recruited through 35 participating General Practices in the rural areas of Norfolk and market towns as well as the city of Norwich, in the United Kingdom [17]. A total of 25639 men and women, aged 40 through 74 years, were enrolled in the study. Participants for EPIC-Denmark were selected from the Copenhagen and Aarhus areas in Denmark, and were identified through the Civil Registration System (CPR) [18]. Selection criteria were being born in Denmark, being between 50 and 64 years of age, and being free of cancer. A total of 57053 men and women were enrolled.
At baseline, all participants underwent a physical examination and filled out a lifestyle questionnaire and a food frequency questionnaire (FFQ). All participants gave written informed consent before enrolment into the study, and ethical approval for the studies was obtained from the Norfolk and Norwich Hospital Ethics Committee (EPIC-Norfolk) and from the relevant Scientific Committees and the Danish Data Protection Agency (EPIC-Denmark).

Population for analysis

We excluded all participants who had a history of cancer or cardiovascular disease at baseline (n = 2481 in EPIC-Norfolk; n = 1474 in EPIC-Denmark); who had missing dietary data (n = 547; n = 91); and who reported implausible energy intakes compared to their estimated basal metabolic rate (n = 266; n = 554). In addition, we excluded all participants who had missing data on covariables, such as smoking, physical activity, BMI and education level (n = 213; n = 331). For this study, 22132 and 54603 participants were left for analysis in EPIC-Norfolk and EPIC-Denmark, respectively.

Dietary assessment

Dietary data were obtained through validated, country-specific FFQs, that allowed the participants to specify the food consumption frequency during the preceding year [19, 20]. Based on these data, the daily intakes of macro- and micronutrients were calculated for each participant with use of FETA [21], based on McCance & Widdowson's food composition tables [22-31] (Norfolk) and with use of the software program FoodCalc [32] (EPIC-Denmark). Data on individual fatty acids intake were calculated based on the fatty acids supplement to the McCance & Widdowson's The Composition of Foods [33] , or McCance and Widdowson's

The Composition of Foods integrated dataset (CoF IDS) [34] and on the Danish food composition tables from 1996 [35].
The FFQs were both previously validated [36-38] against weighed records. The Norfolk FFQ was not validated for its ability to measure SFA, but for total fat the correlation coefficient was 0.55 in women [36] . For the Danish FFQ, the correlation coefficients were 0.67 (men) and 0.48 (women) for total fat intake and 0.46 (men) and 0.39 (women) for saturated fat intake [38].
For this analyses, the intakes of individual saturated fatty acids and of all other macronutrients were expressed as percentages of total energy intake (en%). For both cohorts, we summed the intakes of butyric acid through capric acid, because of very low intakes and because they are all derived from the same food sources, predominantly dairy products. For the same reasons, the intakes of pentadecylic and margaric acid were also summed in EPIC-Norfolk. In EPIC-Denmark, pentadecylic acid was analysed individually, because data on margaric acid intake were not available. Also, the intake of pentadecylic acid was available for 54541 participants. Furthermore, for the Danish cohort trans-fat intake was available only from ruminant sources, and was therefore left out of the analyses.

Outcome assessment

Information on vital status was obtained by flagging the participants for death certification at the United Kingdom Office of National Statistics (EPIC-Norfolk) and through linkage with The Danish National Patient Register [39] and The Danish Register of Causes of Death [40] (EPIC-Denmark). Information on hospital admissions in Norfolk and Denmark was obtained through linkage with the Norfolk Health Authority database (ENCORE) and the Danish National Patient Register, respectively.
The cause of death or hospital admission was coded according to the ninth revision of the International Classification of Diseases (ICD) for Norfolk and according to the eight and tenth ICD revisions for Denmark. The outcome of interest in this study was incident MI. This included both fatal and nonfatal MI events classified with codes 410-410.99 (ICD-8 and ICD-9) and I21.0-121.9 (ICD-10). In addition, in the Danish cohort the cardiac arrest cases (ICD-8 and ICD-9 codes: 427.27, and ICD-10 codes: I46.0–I46.9) were included. Follow-up time was completed until 31 March 2015 (EPIC-Norfolk) and 31 December 2009 (EPIC-Denmark).

Assessment of other variables

Information on baseline non-dietary factors, including medical history, medication use, smoking status, alcohol use, education level and physical activity level was obtained with use of general questionnaires. Smoking status was defined as never, former and current. Education level was categorized as none, 0 level, A level, and having a degree (Norfolk) or according to the number of years one attended school: 0 - 7 years, 8 - 10 years, ≥10 years (Denmark). Alcohol intake, as obtained from the FFQ, was expressed according to the

Table 1. Baseline characteristics across quintiles of the total saturated fatty acids intake (en%) in EPIC-Norfolk

	Q1	Q2	Q3	Q4	Q5
Total saturated fatty acids, *en%*	8.5 (± 1.3)[a]	11.0 (± 0.5)	12.5 (± 0.4)	14.2 (± 0.6)	17.6 (± 2.0)
Participants, *n*	4426	4427	4426	4427	4426
Male, *%*	35	42	46	51	52
Age, *y*	58.3 (± 8.7)	58.5 (± 9.2)	58.4 (± 9.3)	58.6 (± 9.5)	59.7 (± 9.4)
BMI, kg/m^2	26.5 (± 4.0)	26.4 (± 3.9)	26.3 (± 3.8)	26.3 (± 3.9)	26 (± 3.9)
Waist circumference, *cm*	86.6 (± 12.2)	87.8 (± 12.2)	88 (± 12.2)	88.9 (± 12.5)	88.8 (± 12.4)
No education, %	34	35	36	37	39
High education level, %	14	14	13	12	12
Current smoker, *%*	8	9	11	12	20
Physically inactive, *%*	26	28	29	32	32
Systolic blood pressure, *mmHg*	134.7 (± 18.5)	135.4 (± 18.4)	135 (± 18.2)	135.3 (± 18.1)	135.6 (± 18.2)
Diastolic blood pressure, *mmHg*	81.9 (± 11.3)	82.5 (± 11.2)	82.5 (± 11.1)	82.8 (± 11.2)	82.7 (± 11.2)
HDL-cholesterol, *mmol/L*	1.5 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)
LDL- cholesterol, *mmol/L*	3.9 (± 1.0)	3.9 (± 1.0)	4.0 (± 1.0)	4.0 (± 1.0)	4.1 (± 1.1)
Use of lipid lowering medication, *%*	3	1	1	0	0
Diabetes Mellitus, *%*	3	2	2	2	1
Alcohol, *g*	5 (1-13)[b]	5 (1-11)	5 (1-11)	4 (1-10)	3 (1-10)
Energy, *kcal*	1787 (± 502)	1987 (± 555)	2087 (± 581)	2155 (± 617)	2229 (± 642)
Fat, *en%*	25.7 (± 4.1)	30.9 (± 3.1)	33.6 (± 3.1)	36.1 (± 3.3)	39.7 (± 4.0)
Butyric (4:0) - capric (10:0) acid, *en%*	0.4 (0.3-0.5)	0.6 (0.4-0.7)	0.7 (0.5-0.8)	0.9 (0.7-1.1)	1.5 (1.1-1.9)
Lauric acid (12:0), *en%*	0.3 (0.2-0.4)	0.4 (0.3-0.5)	0.5 (0.4-0.5)	0.5 (0.4-0.6)	0.7 (0.6-0.8)

Myristic acid (14:0), *en%*	0.8 (0.6-0.9)	1.1 (0.9-1.2)	1.3 (1.1-1.4)	1.6 (1.4-1.7)	2.1 (1.9-2.4)
Palmitic acid (16:0), *en%*	4.8 (4.3-5.2)	5.9 (5.6-6.2)	6.6 (6.4-6.9)	7.4 (7.1-7.7)	8.6 (8.1-9.2)
Pentadecylic (15:0) & margaric (17:0) acid, *en%*	0.2 (0.1-0.2)	0.2 (0.2-0.3)	0.3 (0.3-0.3)	0.4 (0.3-0.4)	0.5 (0.4-0.6)
Stearic acid (18:0), *en%*	1.9 (1.7-2.1)	2.4 (2.2-2.6)	2.7 (2.5-2.9)	3.0 (2.9-3.3)	3.6 (3.3-3.9)
Cis-monounsaturated fatty acids, *en%*	7.6 (± 1.6)	8.8 (± 1.5)	9.4 (± 1.6)	10.0 (± 1.7)	10.5 (± 1.8)
Cis-polyunsaturated fatty acids, *en%*	5.8 (± 1.9)	6.2 (± 2.1)	6.2 (± 2.1)	5.9 (± 2.0)	4.9 (± 1.8)
Trans-fatty acids, *en%*	1.0 (± 0.4)	1.3 (± 0.4)	1.5 (± 0.5)	1.7 (± 0.5)	1.9 (± 0.6)
Carbohydrates, *en%*	55.4 (± 6.9)	52.2 (± 5.6)	50.3 (± 5.3)	48.5 (± 5.2)	45.5 (± 5.5)
Protein, *en%*	18.3 (± 3.4)	17.2 (± 3.0)	16.5 (± 2.8)	16.1 (± 2.8)	15.4 (± 2.7)
Cholesterol, *mg*	194 (± 77)	244 (± 87)	275 (± 97)	310 (± 109)	363 (± 130)
Fibre, *g*	21 (± 8)	20 (± 6)	19 (± 6)	18 (± 6)	16 (± 6)
Vitamin C, *mg*	149 (± 72)	129 (± 58)	120 (± 53)	113 (± 50)	105 (± 49)

[a] Values expressed in means with SD (all such values)

[b] Values expressed in medians with IQR (all such values)

following categories: none, 0 up to 5 g/d, 5 up to 15 g/d, 15 up to 30 g/d, 30 up to 45 g/d, and ≥ 45 g/d. Physical activity level was obtained with use of a validated questionnaire and expressed according to the Cambridge Physical Activity Index [41] which resulted in the following categories: active, moderately active, moderately inactive and inactive. Height, weight and waist circumference were measured at the physical examination. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) and divided into the following categories: BMI < 18.5, 18.5 ≤ BMI < 23, 23 ≤ BMI < 25, 25 ≤ BMI < 30, 30 ≤ BMI < 35, and BMI ≥ 35.

3.3

Data analysis

We ranked participants from the EPIC-Norfolk and EPIC-Denmark cohorts, separately, according to their intake of each individual (or summed) SFAs (in en%) and created quintiles of the intake distributions. We expressed baseline characteristics across these quintiles as means with SD, as medians with IQR, or as percentages. To evaluate the correlations between the different SFAs, we calculated Pearson correlation coefficients.
For each participant, we calculated the follow-up time in years, which started on the date of study entry and ended on either the date of an MI event, the date of death, the date of loss to follow-up, or the end of follow-up (31 March 2015 or 31 December 2009 for EPIC-Norfolk and EPIC-Denmark, respectively), whichever occurred first.
With use of Cox proportional hazard regression analysis, we calculated hazard ratios (HR) with 95% confidence intervals (CI) for the associations between quintiles intake of the individual (or summed) SFA intakes and the risk of incident MI, for each cohort separately. In all analyses, the lowest quintile of intake was used as the reference category. We ran 2 models to adjust for potential confounders. To the first model we added age, sex, total energy intake (excluding energy from alcohol), BMI, waist circumference, education level, physical activity level, smoking status, and alcohol intake (in categories). In the second model, we additionally included the intakes of energy from of PUFA and protein, intakes of dietary cholesterol, vitamin C, fibre, the sum of all other SFAs and in EPIC-Norfolk also trans fatty acids. We examined whether the associations followed a linear trend across the intake quintiles by assigning each participant the median intake of the quintile they belonged to and running the Cox regression models using this median intake as continuous variable. By inclusion of both linear and squared terms for SFA intake in the Cox models, we explored a potential non-linear association. We checked the Cox proportional hazards assumption by constructing log-log plots and observed no abnormalities.

Sensitivity analyses

Firstly, for the SFAs that were significantly related to MI in EPIC-Norfolk, but not in EPIC-Denmark, we additionally reran the Cox analyses in EPIC-Denmark using intake categories that were regrouped so that the intake ranges were identical to those of the quintiles in EPIC-Norfolk. In this way, we examined whether the intake range of the reference

categories were important. Secondly, one could argue that the baseline dietary measures in this study may not be related to MI events that occurred two decades thereafter. Therefore, we repeated our analyses by ending the follow-up time at 31 March 2001 to examine whether the associations were different for a shorter follow-up time of eight years. Thirdly, to limit the possibility of reverse causation we repeated the analyses after exclusion of the first two years of follow-up. Fourthly, the proportion of participants who at baseline had diabetes was higher among low SFA consumers than among high SFA consumers. Because those participants are likely to have a different baseline risk of MI than participants who are disease free, we repeated all analyses after excluding them (n = 510 in EPIC-Norfolk ; n = 1105 in EPIC-Denmark). Fifthly, the reported use of lipid-lowering medication at baseline was higher among the lower SFA consumers. Therefore, we repeated the analyses after exclusion of all participants who reported the use of lipid-lowering medication at baseline. Additionally, in the participants of EPIC-Norfolk with complete data on their baseline ratio of blood total cholesterol: HDL-cholesterol levels (n = 20046), we repeated the analyses and additionally adjusted the model for the ratio to examine whether this was a potential intermediate. Lastly, we examined the hypothesis that the association between SFA intake and MI may differ depending on the type of macronutrient that is being replaced, by amending the aforementioned Cox regression models as follows. To all Cox models we added the intake of total energy (excluding alcohol) as well as intakes of energy from (cis)-MUFA, (cis-)PUFA, protein, carbohydrates and in EPIC-Norfolk for energy from trans-fat. By excluding one of the latter five macronutrients from the models, the HR for SFA can be interpreted as the difference in MI risk for a higher intake of energy from the individual (or summed) SFA(s) and at the same time a lower intake of an equal amount of energy from the excluded macronutrient.

All statistical analyses were done using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Two sided P values <0.05 were considered statistically significant.

Results

Population characteristics

The mean (± SD) intakes per day of total SFA were 13.3 (± 3.5) en%, and 12.5 (± 2.6) en% in EPIC-Norfolk and EPIC-Denmark, respectively. In both cohorts, the majority of SFA was represented by palmitic acid (~ 52%), stearic acid (~ 22%) and myristic acid (~ 10 en%) (Figure 1). High correlations were observed for the sum of butyric through capric acid with myristic acid (r = 0.89 (Norfolk), and r = 0.92 (Denmark)) and with pentadecylic acid (r = 0.96 (Denmark)) (Supplemental Table S1). Myristic acid was highly correlated with pentadecylic acid and margaric acid (r = 0.88 (Norfolk)). In addition, the correlation coefficients between palmitic and stearic acid were high (r = 0.92 (Norfolk) and 0.90 (Denmark)).

Figure 1. Individual saturated fatty acids expressed as percentages of the mean intake of total saturated fat in EPIC-Norfolk and EPIC-Denmark

Table 2. Baseline characteristics across quintiles of the total saturated fatty acids intake (en%) in EPIC-Denmark

	Q1	Q2	Q3	Q4	Q5
Total saturated fatty acids, *en%*	8.8 (± 1.2)	11.1 (± 0.4)	12.5 (± 0.4)	13.9 (± 0.4)	16.2 (± 1.3)
Participants, *n*	10920	10921	10921	10921	10920
Age, *y*	56.5 (± 4.3)	56.6 (± 4.3)	56.6 (± 4.4)	56.7 (± 4.4)	56.9 (± 4.4)
Male, *%*	42	46	48	50	50
BMI, kg/m^2	26.2 (± 3.9)	26.2 (± 4.0)	26 (± 4.0)	25.9 (± 4.1)	25.7 (± 4.2)
Waist circumference, *cm*	89.4 (± 12.4)	89.3 (± 12.5)	88.8 (±12.7)	87.9 (± 12.5)	87.1 (± 13.0)
≤7 years of education, *%*	30	31	32	34	36
>10 years of education, *%*	23	22	21	20	20
Current smoker, *%*	26	30	33	36	45
Physically inactive, *%*	10	10	10	11	13
Systolic blood pressure, *mmHg*	141 (± 21)	140 (± 21)	140 (± 20)	139 (± 20)	138 (± 20)
Diastolic blood pressure, *mmHg*	84 (± 11)	84 (± 11)	83 (± 10)	83 (± 11)	82 (± 11)
Use of lipid lowering medication at baseline, *%*	3	1	1	1	0
Diabetes at baseline, *%*	3.3	2.2	2.2	2.2	1.1
Alcohol, *g*	17 (7 - 40)	15 (7 - 35)	14 (7 - 32)	12 (6 - 23)	9 (3 - 17)
Energy, *kcal*	2190 (± 590)	2302 (± 616)	2377 (± 645)	2430 (± 674)	2451 (± 705)
Sum of butyric (4:0) - capric (10:0) acid, *en%*	0.6 (0.5 - 0.8)	0.9 (0.7 - 1.1)	1.1 (0.9 - 1.3)	1.3 (1.1 - 1.6)	1.7 (1.4 - 2.0)
Lauric acid (12:0) , *en%*	0.2 (0.2 - 0.3)	0.3 (0.2 - 0.3)	0.3 (0.3 - 0.4)	0.4 (0.3 - 0.5)	0.5 (0.4 - 0.6)
Myristic acid (14:0) , *en%*	0.8 (0.7 - 1.0)	1.1 (1.0 - 1.2)	1.3 (1.1 - 1.4)	1.4 (1.3 - 1.6)	1.7 (1.6 - 1.9)
Palmitic acid (16:0), *en%*	4.9 (4.5 - 5.3)	5.9 (5.7 - 6.1)	6.5 (6.3 - 6.8)	7.1 (6.9 - 7.4)	8.0 (7.7 - 8.5)
Pentadecylic (15:0) acid, *en%*	0.1 (0.0 - 0.1)	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.2)

Stearic acid (18:0), *en%*	2.1 (1.8 - 2.3)	2.5 (2.4 - 2.7)	2.8 (2.7 - 3.0)	3.1 (2.9 - 3.3)	3.5 (3.3 - 3.8)
Monounsaturated fatty acids, *en%*	8.7 (± 1.7)	10.3 (± 1.5)	11.2 (± 1.5)	11.9 (± 1.5)	12.8 (± 1.7)
Polyunsaturated fatty acids, *en%*	5.2 (± 1.6)	5.6 (± 1.5)	5.6 (± 1.4)	5.6 (± 1.3)	5.2 (± 1.2)
Carbohydrates, *en%*	47.9 (± 7.7)	44.7 (± 6.1)	43.3 (± 5.4)	42.1 (± 5.0)	40.4 (± 4.6)
Protein, *en%*	16.2 (± 2.6)	16.5 (± 2.5)	16.5 (± 2.4)	16.6 (± 2.3)	16.7 (± 2.3)
Cholesterol, *mg*	351 (± 156)	417 (± 166)	450 (± 174)	482 (± 189)	514 (± 214)
Fibre, *g*	23 (± 8)	22 (± 7)	21 (± 7)	21 (± 7)	19 (± 6)

[a] Values expressed in means with SD (all such values)

[b] Values expressed in medians with IQR (all such values)

Tables 1 and 2 show the baseline characteristics across quintiles of the total SFA intake (in en%) in Norfolk and Denmark, respectively. In both cohorts, the participants with higher intakes of energy from total SFA, as well as from all the individual SFAs (data not shown), were more often men who smoked, and who had a lower BMI, less education, and less physical activity. Moreover, higher intakes of SFA were associated with higher intakes of total energy, MUFA, trans-fat, and lower intakes of carbohydrates, fibre, vitamin C and alcohol. In both cohorts, the prevalence of Diabetes Mellitus and the use of lipid-lowering medication was more frequent in the low SFA intake quintiles compared to the higher quintiles.

Associations between individual SFAs and MI risk in EPIC-Norfolk

Over a median (IQR) follow-up time of 18.8 (17.4 - 20.2) years, 1209 (5.5 %) incident MI events during were documented. After multivariable adjustment for lifestyle and dietary factors (model 2), the MI risks in the higher intake quintiles of the sum of butyric through capric acid were lower as compared with quintile 1 (Q1) (Table 3) and followed a linear trend (HR Q5 versus Q1: 0.77, 95% CI: 0.59, 1.01, P_{trend} = 0.04). We observed a similar, although borderline statistically significant, linear trend across the HRs of the quintiles of myristic acid (HR Q5 versus Q1: 0.73, 95% CI: 0.53, 1.02, P_{trend} =0.06) and of the sum of pentadecylic acid and margaric acid (HR Q5 versus Q1: 0.74, 95% CI: 0.54, 1.01, P_{trend} = 0.07). Stearic acid was not significantly associated with MI risk in any of the intake quintiles, but the HRs did follow a significant linear trend towards a lower MI risk in the higher intake quintiles compared with the lowest (P = 0.05). Neither lauric acid (12:0) nor palmitic acid were associated with risk of MI.

The quadratic terms for the sum of butyric through capric acid and for myristic acid were statistically significant (both P = 0.001). This appeared to be driven by the participants in the top of the intake distributions. The top 0.5% of these distributions contained participants who, compared with all others, were on average less healthy in terms of the presence of diseases and disease risk factors (Supplemental Tables S2 and S3). After exclusion of the top 0.5% of the distributions the quadratic terms were no longer statistically significant (P = 0.12 for butyric through capric acid and P = 0.31 for myristic acid) (data not shown).

Associations between individual SFAs and MI risk in EPIC-Denmark

During a median (IQR) follow-up time of 13.6 (12.9 - 14.3) years, 2318 (4.2%) incident MI events occurred in EPIC-Denmark. The multivariable adjusted (model 2) HRs for the association between the sum of butyric through capric acid and MI risk in EPIC-Denmark lowered across intake quintiles 2 through 4 (HR Q4 vs. Q1: 0.82, 95% CI: 0.71, 0.95) compared to quintile 1 and went up in quintile 5 (HR 0.92, 95% CI: 0.79, 1.08, P for linear trend = 0.3) (Table 3). A similar pattern of HRs was observed for myristic acid (HR Q4 vs. Q1: 0.85, 95% CI: 0.71, 1.01, and HR Q5 vs. Q1: 0.90, 95% CI: 0.73, 1.02, P_{trend} = 0.3). The

quadratic terms of these SFA were both significant (both p=0.001), which was driven by the higher HR in upper intake quintiles. All other individual SFA were not associated with incident MI risk.

Sensitivity analyses

After we reorganized the SFA intake categories of EPIC-Denmark according to the intake ranges of the quintiles in EPIC-Norfolk, the lower MI risks followed significant linear trends across intake categories 2 through 5 of the sum of butyric acid through capric acid (HR Q5 versus Q1: 0.74, 95% CI: 0.60, 0.91, P_{trend} = 0.02) and of myristic acid (HR Q5 versus Q1: 0.81, 95% CI: 0.63, 1.06, P_{trend} = 0.05) (Table 4).
The overall tendency of an inverse association between the consumption of butyric through capric acid and of myristic acid and MI risk was similar in the analyses where we stopped the follow-up time after 8 years or where we excluded the 2 two years of follow-up including all subjects who suffered an MI event during that time (Supplemental Tables S4 and S5). Exclusion of participants who had diabetes at baseline (Supplemental Table S6), or of participants who used lipid lowering medication at baseline (Supplemental Table S7) did not change the conclusions either. Neither did additional adjustment for the ratio of baseline total cholesterol to HDL-cholesterol levels (EPIC-Norfolk only) (Supplemental Table S8). In addition, the associations did not alter when we statistically modelled the substitution of the individual SFAs for different macronutrients (Supplemental Table S9).

Discussion

In this study in two separate cohorts from the UK and Denmark, a higher baseline consumption of butyric through capric acid and of myristic acid was associated with a lower MI risk during a follow-up time of ~ 14 to 20 years. Furthermore, in the UK a borderline significant inverse association was observed between the consumption of odd-chain SFAs (pentadecylic acid and margaric acid) and MI. The consumption of the other individual SFAs was not associated with MI risk in either population, except for a significant inverse linear trend across quintiles of stearic acid intake in the UK.

Strengths of this study are the large sample size of the included cohorts, with a long follow-up time and a large number of MI events. In addition, the extensive assessment of population characteristics at baseline allowed us to adjust the observed associations for many potential confounders. Furthermore, because both cohorts are part of the international EPIC cohort, they have a similar recruitment period (between 1993 and 1997). Limitations of this study include the use of only baseline dietary measurements, which may not be representative of the dietary intake during follow-up. However, sensitivity analyses with a shortened follow-up time and excluding those likely to change their diet (cases in the first 2 years, diabetes at baseline) yielded similar results. Furthermore, margaric acid was excluded from the analyses in EPIC-Denmark, because the content of that specific SFA

Table 3. Hazard ratios (95% CI) for the associations between individual SFAs (in quintiles) and MI incidence risk in EPIC-Norfolk and in EPIC-Denmark

		Q1	Q2	Q3	Q4	Q5	P for
		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	linear trend
Butyric acid (4:0)-Capric (10:0) acid							
EPIC-Norfolk	Median intake (IQR)	0.3 (0.2-0.4)	0.5 (0.5-0.6)	0.7 (0.7-0.8)	1.0 (0.9-1.1)	1.6 (1.4-2.0)	
	Cases/subjects	238/4426	246/4427	250/4426	221/4427	254/4426	
	Model 1[a]	Ref	0.97 (0.81, 1.16)	0.97 (0.81, 1.16)	**0.81 (0.68, 0.98)**	0.84 (0.70, 1.00)	0.02
	Model 2[b]	Ref	0.95 (0.79, 1.14)	0.95 (0.78, 1.15)	**0.78 (0.63, 0.96)**	0.77 (0.59, 1.01)	**0.04**
EPIC-Denmark	Median intake (IQR)	0.60 (0.47-0.71)	0.95 (0.88-1.02)	1.23 (1.16-1.30)	1.54 (1.45-1.64)	2.05 (1.87-2.33)	
	Cases/subjects	502/10920	453/10921	433/10921	425/10921	505/10920	
	Model 1	Ref	0.93 (0.82, 1.06)	0.90 (0.79, 1.03)	**0.86 (0.75, 0.98)**	0.99 (0.87, 1.12)	0.70
	Model 2	Ref	0.92 (0.80, 1.04)	0.87 (0.76, 1.00)	**0.82 (0.71, 0.95)**	0.92 (0.79, 1.08)	0.32
Lauric acid (12:0)							
EPIC-Norfolk	Median intake (IQR)	0.3 (0.2-0.3)	0.4 (0.4-0.4)	0.5 (0.4-0.5)	0.6 (0.6-0.6)	0.8 (0.7-1.0)	
	Cases/subjects	211/4426	209/4427	257/4426	270/4427	262/4426	
	Model 1	Ref	0.93 (0.77, 1.13)	1.07 (0.89, 1.29)	1.02 (0.85, 1.22)	0.94 (0.78, 1.12)	0.57
	Model 2	Ref	0.94 (0.78, 1.15)	1.10 (0.90, 1.34)	1.05 (0.85, 1.30)	1.00 (0.78, 1.28)	0.91
EPIC-Denmark	Median intake (IQR)	0.22 (0.18-0.25)	0.31 (0.29-0.33)	0.38 (0.36-0.40)	0.46 (0.44-0.48)	0.57 (0.53-0.63)	
	Cases/subjects	485/10920	437/10921	457/10921	493/10921	446/10920	
	Model 1	Ref	0.93 (0.81, 1.05)	0.95 (0.83, 1.08)	1.01 (0.89, 1.15)	0.93 (0.81, 1.06)	0.61
	Model 2	Ref	0.90 (0.79, 1.03)	0.91 (0.79, 1.05)	0.96 (0.82, 1.12)	0.85 (0.71, 1.03)	0.27
Myristic acid (14:0)							
EPIC-Norfolk	Median intake (IQR)	0.8 (0.7-0.9)	1.1 (1.0-1.2)	1.3 (1.3 -1.4)	1.6 (1.6-1.8)	2.2 (2.0-2.5)	

	Cases/subjects	228/4426	237/4427	240/4426	237/4427	267/4426	
	Model 1	Ref	0.92 (0.77, 1.11)	0.90 (0.75, 1.08)	**0.82 (0.68, 0.98)**	**0.83 (0.69, 0.99)**	**0.03**
	Model 2	Ref	0.89 (0.73, 1.08)	0.86 (0.69, 1.06)	**0.76 (0.59, 0.97)**	0.73 (0.53, 1.02)	**0.06**
EPIC-Denmark	Median intake (IQR)	0.93 (0.81-1.01)	1.20 (1.14-1.25)	1.40 (1.35-1.45)	1.62 (1.56-1.69)	1.95 (1.84-2.12)	
	Cases/subjects	447/10920	467/10921	445/10921	451/10921	508/10920	
	Model 1	Ref	1.01 (0.89, 1.15)	0.94 (0.83, 1.08)	0.93 (0.81, 1.06)	1.02 (0.89, 1.16)	0.87
	Model 2	Ref	0.96 (0.84, 1.11)	0.88 (0.75, 1.02)	0.85 (0.71, 1.01)	0.90 (0.73, 1.11)	0.28
Pentadecylic (15:0) & margaric (17:0) acid							
EPIC-Norfolk	Median intake (IQR)	0.2 (0.1-0.2)	0.2 (0.2-0.3)	0.3 (0.3-0.3)	0.4 (0.4-0.4)	0.5 (0.5-0.6)	
	Cases/subjects	229/4426	221/4427	246/4426	242/4427	271/4426	
	Model 1	Ref	0.90 (0.75, 1.08)	0.89 (0.74, 1.07)	**0.82 (0.68, 0.99)**	**0.81 (0.68, 0.97)**	**0.02**
	Model 2	Ref	0.88 (0.72, 1.07)	0.85 (0.69, 1.05)	**0.77 (0.61, 0.98)**	0.74 (0.54, 1.01)	0.07
Pentadecylic acid (15:0)[c]							
	Median intake (IQR)	0.06 (0.05-0.06)	0.08 (0.08-0.09)	0.10 (0.10-0.11)	0.12 (0.12-0.13)	0.16 (0.15-0.18)	
	Cases/subjects	495/10908	465/10908	446/10909	443/10908	468/10908	
	Model 1	Ref	0.96 (0.85, 1.09)	0.92 (0.81, 1.05)	0.90 (0.78, 1.02)	0.94 (0.81, 1.08)	0.69
	Model 2	Ref	0.96 (0.84, 1.10)	0.92 (0.80, 1.07)	0.90 (0.76, 1.06)	0.94 (0.77, 1.15)	0.45
Palmitic acid (16:0)							
EPIC-Norfolk	Median intake (IQR)	5.0 (4.4-5.3)	6.1 (5.9-6.3)	7.0 (6.7-7.1)	7.7 (7.5 -8.0)	9.1 (8.6-9.8)	
	Cases/subjects	211/4426	242/4427	255/4426	241/4427	260/4426	
	Model 1	Ref	0.99 (0.83, 1.20)	1.01 (0.84, 1.22)	0.91 (0.75, 1.09)	0.90 (0.75, 1.09)	0.16
	Model 2	Ref	1.00 (0.82, 1.22)	1.03 (0.83, 1.29)	0.94 (0.72, 1.21)	0.97 (0.70, 1.36)	0.76

Table continues on the next page

Table 3. continued		Q1 HR (95% CI)	Q2 HR (95% CI)	Q3 HR (95% CI)	Q4 HR (95% CI)	Q5 HR (95% CI)	P for linear trend
Palmitic acid (16:0)							
EPIC-Denmark	Median intake (IQR)	5.6 (5.1-5.9)	6.7 (6.4-6.8)	7.4 (7.2-7.5)	8.0 (7.9-8.2)	9.0 (8.7-9.4)	
	Cases/subjects	358/10920	407/10921	432/10921	490/10921	631/10920	
	Model 1	Ref	1.00 (0.87, 1.15)	0.96 (0.83, 1.10)	0.98 (0.85, 1.12)	1.06 (0.93, 1.22)	0.36
	Model 2	Ref	0.98 (0.84, 1.15)	0.94 (0.79, 1.11)	0.96 (0.79, 1.16)	1.05 (0.83, 1.32)	0.79
Stearic acid (18:0)							
EPIC-Norfolk	Median intake (IQR)	1.96 (1.7-2.1)	2.5 (2.4-2.6)	2.8 (2.7-2.9)	3.2 (3.1-3.3)	3.8 (3.6-4.2)	
	Cases/subjects	205/4426	253/4427	253/4426	251/4427	247/4426	
	Model 1	Ref	1.06 (0.88, 1.27)	1.01 (0.84, 1.22)	0.95 (0.79, 1.15)	0.86 (0.71, 1.04)	**0.04**
	Model 2	Ref	1.01 (0.82, 1.24)	0.94 (0.75, 1.18)	0.86 (0.66, 1.12)	0.74 (0.52, 1.05)	**0.05**
EPIC-Denmark	Median intake (IQR)	2.3 (2.1-2.5)	2.8 (2.7-2.9)	3.2 (3.1-3.3)	3.5 (3.4-3.6)	4.0 (3.9-4.3)	
	Cases/subjects	355/10920	407/10921	471/10921	486/10921	599/10920	
	Model 1	Ref	1.00 (0.86, 1.15)	1.04 (0.90, 1.19)	0.98 (0.85, 1.12)	1.08 (0.94, 1.24)	0.28
	Model 2	Ref	0.95 (0.81, 1.11)	0.96 (0.81, 1.14)	0.89 (0.73, 1.07)	0.96 (0.77, 1.19)	0.78

[a] Model 1 is adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake

[b] Model 2 is additionally adjusted for the sum of the other SFAs, intakes of protein, PUFA, cholesterol, vitamin c, fibre, and in EPIC-Norfolk for *trans*-fat

[c] n = 54541

Table 4. HR (95% CI) for incident MI risk across categories[a] of intakes of the sum of butyric through capric acid and of myristic acid.: sensitivity analysis

		Category 1	Category 2	Category 3	Category 4	Category 5	P for linear trend
Butyric acid (4:0)-capric (10:0) acid							
EPIC-Norfolk	Median intake (IQR)	0.3 (0.2-0.4)	0.5 (0.5-0.6)	0.7 (0.7-0.8)	1.0 (0.9-1.1)	1.6 (1.4-2.0)	
	Cases/subjects	238/4426	246/4427	250/4426	221/4427	254/4426	
	Model 1[a]	Ref	0.97 (0.81, 1.16)	0.97 (0.81, 1.16)	**0.81 (0.68, 0.98)**	0.84 (0.70, 1.00)	**0.02**
	Model 2[b]	Ref	0.95 (0.79, 1.14)	0.95 (0.78, 1.15)	**0.78 (0.63, 0.96)**	0.77 (0.59, 1.01)	**0.04**
EPIC-Denmark	Median intake (IQR)	0.4 (0.3-0.4)	0.6 (0.5-0.6)	0.7 (0.7-0.8)	1.0 (0.9-1.1)	1.6 (1.4-1.9)	
	Cases/ subjects	130/2378	171/3702	238/5708	584/14245	1195/28570	
	Model 1	Ref	0.90 (0.72, 1.14)	0.84 (0.68, 1.04)	0.84 (0.69, 1.02)	**0.83 (0.69, 0.99)**	
	Model 2	Ref	0.88 (0.70, 1.11)	0.81 (0.65, 1.01)	**0.79 (0.65, 0.96)**	**0.74 (0.60, 0.91)**	
Myristic acid (14:0)							
EPIC-Norfolk	Median intake (IQR)	0.8 (0.7-0.9)	1.1 (1.0-1.2)	1.3 (1.3 -1.4)	1.6 (1.6-1.8)	2.2 (2.0-2.5)	
	Cases/subjects	228/4426	237/4427	240/4426	237/4427	267/4426	
	Model 1	Ref	0.92 (0.77, 1.11)	0.90 (0.75, 1.08)	**0.82 (0.68, 0.98)**	**0.83 (0.69, 0.99)**	**0.03**
	Model 2	Ref	0.89 (0.73, 1.08)	0.86 (0.69, 1.06)	**0.76 (0.59, 0.97)**	0.73 (0.53, 1.02)	**0.06**
EPIC-Denmark	Median intake (IQR)	0.9 (0.7-0.9)	1.1 (1.1-1.2)	1.4 (1.3-1.4)	1.7 (1.6-1.7)	2.1 (2.0-2.2)	
	Cases/subjects	284/7053	476/10785	547/13481	671/15933	341/7351	
	Model 1	Ref	1.04 (0.90, 1.21)	0.94 (0.82, 1.09)	0.94 (0.82, 1.08)	1.01 (0.86, 1.19)	**0.59**
	Model 2	Ref	0.97 (0.83, 1.13)	**0.84 (0.71, 1.00)**	**0.80 (0.66, 0.98)**	0.81 (0.63, 1.06)	**0.05**

[a] The intake categories are according to the intake ranges of the quintiles in EPIC-Norfolk

[b] Model 1 is adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake

[c] Model 2 is additionally adjusted for the sum of the other SFAs, intakes of protein, PUFA, cholesterol, vitamin c, fibre, and in EPIC-Norfolk for *trans*-fat

was missing in the Danish food composition database. In addition, the baseline intake measurement of trans-fat in EPIC-Denmark was not representative of intake during follow-up, since the intake of trans-fat changed dramatically in Denmark during that period. Therefore, we did not include trans-fat intake as a potential confounder in the analyses in EPIC-Denmark. Although we cannot exclude the possibility of residual confounding, the results in EPIC-Denmark were similar to those in EPIC-Norfolk, which were adjusted for trans-fat. Therefore, we do not expect that additional adjustment for trans-fat would have influenced the results of EPIC-Denmark.

Four other observational cohort studies, two from the Netherlands and two from the US, investigated the association between individual SFAs and CHD risk [12-15] and showed divergent and sometimes conflicting results.
The inverse associations we observed in this study between baseline intakes of the SFAs with chain lengths ≥10 carbons, myristic acid and the odd-chain SFAs with 15 and 17 carbons with MI risk are in line with the results of the Dutch EPIC-NL cohort [13] . However, in the other studies the sum of butyric through capric acid was not associated with IHD risk [12, 14, 15] and neither was the sum of pentadecylic and margaric acid [14]. Myristic acid was either not associated [12, 14] or adversely associated with CHD risk [15].
The lack of association between baseline intakes of lauric acid, palmitic acid and stearic acid and MI risk during follow-up in our study is also in line with the EPIC-NL cohort [13]. But in the Rotterdam Study, palmitic acid was related to an increased CHD risk [14] and in the US cohorts, palmitic and stearic acid were both associated with an increased risk of CHD [12, 15].
The exact explanation for these divergent findings between the cohort studies is not straightforward. We discuss three possibilities.

Firstly, the study populations differ with respect to the consumption of dairy foods and meat, which are the two major sources of SFA. In the US, the major food sources of SFA are meat and mixed meals [42]. These food groups make an important contribution to the dietary intakes of palmitic and stearic acids, which were related to an increased CHD risk in the US cohorts [12, 15] but not in the European EPIC cohorts [13, 14]. On the other hand, dairy products are a major SFA food source in the UK, Denmark and the Netherlands [43, 44]. Butyric through capric acid, myristic acid, pentadecylic acid, and margaric acid, which in these European cohorts were inversely related to CHD, all largely come from dairy food sources. In a previous cohort study, SFA from dairy foods and meat were related to respectively a lower and a higher CHD risk [45]. This difference in SFA food sources may, at least in part, explain why in the European cohorts an inverse association with dairy-derived SFAs and in the US cohorts an adverse association with the long-chain SFAs was observed.

Secondly, there are differences in the data assessment between the cohort studies. In all cohort studies, dietary intakes were measured with a country-specific FFQ. However, in our study, we used baseline measures of SFA intake only, whereas in the US cohorts repeated

measures of diet were used. It is conceivable that dietary intake changes over time, as was observed in the US study [15]. Therefore, the use of repeated measures probably yields a more accurate measure of SFA intake during follow-up and might be another explanation for the divergent findings. However, repeat analysis with a shortened follow-up time in our study did not yield different results, compared to analysis using the complete follow-up time. And even though in our study the associations may have attenuated due to missing dietary data during follow-up, consistent associations were observed in three separate cohorts [13].

Thirdly, there are some differences with respect to the analyses between the studies. Contrary to our study, the Dutch studies [13, 14] and the earliest of the US studies [12], in the most recent US study [15] none of the HRs for the associations between SFA and CHD -without taking into account the substituting macronutrient - were adjusted for dietary factors such as the consumption of trans-fat, PUFA, vitamin C and fibre. Moreover, in this recent US study [15], the HRs for each individual SFA were not adjusted for the other SFAs as was done in the present study and in previous studies [13, 14]. Adjustment for dietary factors and other SFAs altered the associations in our study. Therefore, the discrepancy between the findings of the recent US study and those of our present study, the study in EPIC-NL [13] and the earlier study in the NHS [12] could also be the result of residual confounding.
The presented associations for the individual SFAs with CHD in the NHS study might represent the association for total SFA or just one type of SFA, or even simply an unhealthy dietary pattern. And indeed, in their substitution analyses, where they did adjust the HRs for the other macronutrients as well as the other SFAs, the association between the sum of lauric acid and myristic acid and CHD risk was no longer harmful when it was compared with PUFA, MUFA, whole-grain carbohydrates, or plant protein. On the other hand, because of the high correlations between the SFAs, it is also possible that additional adjustment for other SFAs in our study resulted in over-adjustment, and may explain the discrepant results. Based on the observational evidence alone, it is impossible to know which of the two used methods in the analyses provides the best estimate.

Apart from the aforementioned and discussed divergent outcomes of the various cohort studies, in general there appears to be a consistent difference between the shorter chain SFAs (butyric through capric acid, myristic acid), the odd-chain SFAs (pentadecylic acid and margaric acid) and the longer-chain SFAs (palmitic acid and stearic acid), with respect to their associations with MI or CHD risk. The observed significant associations with MI or CHD in the existing cohort studies [12-15], are either inverse for the short-to medium chain SFAs [13] or adverse for the long-chain SFAs [12, 14, 15]. This could reflect a difference in the underlying dietary pattern, e.g., the difference in consumption of dairy versus meat, but could also reflect actual differences between SFAs with respect to their effect on CHD risk markers. Because of the high correlations between the SFAs, observational cohort studies alone will not suffice in answering the question whether individual SFAs have different associations with MI or CHD. In our study, butyric through capric acid, myristic acid and

the odd-chain SFAs, (pentadecylic acid and margaric acid) were highly correlated because of the shared food sources, which made it impossible to separate them in the analyses. Thus, it is unclear whether the observed associations in our study pertain to all these SFAs or represent the association of one of them. At present, controlled trials have been conducted for myristic acid but not for butyric through capric acid or the odd-chain SFAs. Myristic acid was shown to increase serum LDL-cholesterol as compared with carbohydrates [5], but had little effect on the ratio of total cholesterol to HDL-cholesterol, which is considered to be a stronger CHD risk predictor than LDL-cholesterol levels alone [11]. This could explain why in our study and previous studies [13, 14], myristic acid was not harmfully associated with risk of MI or CHD [13, 14].

In conclusion, based on the results of the present and previous observational cohort studies, the association between SFA and MI or CHD appears to differ for short- to medium-chain SFAs versus the long-chain SFAs. The short- to medium-chain SFAs and the odd-chain SFAs (with 15 and 17 carbons) appear to be inversely related or unrelated to MI risk, whereas the longer-chain SFAs, palmitic and stearic acid, may be adversely or unrelated to MI risk. Whether this difference is caused by the SFAs as such, by the differences in underlying dietary pattern, or by residual confounding in observational studies is unclear and cannot be solved using observational evidence alone. Therefore, for further examination of the effects of the short-to medium-chain SFAs on MI risk, evidence from intervention studies is needed.

Disclosures

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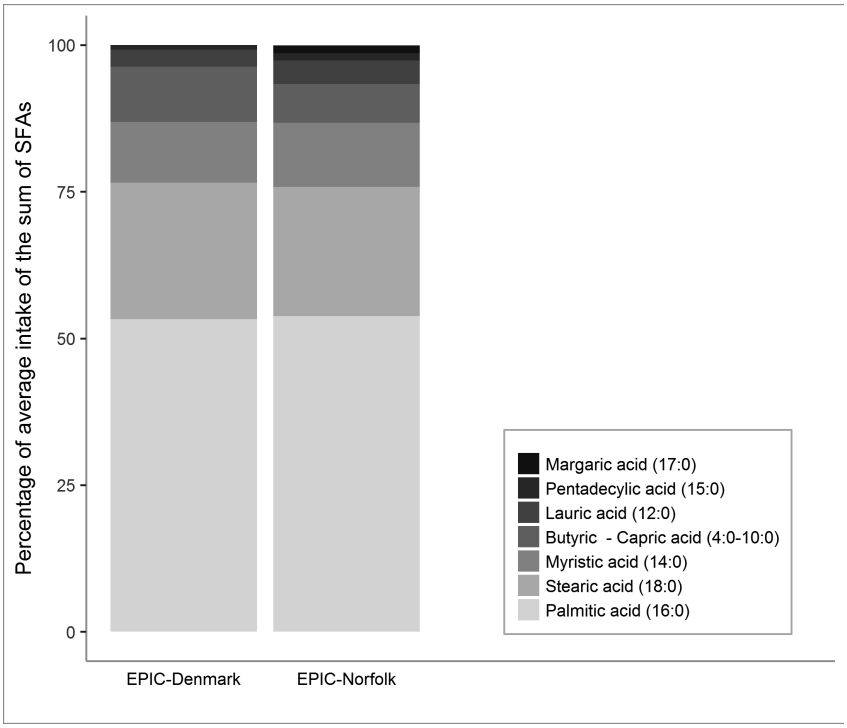
Supplemental Material

Supplemental Table S1. Correlations between the individual dietary saturated fatty acids (in en%) in EPIC-Norfolk and EPIC-Denmark

	(1)	(2)	(3)	(4)	(5)
EPIC-Norfolk					
(1) Butyric acid - capric acid (4:0 -10:0)	1				
(2) Lauric acid (12:0)	0.68	1			
(3) Myristic acid (14:0)	0.89	0.65	1		
(4) Palmitic acid (16:0)	0.66	0.48	0.81	1	
(5) Pentadecylic & margaric acid (15:0 & 17:0)	0.75	0.44	0.88	0.78	1
(6) Stearic acid (18:0)	0.64	0.47	0.77	0.92	0.77
EPIC-Denmark					
(1) Butyric acid - capric acid (4:0 -10:0)	1				
(2) Lauric acid (12:0)	0.95	1			
(3) Myristic acid (14:0)	0.92	0.93	1		
(4) Palmitic acid (16:0)	0.58	0.62	0.75	1	
(5) Pentadecylic acid (15:0)	0.95	0.94	0.95	0.61	1
(6) Stearic acid (18:0)	0.55	0.46	0.58	0.90	0.47



Supplemental figure 1. Individual saturated fatty acids expressed as percentages of the mean intake of the sum of saturated fatty acids in EPIC-Norfolk and EPIC-Denmark

Supplemental Table S2. Baseline characteristics across quintiles and the top 0.5% of the intake distribution of the sum of butyric (4:0) through capric (10:0) acid in EPIC-Norfolk

	Q1	Q2	Q3	Q4	Q5	
						Top 0.5%
Median (IQR) intake, en%	0.3 (0.2 - 0.4)	0.5 (0.5 - 0.6)	0.7 (0.6 - 0.7)	0.9 (0.9 - 1.0)	1.5 (1.3 - 1.8)	2.9 (2.8 - 3.1)
Subjects, n	4426	4427	4426	4427	4315	111
Male, %	44.0	45.1	44.0	45.1	46.2	49.5
Age (y)	57.7 (± 8.7)	58.1 (± 9.2)	58.5 (± 9.2)	58.8 (± 9.4)	60.4 (± 9.4)	62.5 (± 8.8)
BMI (kg/m^2)	26.6 (± 4.0)	26.4 (± 3.9)	26.3 (± 3.7)	26.2 (± 3.9)	25.9 (± 3.8)	25.9 (± 4.2)
Waist circumference (cm)	88.2 (± 12.3)	88.2 (± 12.2)	87.8 (± 12.1)	87.9 (± 12.5)	87.8 (± 12.4)	88.9 (± 13.7)
Education level, high %	12.1	12.1	13.2	14.3	15.4	11
Current smoker, %	8.8	9.9	9.9	12.1	17.6	26.4
Physically active, %	24.2	24.2	22	23.1	22.0	18.7
Blood pressure, mmHg						
Systolic	135 (± 18)	135 (± 18)	135 (± 18)	135 (± 18)	136 (± 19)	139 (± 18)
Diastolic	82 (± 11)	82 (± 11)	82 (± 11)	83 (± 11)	83 (± 11)	84 (± 12)
HDL-cholestol, mmol/L	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.5 (± 0.4)	1.5 (± 0.5)
LDL-cholesterol, mmol/L	3.9 (± 1.0)	3.9 (± 1.0)	3.9 (± 1.0)	4.0 (± 1.0)	4.0 (± 1.1)	4.4 (± 1.1)
Diabetes Mellitus at baseline, %	2.2	2.2	2.2	2.2	1.1	3.3
Use of lipid lowering medication, %	2.2	1.1	1.1	0.0	0.0	0.0
Alcohol, g/d	4.7 (0.8 - 11.3)	4.7 (0.8 - 10.9)	4.3 (0.8 - 10.3)	3.9 (0.8 - 10.6)	4.7 (0.8 - 11.3)	5.1 (0.8 - 19.4)
Energy, kcal	1967 (± 627)	2084 (± 589)	2046 (± 578)	2020 (± 593)	2129 (± 609)	2045 (± 503)
Saturated fatty acids, en%	9.6 (± 2.3)	11.3 (± 2.0)	12.2 (± 1.9)	13.5 (± 1.9)	16.8 (± 2.4)	23.2 (± 2.4)
Lauric acid (12:0), en%	0.3 (0.2 - 0.4)	0.4 (0.3 - 0.4)	0.4 (0.4 - 0.5)	0.5 (0.4 - 0.6)	0.7 (0.6 - 0.8)	1.1 (1.0 - 1.2)

Myristic acid (14:0), en%	0.8 (0.7 – 1.0)	1.1 (0.9 - 1.2)	1.2 (1.1 - 1.4)	1.5 (1.3 - 1.7)	2.1 (1.8 - 2.4)	3.3 (3.0 - 3.5)
Pentadecylic (15:0) & margaric (17:0) acid, en%	0.2 (0.1 - 0.3)	0.2 (0.2 - 0.3)	0.3 (0.2 - 0.3)	0.3 (0.3 - 0.4)	0.5 (0.4 - 0.5)	0.7 (0.7 - 0.8)
Palmitic acid (16:0), en%	5.4 (4.5 - 6.3)	6.2 (5.4 - 7.0)	6.5 (5.7 - 7.2)	6.9 (6.2 - 7.7)	8.2 (7.4 - 9.0)	10.7 (9.8 - 11.2)
Stearic acid (18:0), en%	2.2 (1.8 - 2.6)	2.5 (2.2 - 2.9)	2.6 (2.3 – 3.0)	2.9 (2.5 - 3.2)	3.4 (3.0 - 3.8)	4.4 (4.1 - 4.7)
Cis-MUFA, en%	8.6 (± 2.1)	9.1 (± 2.0)	9.2 (± 1.8)	9.5 (± 1.8)	9.9 (± 1.7)	11.1 (± 1.5)
Cis-PUFA, en%	6.5 (± 2.3)	6.3 (± 2.1)	6 (± 1.9)	5.6 (± 1.8)	4.7 (± 1.6)	3.7 (± 1.1)
Trans fatty acids, en%	1.2 (± 0.6)	1.5 (± 0.6)	1.5 (± 0.6)	1.6 (± 0.5)	1.6 (± 0.5)	1.7 (± 0.3)
Carbohydrates, en%	53 (± 7.2)	51.6 (± 6.4)	50.9 (± 5.9)	49.5 (± 6.0)	47.2 (± 5.9)	40 (± 5.7)
Protein, en%	17.5 (± 3.6)	16.7 (± 3.1)	16.9 (± 3.0)	16.7 (± 3.0)	15.6 (± 2.7)	14.3 (± 2.4)
Cholesterol, mg	234 (± 111)	266 (± 108)	271 (± 107)	281 (± 110)	332 (± 125)	363 (± 115)
Fibre, g	20 (± 8)	20 (± 7)	19 (± 6)	18 (± 6)	17 (± 6)	14 (± 5)
Vitamin C, mg	133 (± 67)	127 (± 61)	124 (± 57)	117 (± 54)	114 (± 53)	94 (± 47)

Supplemental Table S3. Baseline characteristics across quintiles and the top 0.5% of the intake distribution of myristic acid (14:0) in EPIC-Norfolk

	Q1	Q2	Q3	Q4	Q5	
						Top 0.5%
Median (IQR) intake, en%	0.4 (0.3 - 0.5)	0.6 (0.5 - 0.7)	0.7 (0.6 - 0.8)	0.9 (0.7 - 1.1)	1.5 (1.2 - 1.9)	2.9 (2.6 - 3.1)
Subjects, n	4426	4427	4426	4427	4316	110
Male, %	38.5	44	46.2	47.3	48.4	50.6
Age, y	57.7	58 (± 9.2)	58.3 (± 9.2)	58.9 (± 9.4)	60.4 (± 9.3)	63.3 (± 8.9)
BMI, kg/m^2	26.6 (± 4.1)	26.4 (± 3.9)	26.4 (± 3.8)	26.2 (± 3.8)	25.9 (± 3.8)	26.2 (± 3.8)
Waist hip circumference, cm	87.4 (± 12.4)	87.9 (± 12.3)	88.2 (± 12.2)	88.4 (± 12.4)	88.2 (± 12.4)	89.6 (± 13.2)
Education level, high %	13	13	13	12	13	11
Current smoker, %	8	10	10	13	17	25
Physically active, %	20	20	19	18	18	14
Systolic blood pressure, mmHg	135 (± 18)	135 (± 18)	135 (± 18)	135 (± 18)	136 (± 18)	139 (± 17)
Diastolic blood pressure, mmHg	82 (± 11)	82 (± 11)	82 (± 11)	83 (± 11)	83 (± 11)	84 (± 12)
HDL-cholesterol, mmol/L	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.4 (± 0.4)	1.5 (± 0.4)
LDL- cholesterol, mmol/L	3.9 (± 1.0)	3.9 (± 1.0)	3.9 (± 1.0)	4.0 (± 1.0)	4.1 (± 1.1)	4.3 (± 1.2)
Diabetes Mellitus, %	3.3	2.2	2.2	1.1	1.1	3.3
Use of lipid lowering medication, %	3.3	1.1	1.1	0.0	0.0	0.0
Alcohol, g/d	4.7 (0.8 - 12.2)	4.9 (0.8 - 11.2)	4.7 (0.8 - 10.6)	3.6 (0.8 - 10.2)	3.1 (0.8 - 10.1)	2.8 (0.8 - 12.8)
Energy, kcal	1876 (± 558)	2010 (± 566)	2053 (± 578)	2113 (± 628)	2193 (± 628)	2134 (± 570)
Saturated fatty acids, en%	8.9 (± 1.8)	11.1 (± 1.4)	12.4 (± 1.3)	13.9 (± 1.4)	17.1 (± 2.1)	23.6 (± 2.2)
Butyric (4:0) through capric (10:0)	0.4 (0.3 - 0.5)	0.6 (0.5 - 0.7)	0.7 (0.6 - 0.8)	0.9 (0.7 - 1.1)	1.5 (1.2 - 1.9)	2.9 (2.6 - 3.1)

Lauric acid (12:0), en%	0.3 (0.2 - 0.4)	0.4 (0.3 - 0.5)	0.5 (0.4 - 0.5)	0.5 (0.4 - 0.6)	0.7 (0.6 - 0.8)	1.0 (1 - 1.2)
Pentadecylic (15:0) & margaric(17:0) acid, en%	0.8 (0.7 - 0.9)	1.1 (1.0 - 1.1)	1.3 (1.2 - 1.4)	1.6 (1.5 - 1.7)	2.1 (1.9 - 2.4)	3.3 (3.2 - 3.5)
Palmitic acid (16:0), en%	0.2 (0.1 - 0.2)	0.2 (0.2 - 0.3)	0.3 (0.3 - 0.3)	0.4 (0.3 - 0.4)	0.5 (0.4 - 0.6)	0.8 (0.7 - 0.8)
Stearic acid (18:0), en%	5.0 (4.3 - 5.7)	6.0 (5.4 - 6.6)	6.6 (6 - 7.1)	7.2 (6.6 - 7.7)	8.4 (7.7 - 9.1)	10.8 (10.4 - 11.2)
Cis-MUFA, en%	8.2 (± 2.0)	9.1 (± 1.8)	9.3 (± 1.7)	9.6 (± 1.8)	10.0 (± 1.8)	11.0 (± 1.9)
Cis-PUFA, en%	6.5 (± 2.3)	6.4 (± 2.1)	6.0 (± 1.9)	5.5 (± 1.7)	4.6 (± 1.5)	3.6 (± 1.1)
Trans-fat, en%	1.0 (± 0.4)	1.3 (± 0.4)	1.5 (± 0.5)	1.7 (± 0.5)	1.9 (± 0.6)	2.1 (± 0.7)
Carbohydrates, en%	54.2 (± 7.1)	51.7 (± 6.2)	50.5 (± 5.8)	49.1 (± 5.7)	46.7 (± 5.6)	39.0 (± 5.3)
Protein, en%	17.8 (± 3.5)	17.1 (± 3.1)	16.8 (± 2.9)	16.3 (± 2.9)	15.4 (± 2.6)	14.2 (± 2.4)
Cholesterol, mg	204 (± 86)	244 (± 90)	272 (± 96)	307 (± 114.1)	357 (± 128.4)	397 (± 134.9)
Fibre, g/d	21 (± 8)	20 (± 6.5)	19 (± 6.1)	18 (± 5.9)	17 (± 5.7)	13 (± 4.9)
Vitamin C, mg	140 (± 71)	127 (± 58.6)	120 (± 53.5)	116 (± 53.1)	110 (± 51.5)	89 (± 42.6)

Supplemental Table S4. Hazard ratios (95% CI) for the associations between individual SFAs (in quintiles) and MI incidence in EPIC-Norfolk and EPIC-Denmark during the first eight years of follow-up.

		Q1	Q2	Q3	Q4	Q5	*P* for trend
Butyric acid (4:0) – Capric acid (10:0)							
EPIC-Norfolk	Median intake (IQR)	0.32 (0.24 - 0.38)	0.54 (0.49 - 0.58)	0.71 (0.67 - 0.76)	0.96 (0.88 - 1.07)	1.62 (1.38 - 2.00)	
	Cases/ subjects	52/ 4426	49/ 4427	48/ 4426	40/ 4427	50/ 4426	
	HR (95% CI)	Ref	0.87 (0.59, 1.31)	0.84 (0.55, 1.27)	**0.62 (0.39, 1.00)**	0.70 (0.38, 1.27)	0.22
EPIC-Denmark	Median intake (IQR)	0.60 (0.47 - 0.71)	0.95 (0.88 - 1.02)	1.23 (1.16 - 1.30)	1.54 (1.45 - 1.64)	2.05 (1.87 - 2.33)	
	Cases/ subjects	196/ 10920	157/ 10921	159/ 10921	135/ 10921	193/ 10920	
	HR (95% CI)	Ref	0.83 (0.67, 1.04)	0.86 (0.68, 1.07)	**0.70 (0.55, 0.90)**	0.94 (0.71, 1.24)	0.57
Lauric acid (12:0)							
EPIC-Norfolk	Median intake (IQR)	0.26 (0.21 - 0.30)	0.38 (0.36 - 0.41)	0.48 (0.46 - 0.50)	0.59 (0.56 - 0.63)	0.83 (0.74 - 0.97)	
	Cases/ subjects	49/ 4426	41/ 4427	51/ 4426	49/ 4427	49/ 4426	
	HR (95% CI)	Ref	0.80 (0.52, 1.23)	0.96 (0.62, 1.47)	0.81 (0.51, 1.29)	0.77 (0.44, 1.36)	0.44
EPIC-Denmark	Median intake (IQR)	0.22 (0.18 - 0.25)	0.31 (0.29 - 0.33)	0.37 (0.36 - 0.40)	0.46 (0.44 - 0.48)	0.57 (0.53 - 0.63)	
	Cases/ subjects	190/ 10920	156/ 10921	171/ 10921	157/ 10921	166/ 10920	
	HR (95% CI)	Ref	0.84 (0.67, 1.04)	0.89 (0.70, 1.12)	0.80 (0.62, 1.04)	0.84 (0.61, 1.15)	0.28
Myristic acid (14:0)							
EPIC-Norfolk	Median intake (IQR)	0.79 (0.66 - 0.88)	1.08 (1.02 - 1.14)	1.32 (1.26 - 1.39)	1.62 (1.54 - 1.73)	2.22 (2.01 - 2.53)	
	Cases/ subjects	48/ 4426	49/ 4427	42/ 4426	49/ 4427	51/ 4426	
	HR (95% CI)	Ref	0.81 (0.53, 1.24)	0.62 (0.38, 1.00)*	**0.59 (0.35, 1.00)**	**0.46 (0.22, 0.94)**	**0.04**
EPIC-Denmark	Median intake (IQR)	0.93 (0.81 - 1.01)	1.20 (1.14 - 1.25)	1.40 (1.35 - 1.45)	1.62 (1.56 - 1.69)	1.95 (1.84 - 2.12)	
	Cases/ subjects	169/ 10920	175/ 10921	154/ 10921	157/ 10921	185/ 10920	
	HR (95% CI)	Ref	0.96 (0.76, 1.20)	0.81 (0.63, 1.05)	0.79 (0.59, 1.05)	0.87 (0.61, 1.25)	0.31

Pentadecylic (15:0) and margaric (17:0) acid							
EPIC-Norfolk	Median intake (IQR)	0.16 (0.14 - 0.19)	0.23 (0.22 - 0.24)	0.29 (0.27 - 0.30)	0.36 (0.34 - 0.38)	0.49 (0.45 - 0.57)	
	Cases/ subjects	54/ 4426	43/ 4427	50/ 4426	47/ 4427	45/ 4426	
	HR (95% CI)	Ref	0.90 (0.59, 1.39)	0.77 (0.48, 1.23)	**0.57 (0.33, 0.97)**	0.53 (0.27, 1.06)	**0.05**
Pentadecylic acid (15:0)*							
EPIC-Denmark	Median intake (IQR)	0.06 (0.05 - 0.06)	0.08 (0.08 - 0.09)	0.10 (0.10 - 0.11)	0.12 (0.12 - 0.13)	0.16 (0.15 - 0.18)	
	Cases/ subjects	199/ 10908	156/ 10908	164/ 10909	146/ 10908	175/ 10908	
	HR (95% CI)	Ref	0.84 (0.67, 1.04)	0.90 (0.71, 1.14)	0.80 (0.61, 1.05)	0.97 (0.70, 1.34)	0.80
Palmitic acid (16:0)							
EPIC-Norfolk	Median intake (IQR)	4.89 (4.33 - 5.28)	6.05 (5.82 - 6.27)	6.87 (6.67 - 7.07)	7.71 (7.48 - 7.95)	9.05 (8.60 - 9.74)	
	Cases/ subjects	39/ 4426	53/ 4427	48/ 4426	51/ 4427	48/ 4426	
	HR (95% CI)	Ref	1.14 (0.73, 1.79)	0.98 (0.59, 1.64)	0.98 (0.55, 1.76)	0.83 (0.38, 1.81)	0.57
EPIC-Denmark	Median intake (IQR)	5.59 (5.07 - 5.94)	6.65 (6.44 - 6.84)	7.36 (7.20 - 7.53)	8.03 (7.86 - 8.22)	8.95 (8.66 - 9.40)	
	Cases/ subjects	123/ 10920	145/ 10921	171/ 10921	173/ 10921	228/ 10920	
	HR (95% CI)	Ref	0.97 (0.75, 1.27)	1.01 (0.75, 1.35)	0.89 (0.63, 1.23)	0.93 (0.62, 1.39)	0.62
Stearic acid (18:0)							
EPIC-Norfolk	Median intake (IQR)	1.95 (1.70 - 2.11)	2.47 (2.37 - 2.57)	2.85 (2.76 - 2.94)	3.25 (3.14 - 3.36)	3.88 (3.66 - 4.20)	
	Cases/ subjects	42/ 4426	46/ 4427	45/ 4426	59/ 4427	47/ 4426	
	HR (95% CI)	Ref	0.86 (0.54, 1.37)	0.79 (0.47, 1.32)	0.94 (0.52, 1.67)	0.63 (0.29, 1.39)	0.38
EPIC-Denmark	Median intake (IQR)	2.32 (2.08 - 2.49)	2.84 (2.74 - 2.93)	3.18 (3.10 - 3.26)	3.52 (3.43 - 3.62)	4.03 (3.86 - 4.32)	
	Cases/ subjects	122/ 10920	153/ 10921	171/ 10921	172/ 10920	222/ 10920	
	HR (95% CI)	Ref	1.01 (0.78, 1.31)	0.99 (0.74, 1.31)	0.86 (0.63, 1.19)	0.95 (0.66, 1.36)	0.63

Model 2 is adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake, intakes of protein, PUFA, cholesterol, vitamin C, fibre and the sum of the other SFAs, and in EPIC-Norfolk for *trans*-fat

*n=54541

Supplemental Table S5. Hazard ratios (95% CI) for the associations between individual saturated fatty acids (in quintiles) and MI incidence risk in EP-IC-Norfolk (n = 21968) and EPIC-Denmark (n = 54039), after exclusion of the first two years of follow-up.

		Q1	Q2	Q3	Q4	Q5	*P* for trend
Butyric acid (4:0) - capric acid (10:0)							
EPIC-Norfolk	Median intake (IQR)	0.33 (0.25 - 0.40)	0.55 (0.50 - 0.59)	0.72 (0.67 - 0.77)	0.96 (0.89 - 1.06)	1.62 (1.38 - 1.98)	
	Cases/ subjects	229/4393	239/4394	240/4394	216/4394	247/4393	
	HR (95% CI)	Ref	0.96 (0.80, 1.16)	0.95 (0.78, 1.16)	**0.80 (0.65, 0.99)**	0.80 (0.61, 1.05)	**0.05**
EPIC-Denmark	Median intake (IQR)	0.60 (0.47 - 0.71)	0.95 (0.88 - 1.02)	1.23 (1.16 - 1.30)	1.54 (1.45 - 1.64)	2.05 (1.87 - 2.33)	
	Cases/ subjects	448/ 10807	403/ 10808	383/ 10808	386/ 10808	462/ 10808	
	HR (95% CI)	Ref	0.96 (0.83, 1.11)	0.89 (0.75, 1.04)	0.87 (0.73, 1.03)	0.96 (0.79, 1.17)	0.58
Lauric acid (12:0)							
EPIC-Norfolk	Median intake (IQR)	0.26 (0.21 - 0.30)	0.38 (0.35 - 0.40)	0.47 (0.45 - 0.50)	0.59 (0.55 - 0.62)	0.82 (0.73 - 0.96)	
	Cases/ subjects	205/ 4393	200/ 4394	250/ 4394	261/ 4394	255/ 4393	
	HR (95% CI)	Ref	0.94 (0.77, 1.14)	1.11 (0.90, 1.35)	1.06 (0.85, 1.31)	1.01 (0.79, 1.31)	0.79
EPIC-Denmark	Median intake (IQR)	0.22 (0.18 - 0.25)	0.31 (0.29 - 0.33)	0.38 (0.36 - 0.40)	0.46 (0.44 - 0.48)	0.57 (0.53 - 0.63)	
	Cases/ subjects	431/ 10807	391/ 10808	403/ 10808	443/ 10808	414/ 10808	
	HR (95% CI)	Ref	0.96 (0.82, 1.12)	0.95 (0.81, 1.13)	1.02 (0.86, 1.22)	0.92 (0.74, 1.15)	0.68
Myristic acid (14:0)							
EPIC-Norfolk	Median intake (IQR)	0.81 (0.68 - 0.91)	1.11 (1.04 - 1.16)	1.34 (1.28 - 1.41)	1.65 (1.56 - 1.75)	2.23 (2.02 - 2.54)	
	Cases/ subjects	220/ 4393	229/ 4394	230/ 4394	234/ 4394	258/ 4393	
	HR (95% CI)	Ref	0.90 (0.74, 1.10)	0.87 (0.70, 1.08)	0.79 (0.62, 1.02)	0.76 (0.55, 1.06)	0.11
EPIC-Denmark	Median intake (IQR)	0.93 (0.81 - 1.01)	1.20 (1.14 - 1.25)	1.40 (1.35 - 1.45)	1.62 (1.56 - 1.69)	1.95 (1.84 - 2.12)	
	Cases/ subjects	407/ 10807	407/ 10808	401/ 10808	400/ 10808	467/ 10808	
	HR (95% CI)	Ref	0.94 (0.80, 1.10)	0.92 (0.77, 1.10)	0.84 (0.68, 1.02)	0.92 (0.72, 1.18)	0.40

Pentadecylic (15:0) & margaric (17:0) acid							
EPIC-Norfolk	Median intake (IQR)	0.18 (0.14 - 0.20)	0.25 (0.23 - 0.26)	0.31 (0.29 - 0.32)	0.38 (0.35 - 0.41)	0.52 (0.47 - 0.60)	
	Cases/ subjects	221/ 4393	213/ 4394	237/ 2394	237/ 4394	263/ 4393	
	HR (95% CI)	Ref	0.89 (0.73, 1.08)	0.87 (0.70, 1.07)	0.81 (0.63, 1.03)	0.78 (0.57, 1.07)	0.13
Pentadecylic acid* (15:0)							
EPIC-Denmark	Median intake (IQR)	0.06 (0.05 - 0.06)	0.08 (0.08 - 0.09)	0.10 (0.09 - 0.08)	0.12 (0.12 - 0.13)	0.16 (0.15 - 0.18)	
	Cases/ subjects	439/ 10795	415/ 10795	396/ 10795	395/ 10795	436/ 10795	
	HR (95% CI)	Ref	1.02 (0.88, 1.19)	0.94 (0.80, 1.12)	0.93 (0.77, 1.12)	0.97 (0.77, 1.22)	0.62
Palmitic acid (16:0)							
EPIC-Norfolk	Median intake (IQR)	4.98 (4.43 - 5.34)	6.11 (5.88 - 6.32)	6.92 (6.72 - 7.11)	7.74 (7.52 - 7.98)	9.08 (8.62 - 9.75)	
	Cases/ subjects	208/ 4393	230/ 4394	248/ 4394	233/ 4394	252/ 4393	
	HR (95% CI)	Ref	0.96 (0.79, 1.18)	1.02 (0.81, 1.27)	0.91 (0.70, 1.18)	0.95 (0.68, 1.34)	0.72
EPIC-Denmark	Median intake (IQR)	5.59 (5.07 - 5.94)	6.65 (6.44 - 6.84)	7.36 (7.20 - 7.53)	8.03 (7.85 - 8.22)	8.95 (8.66 - 9.40)	
	Cases/ subjects	323/ 10807	364/ 10808	386/ 10808	436/ 10808	573/ 10808	
	HR (95% CI)	Ref	0.98 (0.82, 1.17)	0.88 (0.72, 1.08)	0.93 (0.74, 1.16)	1.00 (0.76, 1.32)	0.95
Stearic acid (18:0)							
EPIC-Norfolk	Median intake (IQR)	1.96 (1.72 - 2.12)	2.46 (2.36 - 2.55)	2.82 (2.73 - 2.91)	3.21 (3.10 - 3.33)	3.84 (3.63 - 4.16)	
	Cases/ subjects	201/ 4393	242/ 4394	248/ 4394	241/ 4394	239/ 4393	
	HR (95% CI)	Ref	0.99 (0.80, 1.21)	0.94 (0.75, 1.19)	0.84 (0.64, 1.10)	0.73 (0.51, 1.04)	0.04
EPIC-Denmark	Median intake (IQR)	2.32 (2.08 - 2.49)	2.83 (2.73 - 2.93)	3.18 (3.10 - 3.26)	3.52 (3.43 - 3.62)	4.03 (3.86 - 4.32)	
	Cases/ subjects	322/ 10807	360/ 10808	418/ 10808	447/ 10808	535/ 10808	
	HR (95% CI)	Ref	0.91 (0.77, 1.09)	0.95 (0.78, 1.15)	0.88 (0.71, 1.09)	0.94 (0.73, 1.20)	0.71

For this analyses follow-up time was shortened by 2 years and all subjects who suffered from an MI event during the first two years were excluded

Model 2 is adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake, intakes of protein, PUFA, cholesterol, vitamin c, fibre and the sum of the other SFAs, and in EPIC-Norfolk for *trans*-fat

*n=53977

Supplemental Table S6. Hazard ratios (95% CI) for the associations between individual SFAs (in quintiles) and MI incidence risk in subjects of EPIC-Norfolk (n=21622) and EPIC-Denmark (n=53498) without diabetes at baseline.

		Q1	Q2	Q3	Q4	Q5	
			HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	*P* for trend
Butyric acid (4:0) - capric acid (10:0)							
EPIC-Norfolk	Median intake (IQR)	0.33 (0.25 - 0.40)	0.55 (0.50 - 0.59)	0.72 (0.67 - 0.77)	0.97 (0.89 - 1.07)	1.63 (1.38 - 1.99)	
	Cases/ subjects	221/ 4324	228/ 4325	236/ 4324	201/ 4325	241/ 4324	
	HR (95% CI)	Ref	0.92 (0.76, 1.12)	0.94 (0.77, 1.14)	**0.74 (0.59, 0.91)**	**0.74 (0.56, 0.98)**	**0.02**
EPIC-Denmark	Median intake (IQR)	0.61 (0.47 - 0.71)	0.95 (0.88 - 1.02)	1.23 (1.16 - 1.30)	1.54 (1.46 - 1.64)	2.05 (1.87 - 2.33)	
	Cases/ subjects	470/ 10699	422/ 10700	413/ 10700	407/ 10700	489/ 10699	
	HR (95% CI)	Ref	0.91 (0.79, 1.04)	0.88 (0.76, 1.01)	0.83 (0.71, 0.97)*	0.94 (0.79, 1.12)	0.79
Lauric acid (12:0)							
EPIC-Norfolk	Median intake (IQR)	0.26 (0.21 - 0.30)	0.38 (0.35 - 0.40)	0.48 (0.45 - 0.50)	0.59 (0.55 - 0.63)	0.82 (0.73 - 0.97)	
	Cases/ subjects	192/ 4324	191/ 4325	237/ 4324	259/ 4325	248/ 4324	
	HR (95% CI)	Ref	0.93 (0.76, 1.15)	1.08 (0.88, 1.32)	1.06 (0.85, 1.32)	0.98 (0.76, 1.27)	0.96
EPIC-Denmark	Median intake (IQR)	0.22 (0.18 - 0.25)	0.31 (0.29 - 0.33)	0.38 (0.36 - 0.40)	0.46 (0.44 - 0.48)	0.47 (0.53 - 0.63)	
	Cases/ subjects	457/ 10699	413/ 10700	427/ 10700	476/ 10700	428/ 10699	
	HR (95% CI)	Ref	0.89 (0.78, 1.02)	0.88 (0.76, 1.02)	0.95 (0.81, 1.12)	0.84 (0.69, 1.03)	0.61
Myristic acid (14:0)							
EPIC-Norfolk	Median intake (IQR)	0.82 (0.69 - 0.91)	1.11 (1.05 - 1.17)	1.35 (1.29 - 1.42)	1.65 (1.56 - 1.76)	2.24 (2.03 - 2.54)	
	Cases/ subjects	205/ 4324	226/ 4324	222/ 4324	221/ 4325	253/ 4324	
	HR (95% CI)	Ref	0.91 (0.74, 1.11)	0.84 (0.67, 1.05)	**0.73 (0.57, 0.94)**	**0.70 (0.50, 0.98)**	**0.03**
EPIC-Denmark	Median intake (IQR)	0.93 (0.81 - 1.01)	1.20 (1.14 - 1.25)	1.40 (1.35 - 1.46)	1.62 (1.57 - 1.69)	1.95 (1.84 - 2.13)	
	Cases/ subjects	423/ 10699	426/ 10700	424/ 10700	437/ 10700	491/ 10699	
	HR (95% CI)	Ref	0.92 (0.79, 1.06)	0.87 (0.74, 1.02)	0.85 (0.71, 1.02)	0.91 (0.73, 1.13)	0.44

Pentadecylic (15:0) & margaric (17:0) acid							
EPIC-Norfolk	Median intake (IQR)	0.18 (0.15 - 0.20)	0.25 (0.23 - 0.26)	0.31 (0.29 - 0.32)	0.38 (0.36 - 0.41)	0.52 (0.47 - 0.60)	
	Cases/ subjects	208/ 4324	202/ 4324	230/ 4324	228/ 4325	259/ 4324	
	HR (95% CI)	Ref	0.86 (0.70, 1.06)	0.85 (0.68, 1.06)	**0.77 (0.60, 0.99)**	0.75 (0.54, 1.03)	0.1
Pentadecylic acid (15:0)							
EPIC-Denmark	Median intake (IQR)	0.06 (0.05 - 0.06)	0.08 (0.08 - 0.09)	0.10 (0.10 - 0.11)	0.12 (0.12 - 0.13)	0.16 (0.15 - 0.18)	
	Cases/ subjects	463/ 10687	430/ 10688	428/ 10688	427/ 10688	452/ 10688	
	HR (95% CI)	Ref	0.95 (0.83, 1.09)	0.94 (0.81, 1.09)	0.92 (0.78, 1.09)	0.97 (0.79, 1.19)	0.82
Palmitic acid (16:0)							
EPIC-Norfolk	Median intake (IQR)	4.99 (4.44 - 5.35)	6.12 (5.89 - 6.33)	6.93 (6.73 - 7.12)	7.75 (7.53 - 7.99)	9.08 (8.62 - 9.76)	
	Cases/ subjects	190/ 4324	223/ 4325	239/ 4324	226/ 4325	249/ 4324	
	HR (95% CI)	Ref	0.99 (0.80, 1.22)	1.01 (0.81, 1.28)	0.91 (0.70, 1.19)	0.95 (0.67, 1.35)	0.65
EPIC-Denmark	Median intake (IQR)	5.60 (5.08 - 5.94)	6.66 (6.45 - 6.85)	7.37 (7.20 - 7.53)	8.03 (7.86 - 8.22)	8.96 (8.66 - 9.40)	
	Cases/ subjects	331/ 10699	384/ 10700	411/ 10700	462/ 10700	613/ 10699	
	HR (95% CI)	Ref	0.99 (0.84, 1.16)	0.93 (0.78, 1.12)	0.93 (0.76, 1.15)	1.05 (0.82, 1.35)	0.76
Stearic acid (18:0)							
EPIC-Norfolk	Median intake (IQR)	1.96 (1.73 - 2.12)	2.46 (2.37 - 2.56)	2.83 (2.74 - 2.92)	3.21 (3.11 - 3.34)	3.84 (3.63 - 4.16)	
	Cases/ subjects	187/ 4324	232/ 4325	236/ 4324	233/ 4325	239/ 4324	
	HR (95% CI)	Ref	0.98 (0.79, 1.21)	0.90 (0.71, 1.15)	0.81 (0.62, 1.07)	0.72 (0.50, 1.03)	**0.04**
EPIC-Denmark	Median intake (IQR)	2.33 (2.08 - 2.49)	2.84 (2.74 - 2.93)	3.18 (3.10 - 3.26)	3.52 (3.43 - 3.62)	4.04 (3.87 - 4.32)	
	Cases/ subjects	331/ 10699	378/ 10700	450/ 10700	461/ 10700	581/ 10699	
	HR (95% CI)	Ref	0.93 (0.79, 1.09)	0.95 (0.80, 1.13)	0.86 (0.71, 1.05)	0.94 (0.76, 1.17)	0.68

All HRs are adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake, intakes of protein, PUFA, cholesterol, vitamin c, fibre, and the sum of the other SFAs, and in EPIC-Norfolk for *trans*-fat

Supplemental Table S7. HR with 95% CI for the association between individual SFAs and incident MI in subjects who did not use of lipid lowering medication at baseline

		Q1	Q2	Q3	Q4	Q5	*P* for trend
Butyric acid (4:0) - capric acid (10:0)							
EPIC - Norfolk	Cases/ subjects	232/ 4377	242/ 4378	240/ 4378	215/ 4378	254/ 4378	
	HR (95% CI)	Ref	0.95 (0.79, 1.15)	0.94 (0.77, 1.13)	**0.77 (0.62, 0.96)**	0.79 (0.60, 1.03)	0.06
EPIC - Denmark	Cases/ subjects	467/ 10760	439/ 10761	423/ 10761	412/ 10761	491/ 10761	
	HR (95% CI)	Ref	0.95 (0.83, 1.08)	0.90 (0.78, 1.04)	**0.84 (0.72, 0.98)**	0.94 (0.79, 1.12)	0.34
Lauric acid (12:0)							
EPIC - Norfolk	Cases/ subjects	202/ 4377	207/ 4378	253/ 4378	264/ 4378	257/ 4378	
	HR (95% CI)	Ref	0.97 (0.79, 1.18)	1.11 (0.91, 1.35)	1.06 (0.85, 1.31)	0.99 (0.77, 1.28)	0.98
EPIC - Denmark	Cases/ subjects	452/ 10760	422/ 10761	447/ 10761	475/ 10761	436/ 10761	
	HR (95% CI)	Ref	0.93 (0.81, 1.07)	0.94 (0.82, 1.09)	0.97 (0.83, 1.14)	0.88 (0.72, 1.07)	0.33
Myristic acid (14:0)							
EPIC - Norfolk	Cases/ subjects	221/ 4377	233/ 4378	231/ 4378	237/ 4378	261/ 4378	
	HR (95% CI)	Ref	0.90 (0.74, 1.09)	0.85 (0.69, 1.06)	**0.78 (0.61, 0.99)**	0.74 (0.53, 1.02)	0.07
EPIC - Denmark	Cases/ subjects	418/ 10760	446/ 10761	435/ 10761	438/ 10761	495/ 10761	
	HR (95% CI)	Ref	0.97 (0.84, 1.12)	0.90 (0.76, 1.05)	0.85 (0.71, 1.02)	0.91 (0.73, 1.13)	0.25
Palmitic acid (16:0)							
EPIC - Norfolk	Cases/ subjects	207/ 4377	234/ 4378	251/ 4378	246/ 4378	242/ 4378	
	HR (95% CI)	Ref	0.99 (0.81, 1.21)	1.02 (0.82, 1.27)	0.93 (0.72, 1.20)	0.98 (0.70, 1.37)	0.79

EPIC - Denmark	Cases/ subjects	330/ 10760	393/ 10761	248/ 10761	236/ 10761	258/ 10761	
	HR (95% CI)	Ref	1.02 (0.86, 1.19)	0.95 (0.80, 1.15)	0.98 (0.80, 1.20)	1.08 (0.85, 1.38)	0.60
Pentadecylic (15:0) & margaric (17:0) acid							
EPIC - Norfolk	Cases/ subjects	221/ 4377	233/ 4378	231/ 4378	237/ 4378	261/ 4378	
	HR (95% CI)	Ref	0.86 (0.71, 1.05)	0.86 (0.69, 1.06)	**0.77 (0.60, 0.98)**	0.74 (0.54, 1.02)	0.08
Pentadecylic acid (15:0)							
EPIC - Denmark	Cases/ subjects	2/ 10748	450/ 10749	432/ 10748	429/ 10749	458/ 10748	
	HR (95% CI)	Ref	0.99 (0.86, 1.13)	0.94 (0.81, 1.09)	0.91 (0.77, 1.08)	0.95 (0.77, 1.17)	0.48
Stearic acid (16:0)							
EPIC - Norfolk	Cases/ subjects	199/ 4377	245/ 4378	251/ 4378	246/ 4378	242/ 4378	
	HR (95% CI)	Ref	0.99 (0.81, 1.22)	0.93 (0.74, 1.18)	0.84 (0.64, 1.10)	0.71 (0.50, 1.01)	**0.04**
EPIC - Denmark	Cases/ subjects	327/ 10760	391/ 10761	463/ 10761	465/ 10761	586/ 10761	
	HR (95% CI)	Ref	0.97 (0.83, 1.14)	1.00 (0.84, 1.19)	0.89 (0.73, 1.08)	0.98 (0.78, 1.22)	0.76

All HRs are adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake, intakes of protein, PUFA, cholesterol, vitamin c, fibre, and the sum of the other SFAs, and in EPIC-Norfolk for *trans*-fat

EPIC-Norfolk: n = 21889

EPIC-Denmark: n = 53804

Supplemental Table S8. Hazard ratios (95% CI) for the associations between the individual SFAs (in quintiles) and MI incidence risk in 20046 participants* of EPIC-Norfolk, after additional adjustment for the ratio of total: HDL cholesterol levels.

	Q1	Q2	Q3	Q4	Q5	*P* for trend
Butyric acid (4:0) - capric acid (10:0)						
Cases/ subjects	212/ 3991	210/ 3997	223/ 4034	194/ 4017	217/ 4007	
Model 1	Ref	0.90 (0.74, 1.10)	0.93 (0.76, 1.14)	**0.76 (0.61, 0.95)**	**0.74 (0.55, 0.98)**	**0.03**
Model 2	Ref	0.91 (0.75, 1.11)	0.94 (0.77, 1.15)	**0.77 (0.61, 0.96)**	**0.73 (0.55, 0.97)**	**0.02**
Lauric acid (12:0)						
Cases/ subjects	189/ 3997	181/ 4003	218/ 4006	241/ 4029	227/ 4011	
Model 1	Ref	0.91 (0.74, 1.12)	1.04 (0.84, 1.29)	1.05 (0.83, 1.31)	0.97 (0.74, 1.27)	0.95
Model 2	Ref	0.91 (0.74, 1.13)	1.05 (0.85, 1.30)	1.05 (0.84, 1.31)	0.97 (0.75, 1.27)	0.95
Myristic acid (14:0)						
Cases/ subjects	197/ 3986	210/ 4028	217/ 4014	206/ 4016	2626/ 4002	
Model 1	Ref	0.90 (0.73, 1.11)	0.90 (0.71, 1.13)	**0.76 (0.58, 0.99)**	0.73 (0.51, 1.04)	0.06
Model 2	Ref	0.90 (0.73, 1.11)	0.90 (0.72, 1.13)	**0.76 (0.58, 0.99)**	0.72 (0.51, 1.02)	**0.05**
Palmitic acid (16:0)						
Cases/ subjects	187/ 4012	210/ 4020	231/ 4035	209/ 4003	219/ 3976	
Model 1	Ref	0.99 (0.80, 1.23)	1.07 (0.84, 1.35)	0.94 (0.71, 1.24)	0.97 (0.68, 1.39)	0.80
Model 2	Ref	1.00 (0.81, 1.24)	1.07 (0.85, 1.36)	0.95 (0.72, 1.25)	0.98 (0.68, 1.40)	0.82
Pentadecylic (15:0) & margaric (17:0) acid						
Cases/ subjects	201/ 4010	191/ 4022	223/ 4009	205/ 4031	236/ 3974	
Model 1	Ref	0.89 (0.72, 1.09)	0.92 (0.74, 1.16)	0.78 (0.61, 1.02)	0.82 (0.59, 1.15)	0.27
Model 2	Ref	0.89 (0.72, 1.10)	0.93 (0.74, 1.17)	0.79 (0.61, 1.02)	0.82 (0.59, 1.14)	0.23

Stearic acid (18:0)						
Cases/ subjects	186/ 4015	221/ 4024	221/ 4006	220/ 3992	208/ 4009	
Model 1	Ref	0.95 (0.77, 1.18)	0.90 (0.70, 1.15)	0.81 (0.61, 1.07)	**0.67 (0.46, 0.97)**	**0.02**
Model 2	Ref	0.96 (0.77, 1.19)	0.90 (0.71, 1.15)	0.82 (0.62, 1.09)	**0.67 (0.46, 0.98)**	**0.03**

These analyses were conducted in participants with complete data on baseline levels of total- and HDL-cholesterol levels only.

Model 1 is adjusted the HRs HRs are adjusted for age, sex, total energy intake, BMI, waist circumference, physical activity, smoking, education level, alcohol intake, intakes of protein, PUFA, cholesterol, vitamin c, fibre, and the sum of the other SFAs, and for *trans*-fat

Model 2 is additionally adjusted for the ratio of baseline total: HDL-cholesterol levels

Supplemental Table S9. Hazard ratios (95% CI) for the associations between the substitution individual SFAs (in quintiles) for carbohydrates, PUFA, MUFA and protein, and MI incidence risk in EPIC-Norfolk and EPIC-Denmark

	Q1	Q2	Q3	Q4	Q5	*P* for trend
Butyric acid (4:0) - capric acid (10:0)						
EPIC-Norfolk						
For carbohydrates	Ref	0.95 (0.79, 1.14)	0.95 (0.78, 1.15)	**0.79 (0.63, 0.98)**	0.79 (0.59, 1.06)	0.09
For *cis*-PUFA	Ref	0.95 (0.79, 1.14)	0.95 (0.78, 1.15)	**0.78 (0.63, 0.97)**	0.78 (0.59, 1.04)	0.06
For *cis*-MUFA	Ref	0.95 (0.79, 1.15)	0.96 (0.79, 1.16)	**0.80 (0.64, 0.99)**	0.81 (0.60, 1.09)	0.13
For protein	Ref	0.95 (0.79, 1.14)	0.95 (0.79, 1.16)	**0.79 (0.63, 0.98)**	0.80 (0.59, 1.07)	0.10
EPIC-Denmark						
For carbohydrates	Ref	0.92 (0.81, 1.05)	0.87 (0.76, 1.01)	**0.82 (0.71, 0.96)**	0.94 (0.78, 1.12)	0.36
For PUFA	Ref	0.92 (0.81, 1.05)	0.87 (0.76, 1.01)	**0.82 (0.71, 0.96)**	0.94 (0.78, 1.12)	0.35
For MUFA	Ref	0.92 (0.80, 1.04)	0.87 (0.76, 1.00)	**0.82 (0.71, 0.95)**	0.93 (0.79, 1.10)	0.33
For protein	Ref	0.92 (0.80, 1.04)	0.87 (0.76, 1.00)	**0.82 (0.70, 0.96)**	0.93 (0.78, 1.11)	0.32
Lauric acid (12:0)						
EPIC-Norfolk						
For carbohydrates	Ref	0.92 (0.76, 1.13)	1.06 (0.87, 1.30)	1.01 (0.81, 1.25)	0.96 (0.74, 1.24)	0.86
For *cis*-PUFA	Ref	0.92 (0.75, 1.12)	1.05 (0.86, 1.28)	0.99 (0.80, 1.24)	0.94 (0.72, 1.22)	0.73
For *cis*-MUFA	Ref	0.93 (0.76, 1.13)	1.07 (0.88, 1.31)	1.02 (0.82, 1.26)	0.98 (0.76, 1.26)	0.99
For protein	Ref	0.92 (0.76, 1.13)	1.06 (0.87, 1.30)	1.01 (0.81, 1.25)	0.96 (0.74, 1.25)	0.89
EPIC-Denmark						
For carbohydrates	Ref	0.90 (0.78, 1.03)	0.90 (0.78, 1.05)	0.95 (0.80, 1.12)	0.85 (0.68, 1.06)	0.29
For PUFA	Ref	0.90 (0.78, 1.03)	0.90 (0.78, 1.05)	0.95 (0.80, 1.12)	0.85 (0.68, 1.06)	0.29
For MUFA	Ref	0.90 (0.78, 1.03)	0.90 (0.78, 1.04)	0.94 (0.80, 1.12)	0.84 (0.68, 1.05)	0.26
For protein	Ref	0.90 (0.78, 1.03)	0.90 (0.78, 1.05)	0.95 (0.80, 1.12)	0.85 (0.68, 1.06)	0.28

Myristic acid (14:0)						
EPIC-Norfolk						
For carbohydrates	Ref	0.89 (0.73, 1.08)	0.85 (0.68, 1.06)	**0.75 (0.59, 0.97)**	0.72 (0.51, 1.03)	0.07
For *cis*-PUFA	Ref	0.88 (0.73, 1.07)	0.84 (0.68, 1.05)	**0.74 (0.58, 0.96)**	0.71 (0.50, 1.00)	0.05
For *cis*-MUFA	Ref	0.89 (0.73, 1.08)	0.86 (0.69, 1.07)	**0.77 (0.60, 0.99)**	0.75 (0.53, 1.06)	0.10
For protein	Ref	0.89 (0.73, 1.08)	0.85 (0.69, 1.06)	**0.76 (0.59, 0.97)**	0.73 (0.51, 1.03)	0.07
EPIC-Denmark						
For carbohydrates	Ref	0.96 (0.84, 1.11)	0.88 (0.75, 1.03)	0.85 (0.71, 1.01)	0.91 (0.73, 1.14)	0.27
For PUFA	Ref	0.96 (0.84, 1.11)	0.88 (0.75, 1.02)	0.85 (0.71, 1.01)	0.91 (0.73, 1.13)	0.25
For MUFA	Ref	0.96 (0.84, 1.11)	0.88 (0.75, 1.02)	0.85 (0.71, 1.01)	0.91 (0.73, 1.13)	0.25
For protein	Ref	0.96 (0.84, 1.11)	0.88 (0.75, 1.02)	0.85 (0.71, 1.01)	0.91 (0.73, 1.13)	0.25
Pentadecylic (15:0) & margaric (17:0) acid						
EPIC-Norfolk						
For carbohydrates	Ref	0.88 (0.72, 1.06)	0.85 (0.69, 1.06)	**0.78 (0.61, 0.99)**	0.75 (0.54, 1.03)	0.09
For *cis*-PUFA	Ref	0.87 (0.72, 1.06)	0.85 (0.69, 1.05)	**0.77 (0.61, 0.99)**	0.74 (0.54, 1.02)	0.08
For *cis*-MUFA	Ref	0.88 (0.72, 1.07)	0.86 (0.69, 1.06)	**0.78 (0.61, 1.00)**	0.75 (0.55, 1.03)	0.10
For protein	Ref	0.88 (0.72, 1.06)	0.85 (0.69, 1.06)	**0.78 (0.61, 0.99)**	0.75 (0.54, 1.03)	0.09
Pentadecylic acid (15:0)						
EPIC-Denmark						
For carbohydrates	Ref	0.97 (0.84, 1.10)	0.93 (0.80, 1.07)	0.90 (0.76, 1.06)	0.95 (0.76, 1.17)	0.48
For PUFA	Ref	0.96 (0.84, 1.10)	0.93 (0.80, 1.07)	0.90 (0.76, 1.06)	0.94 (0.76, 1.17)	0.47
For MUFA	Ref	0.96 (0.84, 1.10)	0.92 (0.80, 1.07)	0.90 (0.76, 1.06)	0.94 (0.76, 1.17)	0.46
For protein	Ref	0.97 (0.84, 1.10)	0.93 (0.80, 1.07)	0.90 (0.76, 1.07)	0.95 (0.77, 1.17)	0.49

table continues on next page

Supplemental Table S9 continued

	Q1	Q2	Q3	Q4	Q5	*P* for trend
Palmitic acid (16:0)						
EPIC-Norfolk						
For carbohydrates	Ref	0.99 (0.80, 1.22)	1.01 (0.79, 1.29)	0.91 (0.69, 1.22)	0.95 (0.65, 1.39)	0.68
For *cis*-PUFA	Ref	0.96 (0.78, 1.20)	0.97 (0.76, 1.25)	0.87 (0.64, 1.17)	0.87 (0.59, 1.30)	0.42
For *cis*-MUFA	Ref	1.00 (0.80, 1.24)	1.03 (0.80, 1.32)	0.94 (0.69, 1.27)	0.99 (0.66, 1.48)	0.84
For protein	Ref	0.99 (0.80, 1.22)	1.01 (0.80, 1.29)	0.92 (0.69, 1.23)	0.96 (0.66, 1.41)	0.73
EPIC-Denmark						
For carbohydrates	Ref	0.97 (0.82, 1.14)	0.92 (0.76, 1.12)	0.94 (0.75, 1.17)	1.03 (0.78, 1.36)	0.88
For PUFA	Ref	0.97 (0.82, 1.14)	0.92 (0.75, 1.12)	0.93 (0.74, 1.17)	1.02 (0.76, 1.35)	0.91
For MUFA	Ref	0.97 (0.82, 1.14)	0.92 (0.75, 1.12)	0.93 (0.73, 1.18)	1.01 (0.75, 1.37)	0.96
For protein	Ref	0.97 (0.82, 1.14)	0.92 (0.76, 1.11)	0.93 (0.74, 1.16)	1.01 (0.76, 1.34)	0.96
Stearic acid (18:0)						
EPIC-Norfolk						
For carbohydrates	Ref	1.01 (0.82, 1.24)	0.94 (0.74, 1.19)	0.86 (0.66, 1.13)	0.76 (0.53, 1.08)	0.08
For *cis*-PUFA	Ref	1.00 (0.81, 1.23)	0.93 (0.73, 1.17)	0.84 (0.64, 1.11)	0.73 (0.51, 1.04)	0.05
For *cis*-MUFA	Ref	1.02 (0.83, 1.25)	0.96 (0.75, 1.21)	0.88 (0.67, 1.17)	0.79 (0.54, 1.14)	0.14
For protein	Ref	1.01 (0.82, 1.24)	0.94 (0.74, 1.19)	0.87 (0.66, 1.14)	0.76 (0.53, 1.09)	0.09
EPIC-Denmark						
For carbohydrates	Ref	0.94 (0.80, 1.10)	0.95 (0.80, 1.14)	0.88 (0.72, 1.07)	0.94 (0.75, 1.19)	0.68
For PUFA	Ref	0.94 (0.80, 1.10)	0.95 (0.80, 1.14)	0.87 (0.71, 1.07)	0.94 (0.74, 1.19)	0.66
For MUFA	Ref	0.94 (0.80, 1.10)	0.95 (0.79, 1.14)	0.87 (0.71, 1.08)	0.94 (0.73, 1.20)	0.67
For protein	Ref	0.94 (0.80, 1.10)	0.95 (0.80, 1.13)	0.87 (0.71, 1.06)	0.93 (0.74, 1.17)	0.61

HRs are adjusted for energy intake from all macronutrients including the sum of the other SFAs and except for the macronutrient that is substituted, for total energy (excluding alcohol), age, sex, BMI, waist circumference, physical activity, smoking, education level, alcohol intake, cholesterol, vitamin c, and fibre.

Chapter 4.

Fish intake and CVD in the EPIC-NL cohort

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Abstract

Background: Consumption of one portion of fish per week is suggested to reduce the risk of incident stroke and fatal coronary heart disease (CHD). However, evidence is limited for associations of *1*) intakes of less than one portion per week as compared with no fish consumption, and *2*) types of fish with risk of cardiovascular disease (CVD).

Objective: To investigate the associations of a very low intake compared with no intake of total fish, fatty fish, and lean fish with incidences of total and subtypes of stroke, CHD, myocardial infarction (MI), and CVD mortality.

Methods: Data were used from 34033 participants, aged 20-70 years, of the EPIC-NL cohort. Baseline fish consumption was estimated with use of a validated food frequency questionnaire. With Cox regression models hazard ratios (HR) were calculated for the associations between consumption of total and subtypes of fish and incident CVD events. We compared any fish consumption, <1 portion (<100 grams) fish/week and ≥1 portion fish/week to fish non-consumers.

Results: During 15.1 years of follow-up, 753 stroke events, 2134 CHD events and 540 CVD deaths occurred. Among the fish consumers (~92%) median intakes of total, lean and fatty fish were 57.9, 32.9 and 10.7 grams/week, respectively. Compared with the fish non-consumers, fish consumption was not associated with total stroke (HR: 0.93, 95% CI: 0.82 – 1.05). Lower risks of ischaemic stroke were observed in participants who consumed ≥ 1 portion/week of fatty fish (HR: 0.63, 95% CI: 0.39 – 1.02) and ≥ 1 portion/week of lean fish (HR: 0.70, 95% CI: 0.57 – 0.86), but not in those who consumed less (HR: 0.92, 95% CI: 0.77 – 1.10). In participants who consumed only fatty fish, a lower risk of incident CHD (HR: 0.82, 95% CI: 0.67 – 0.99) was observed, but not in participants who consumed both fatty and lean fish. No associations were observed between total fish consumption and risks of incident MI or CVD mortality.

Conclusion: In this study, an inverse association between fish consumption and ischaemic stroke was observed, although only for the consumption of at least one portion per week of lean fish and fatty fish. A potential protective association of fatty fish with the risk of incident CHD cannot be ruled out by the present study.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide [1, 2]. A healthy diet is one of the modifiable lifestyle factors that contribute to a reduction in CVD risk [3]. International dietary guidelines [4-6] advocate that a healthy diet should contain at least one to two servings of fish, preferably fatty fish, per week. According to the recently published dietary guidelines of the Health Council of the Netherlands [7], sufficient scientific evidence indicates that the consumption of one serving (100 grams) of fish per week lowers the risk of incident total (fatal plus nonfatal) stroke [8, 9] and fatal coronary heart disease (CHD) [10]. To reduce the risk of incident CHD, the consumption of at least five portions per week would be necessary [11, 12].

Yet, according to the Dutch National Food Consumption Survey 2007-2010 [13] the average fish intake in the Netherlands is insufficient. The recommendation to consume fish at least once per week is met by approximately one-third of the people up to 30 years old and by nearly two-thirds of those older than 51 years of age [13]. With this knowledge, it would be of interest to know whether very low intakes of fish (i.e., less than one portion per week), which is typical in the Dutch population, is associated with a reduced risk of CVD, but this is currently unclear.

To our knowledge, a limited number of studies investigated the associations of a very low fish consumption with the risk of stroke and CHD [14-18]. Moreover, the findings of these studies are conflicting: three studies observed no associations of very low fish intake with incident total stroke [14] and mortality due to stroke [14], CHD [15] or myocardial infarction (MI) [16]. Two other studies in the Dutch MORGEN cohort observed lower risks of incident stroke in women, but not in men [17], and a lower risk of CHD mortality [18]. However, both studies in MORGEN combined non-consumers with consumers of very low amounts of fish (i.e., less than one portion of fish per week) as the reference category, whereas it may be important to evaluate the very low fish consumers separately.

One of the factors thought to be responsible for the inverse association between fish consumption and CVD risk is omega-3 polyunsaturated fatty acids (*n-3* PUFAs) [19-21]. Since *n-3* PUFAs are more abundant in fatty fish than in lean fish, fatty fish might be more protective against CVD than lean fish [19]. However, the evidence for different types of fish in general is inconclusive [22, 23]. In addition, to our knowledge, none of the studies that addressed very low fish intakes versus no fish intake considered the type of fish [14, 16].

The aim of the present study is to investigate the associations of very low intake compared with no intake of total fish, fatty fish and lean fish with incidences of total stroke, haemorrhagic stroke, ischaemic stroke, CHD, MI, and with CVD mortality in a Dutch population that typically consumes very low amounts of fish. Data were used from the European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL) cohort. Because the MORGEN cohort is one of the two centers of the EPIC-NL cohort, we partly repeated previous analyses in this cohort [17, 18], with the advantage of a longer follow-up time, more cases, and a larger sample size.

Methods

Study population

The EPIC-NL cohort is the Dutch part of the EPIC (European Prospective Investigation into Cancer and Nutrition) study, which started in 1993 with the aim to investigate the role of nutrition in the occurrence of cancer [24, 25]. Details about the design and rationale of EPIC-NL can be found elsewhere [26]. In brief, EPIC-NL consists of two ongoing Dutch cohorts: the Prospect cohort and the MORGEN (Monitoring Project on Chronic Disease Risk Factors) cohort. Both cohorts were set up simultaneously between 1993 and 1997. The Prospect cohort included 17357 women who participated in the nationwide Dutch breast cancer screening program and lived in Utrecht or its surroundings. The MORGEN cohort included 22654 men and women, aged 20 through 65 years, who were randomly selected from a general population sample of three Dutch towns (Amsterdam, Doetinchem and Maastricht). In total, the EPIC-NL cohort comprised 40011 participants. All participants signed informed consent before inclusion. The present study complied with the Declaration of Helsinki and was approved by the Institutional Board of the University Medical Center Utrecht (Prospect) and the Medical Ethics Committee of the Netherlands Organisation for Applied Scientific Research (TNO) Nutrition and Food Research (MORGEN).
For this study, participants were excluded if they withheld consent for linkage with disease and vital status registries (n = 1304), if their vital status (n = 417) or cause of death (n = 143) was unknown, if they had CVD (n = 1470), diabetes (n = 681), or cancer (n = 1531) at baseline, if they had missing dietary data (n = 117), or if their reported energy intake was implausible compared with their estimated basal metabolic rate (i.e., the bottom and top 0.5% of the energy intake to basal metabolic rate ratio distribution; n = 315). Finally, 34033 participants were included in this study.

Assessment of dietary intake

The average daily intake of 178 foods was assessed by a validated self-administered food frequency questionnaire (FFQ). The FFQ consisted of 213 questions on the average amount, frequency and type of 79 main food items that were consumed in the year preceding enrolment. The average intakes of energy and nutrients were calculated using a digital update of the Dutch food consumption database of 1996 [27].
To determine fish intake, the participants were asked to specify the frequency of consumption of fish, mussels and prawns in times per day, week, month or year. Subsequently, they could specify the type of fish they consumed by indicating the consumption frequency of fish from the three following categories: *1*) plaice, cod, fish fingers and fried fillet of haddock; *2*) mussels and prawns; and *3*) eel, mackerel, fresh herring, herring and canned fish.
For this study, fish was divided into 3 types of fish: fatty fish (eel, mackerel, fresh herring, herring, and canned fish), lean fish (plaid, cod, fish fingers, fried fillet, and haddock) and

shellfish (mussels and prawns). Total fish is the sum of fatty fish, lean fish and shellfish. Fish consumption and consumption of other food groups, products and nutrients, except for alcohol, were adjusted for total energy intake with use of the nutrient residual model [28]. Before the start of the EPIC-NL study, the FFQ was validated against 12 non-consecutive 24-hour recalls among 121 Dutch men and women. For total fish consumption, Spearman rank correlation coefficients were 0.32 for men and 0.37 for women [29].

Assessment of cardiovascular events

Data on the occurrence of mortality during follow-up were obtained through linkage with the municipal population registries, and causes of death were obtained through linkage with 'Statistics Netherlands' (CBS). Data on the occurrence of CVD morbidity were obtained from the Dutch Hospital Association and Order of Medical Specialists which holds a register of diagnoses at hospital discharge. In addition, admission files have been stored from hospitals in the Netherlands since 1990. Participants with prevalent CVD and/or diabetes at baseline were identified through linkage with the National Medical Registry (NMR) or by the participants' self-reported diagnosis in the baseline general questionnaire. For this analysis, incident fatal or nonfatal CVD events were divided into several types, according to the International Classification of Diseases, Tenth Revision (ICD-10). The main outcomes included stroke (I60-I66), haemorrhagic stroke (I60-I62), ischaemic stroke (I63 and I65), CHD (I20-I25, I46 and R96), MI (I21 and I22) and CVD mortality (I20-I26, I46, R96, G45, I60-I67, I69, I70-I74 and I50). For this analysis, follow-up was complete until 1 January 2011.

Assessment of covariates

At baseline, all participants were administered a general questionnaire which gathered information on demographics, lifestyle factors and presence of chronic diseases. Physical activity was calculated with the Cambridge Physical Activity Index [30], that divided participants into four categories: inactive, moderately inactive, moderately active and active. Smoking status was defined as current, former and never. Education level was categorized as low (primary education up to finishing intermediate vocational education), medium (higher general secondary education) and high (higher vocational education and university). Alcohol intake was measured by the FFQ and categorised as follows: 0, 0.1-6.0, 6.1-12.0, 12.1-24.0, and >24 g/d of ethanol for women and 0, 0.1-6.0, 6.1-12.0, 12.1-24.0, 24.1-60.0, and >60 g/d of ethanol for men [31]. During the physical examination at baseline, body height was measured to the nearest 0.5 cm by a tapeline fixed to the wall while the participant was without shoes. Body weight was measured to the nearest 0.5 kg with a floor scale while the participant was in light indoor clothing and without shoes. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2). Blood pressure was measured twice on the left arm with use of a Boso Oscillomat (Bosh & Son, Jungingen, Germany) (Prospect) or a random zero sphygmomanometer (MORGEN). The

Table 1 Baseline characteristics of fish non-consumers and fish consumers in the EPIC-NL cohort.

	Categories of total fish consumption			
	Median (5th – 95th percentiles) total fish intake (g/week)			
	Non-consumers	**All consumers**	**<1 portion/week** [a]	**≥1 portion/week**
		57.9 (7.0 – 207.1)	36.6 (20.1–64.0)	130.1 (102.9–313.1)
Participants, n	2593	31440	22609	8831
Male, %	27	25	29	17
Age, *y*	48.3 (± 13.7) [b]	48.7 (± 11.8)	47.6 (± 12.0)	51.4 (± 10.9)
Physically active %	41	42	43	41
Current smoker, %	30	30	31	29
High education level, %	11	22	21	23
Body Mass Index, *kg/m²*	25.8 (± 4.1)	25.6 (± 3.9)	25.5 (± 3.9)	25.7 (± 4.0)
Waist circumference, *cm*	86 (± 12)	85 (± 11)	85 (± 11)	85 (± 11)
Systolic blood pressure, *mmHg*	127 (± 20)	126 (± 19)	125 (± 18)	127 (± 20)
Ratio total-: HDL-cholesterol	4.1 (3.3 – 5.2) [c]	4.0 (3.2 – 5.0)	4.0 (3.2 – 5.0)	4.0 (3.2 – 5.0)
Dietary intake [d]				
Total energy, *kcal/d*	1907 (1571 – 2407)	1971 (1643 – 2388)	2036 (1690 – 2483)	1825 (1553 – 2156)
Fatty fish, *g/wk*	– –	10.7 (4.7 – 24.2)	7.2 (3.3 – 15.7)	28.7 (13.6 – 48.6)
Lean fish, *g/wk*	– –	32.9 (13.6 – 66.4)	21.4 (9.7 – 38.1)	93.7 (70.5 – 127.1)
Shellfish, *g/wk*	– –	4.8 (2.1 – 11.3)	3.3 (1.5 – 7.9)	11.6 (5.2 – 21.0)
EPA, *mg/d*	– –	35 (15 – 63)	22 (11 – 39)	80 (63 – 104)
DHA, *mg/d*	11 (7 – 17)	79 (42 – 133)	56 (35 – 85)	161 (139 – 199)
ALA, *mg/d*	874 (692 – 1130)	911 (741 – 1132)	909 (742 – 1127)	915 (740 – 1145)
Alcohol, *g/d*	1.4 (0.0 – 9.2)	5.7 (0.9 – 16.4)	5.5 (0.9 – 16.1)	6.1 (1.0 – 17.4)
Fruit, *g/d*	245 (± 170)	261 (± 166)	251 (± 163)	286 (± 169)
Vegetables, *g/d*	130 (± 57)	138 (± 54)	135 (± 52)	147 (± 57)

Saturated fatty acids, *g/d*	33.4 (± 6.2)	32.6 (± 5.8)	33 (± 5.7)	31.8 (± 6.0)
Trans fatty acids, *g/d*	3.1 (± 1.2)	2.9 (± 1.1)	2.9 (± 1.1)	2.7 (± 1.0)
Fibre, *g/d*	23.8 (± 5.2)	23.3 (± 4.7)	23.3 (± 4.8)	23.5 (± 4.7)

[a] 1 portion equals 100 grams.

[b] Data are presented as mean ± standard deviation (all such values).

[c] Data are presented as median with interquartile range (all such values).

[d] All nutrients and foods were adjusted for total energy intake, except for alcohol intake.

Table 2. Associations between total fish consumption and risk of incident total stroke, haemorrhagic stroke, ischaemic stroke, coronary heart disease, myocardial infarction, and of cardiovascular mortality.

	Categories of total fish consumption			
	Median (5th – 95th percentiles) total fish intake (g/week)			
	No consumption	**All consumption**	**<1 portion [a] /week**	**≥1 portion/week**
		57.9 (7.0 - 144.9)	36.6 (5.6 – 92.8)	130.1 (102.9 – 313.1)
	HR	HR (95% CI)	HR (95% CI)	HR (95% CI)
No. participants	2593	31440	22609	8831
Total stroke				
Cases	69	684	470	214
Model 1 [b]	Ref	0.92 (0.81 – 1.05)	0.93 (0.82 – 1.06)	0.91 (0.79 – 1.04)
Model 2 [c]	Ref	0.93 (0.82 – 1.05)	0.93 (0.82 – 1.06)	0.91 (0.79 – 1.05)
Haemorrhagic stroke				
Cases	19	201	144	57
Model 1	Ref	0.91 (0.71 – 1.16)	0.96 (0.75 – 1.22)	0.80 (0.61 – 1.04)
Model 2	Ref	0.90 (0.71 – 1.15)	0.95 (0.74 – 1.22)	0.79 (0.60 – 1.03)
Ischaemic stroke				
Cases	39	374	260	114
Model 1	Ref	0.89 (0.75 – 1.06)	0.91 (0.77 – 1.09)	0.84 (0.63 – 1.13)
Model 2	Ref	0.91 (0.76 – 1.07)	0.92 (0.77 – 1.10)	0.87 (0.72 – 1.05)
Coronary heart disease				
Cases	178	1956	1388	568
Model 1	Ref	1.03 (0.96 – 1.12)	1.04 (0.96 – 1.12)	1.04 (0.95 – 1.13)
Model 2	Ref	1.03 (0.95 – 1.11)	1.03 (0.95 – 1.12)	1.03 (0.94 – 1.12)
Myocardial infarction				
Cases	62	631	459	172
Model 1	Ref	1.00 (0.87 – 1.14)	1.02 (0.89 – 1.17)	0.96 (0.83 – 1.11)
Model 2	Ref	1.00 (0.88 – 1.15)	1.02 (0.89 – 1.17)	0.97 (0.83 – 1.13)
Cardiovascular mortality				
Cases	54	486	339	147
Model 1	Ref	0.94 (0.82 – 1.09)	0.96 (0.83 – 1.11)	0.91 (0.67 – 1.24)
Model 2	Ref	0.96 (0.83 – 1.11)	0.97 (0.83 – 1.12)	0.94 (0.80 – 1.10)

[a] 1 portion equals 100 grams.

[b] Model 1 is adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake and total energy intake.

[c] Model 2 is adjusted for model 1 and for intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

first measurement was performed 5 to 15 minutes after arrival and the second measurement 10 minutes thereafter while the participant was in a supine position. Mean systolic and diastolic blood pressure were calculated. The presence of hypertension was determined by the participants' self-reported presence of hypertension or use of anti-hypertensive medication, measured diastolic blood pressure >90 mm Hg or measured systolic blood pressure >140 mmHg [32]. Blood samples were taken and stored. Measurements of serum concentrations of total cholesterol and high-density lipoprotein (HDL)-cholesterol were performed on an auto analyser (LX20, Beckman Coulter, Mijdrecht, The Netherlands).

Data analysis

Baseline characteristics of the participants were calculated as means with SD, as medians with IQR, or as percentages. Pearson correlation coefficients were calculated between the energy-adjusted intakes of total fish, fatty fish, lean fish and shellfish.
Missing data were present on 8 covariates. The percentages of missing values ranged from 0.05% (BMI) to 2.93% (HDL cholesterol). Multiple imputation was used to deal with these missing data (**Supplemental Table S1**). Ten imputed datasets were constructed, and reported results were based on values pooled using Rubin's rule.
Cox proportional hazards models, stratified by cohort, were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations between fish consumption and risks of incident total stroke, haemorrhagic stroke, ischaemic stroke, CHD, MI and CVD mortality. Participants were categorized into fish non-consumers (participants who reported no fish consumption; 0 g/week) and fish consumers (participants who reported any fish consumption; >0 g/week). The fish consumers were further categorized according to the consumption frequency of total fish into participants who consumed less than one portion per week (<100 g/week) and participants who consumed at least one portion of fish per week (≥100 g/week). Because none of the participants reported consumption of only lean fish, we used two different approaches to categorize the fish consumers according to the types of fish they consumed. Firstly, we separated the fish consumers into those who consumed only fatty fish and those who consumed both fatty and lean fish. Secondly, fish consumers were categorized according to the consumption frequency (<1 portion/week versus ≥1 portion/week) of fatty fish and of lean fish, separately. In all analyses, the fish non-consumers served as the reference category.
Two sequential Cox models were build to adjust for potential confounding. The first model was adjusted for age, sex, total energy intake, physical activity, smoking status, education level, BMI and alcohol intake. Model 2 was additionally adjusted for energy-adjusted intakes of saturated fatty acids, *trans*-fatty acids, fruit, vegetables and dietary fibre. The proportional hazards assumption was examined by calculating Schoenfeld residuals and visual examination of log-log plots. No significant violations were observed.

Table 3. Associations between types of fish consumption and risk of incident total stroke, haemorrhagic stroke, ischaemic stroke, coronary heart disease, myocardial infarction, and of cardiovascular mortality

	Categories of fish consumption		
	Median (5th – 95th percentiles) intake (g/week)		
	No fish	**Only fatty fish**	**Fatty and lean fish**
		3.6 (1.8 – 7.2)	59.3 (26.7 – 106.7)
	HR	HR (95% CI)	HR (95% CI)
No. of participants	2593	595	30845
Total stroke			
Cases	69	16	668
Model 1 [a]	Ref	0.85 (0.65 – 1.13)	0.92 (0.81 – 1.05)
Model 2 [b]	Ref	0.86 (0.65 – 1.14)	0.93 (0.82 – 1.06)
Haemorrhagic stroke			
Cases	19	4	197
Model 1	Ref	0.71 (0.41 – 1.24)	0.91 (0.71 – 1.16)
Model 2	Ref	0.71 (0.41 – 1.24)	0.91 (0.71 – 1.16)
Ischaemic stroke			
Cases	39	12	362
Model 1	Ref	1.10 (0.79 – 1.54)	0.88 (0.75 – 1.05)
Model 2	Ref	1.11 (0.80 – 1.56)	0.90 (0.76 – 1.07)
Coronary heart disease			
Cases	178	32	1924
Model 1	Ref	0.82 (0.67 – 0.99)	1.04 (0.96 – 1.13)
Model 2	Ref	0.82 (0.67 – 0.99)	1.04 (0.96 – 1.12)
Myocardial infarction			
Cases	62	6	625
Model 1	Ref	0.51 (0.33 – 0.78)	1.01 (0.88 – 1.16)
Model 2	Ref	0.51 (0.33 – 0.78)	1.02 (0.89 – 1.16)
Cardiovascular mortality			
Cases	54	5	481
Model 1	Ref	0.39 (0.25 – 0.63)	0.96 (0.83 – 1.11)
Model 2	Ref	0.39 (0.25 – 0.63)	0.97 (0.84 – 1.13)

[a] Model 1 is adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake and total energy intake.

[b] Model 2 is adjusted for model 1 and for intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

Sensitivity analyses

Because there are differences between studies in whether or not shellfish is included in the analyses of total fish intake, we additionally categorized the fish consumers according to the consumption frequency of total fish excluding shellfish, i.e., the sum of fatty fish and lean fish. Furthermore, to minimize the possibility of reverse causation, we performed a sensitivity analysis in which we repeated all abovementioned analyses after exclusion of the first two years of follow-up.
All analyses were performed using SPSS Statistics version 21 (IBM, Armonk, NY, USA). Results were considered statistically significant when *P* values <0.05 (2-sided).

Results

Of the 34033 participants, 2593 (7.6%) reported not to consume any fish. The median (IQR) total fish intake of the fish consumers was 57.9 (25.7–105.9) grams per week. Of the fish consumers, 71.9% consumed less than one portion of fish per week (<100 g/week), 22.8% consumed one to two portions per week and 5.3% ate more than two portions per week. Regarding types of fish, 595 (1.9%) of the fish consumers consumed only fatty fish and 30845 (98.1%) fish consumers consumed both fatty fish and lean fish. No participants consumed only lean fish.
Table 1 shows the baseline characteristics of the 34033 participants for consumers versus non-consumers of fish. On average, the fish consumers were more educated and had higher intakes of alcohol, fruit, vegetables, and EPA and DHA, compared with the fish non-consumers. When we split the fish consumers into those who did and did not consume at least one portion of fish per week, those who consumed at least one portion per week were more often women, were slightly older and had higher intakes of EPA and DHA compared with the participants who consumed less than one portion per week. Baseline characteristics of the fish consumers (n = 31440) in categories of types of fish and consumption frequencies of fatty fish and lean fish are shown in **Supplemental Tables S2 through S4**. Pearson correlation coefficients between types of fish ranged from 0.46 for lean fish versus fatty fish to 0.92 for total fish versus lean fish (**Supplemental Table S5**).

Consumption of total fish and CVD risk

During a median follow-up time of 15.1 years, 753 stroke events were documented of which 413 were ischaemic, 220 were haemorrhagic, and 120 were of unknown origin. Furthermore, 2134 CHD events of which 693 MI events, and 540 deaths due to a cardiovascular event were reported. **Table 2** shows the associations of total fish consumption with incident events of total stroke, haemorrhagic stroke, ischaemic stroke, CHD, MI and with CVD mortality. After adjustment for demographic, lifestyle-related and dietary factors (model 2) a non-significantly lower stroke risk was observed in the fish consumers compared with the non-consumers (HR: 0.93, 95% CI: 0.82 – 1.05). We

observed similar HRs in those who consumed <1 portion of fish per week (HR: 0.93, 95% CI: 0.82 – 1.06) and in those who consumed ≥1 portion of fish per week (HR: 0.91, 95% CI: 0.79 – 1.05), as compared with the non-consumers. For haemorrhagic stroke and ischemic stroke, the findings were similar to those for total stroke. Furthermore, no significant associations were observed between total fish and incident CHD (HR: 1.03, 95% CI: 0.95 – 1.11), MI (HR: 1.00, 95% CI: 0.88 – 1.15) and CVD mortality (HR: 0.96, 95% CI: 0.83 – 1.11), regardless of the consumption frequency.

Consumption of fatty fish and lean fish and CVD risk

No associations were observed between the consumption of only fatty fish or of both fatty and lean fish, as compared with no fish consumption, and total or subtypes of stroke (**Table 3**). The consumers of only fatty fish had a lower risk of incident CHD (HR: 0.82, 95% CI: 0.67 – 0.99), of incident MI (HR: 0.51 – 95% CI: 0.33 – 0.78) and of CVD mortality (HR: 0.39, 95% CI: 0.25 – 0.63). In the consumers of both fatty and lean fish, no associations with incident CHD, MI or CVD mortality were observed.
When we distinguished participants based on the consumption frequency of fatty fish (**Table 4**) we observed a significantly lower risk of total stroke (HR: 0.64, 95% CI: 0.45 – 0.92) and a non-significantly lower risk of ischaemic stroke (HR: 0.63, 95% CI: 0.39 – 1.02) in the participants who consumed ≥1 portion of fatty fish per week, as compared with those who consumed no fatty fish. Similarly, we observed that the consumers of ≥1 portion of lean fish per week had a significantly lower risk of ischaemic stroke (HR: 0.70, 95% CI: 0.57 – 0.86), as compared with participants who did not consume any lean fish. We observed no associations between portions of fatty or lean fish and CHD or MI incidence or CVD mortality.

Sensitivity analysis

The results of the analyses using portion sizes that were based on the intake of total fish excluding shellfish (i.e., the sum of only fatty fish and lean fish) are shown in **Supplemental Table S4**. A significantly lower ischaemic stroke risk was observed in participants who consumed ≥1 portion of fatty and lean fish per week (HR: 0.79, 95% CI: 0.65 – 0.97) but not in those who consumed <1 portion per week (HR: 0.94, 95% CI:0.79 – 1.12), as compared with the fish non-consumers. For all other CVD types, the results were similar to the results observed for portion sizes based on total fish, including shellfish. The exclusion of the first 2 years of follow-up in a sensitivity analysis did not yield different results (**Supplemental Tables S5 through S8**).

Discussion

In the present prospective cohort study in 34033 Dutch men and women, compared with fish non-consumption, fish consumption of less than one portion per week was unrelated

to risk of stroke. In participants who consumed at least one portion of fatty or lean fish per week, a lower risk of ischaemic stroke, but not haemorrhagic stroke, was observed. As compared with the fish non-consumers, lower risks of incident CHD, MI and of CVD mortality were observed in consumers of only fatty fish but not in those who consumed both fatty and lean fish.

The strengths of this study include its long follow-up period, its large sample size and the high number of CVD events that occurred. This enabled us to categorize the fish consumers and to address a wide range of CVD endpoints, including those with a relatively low incidence (e.g., stroke) without losing much statistical power. One of the limitations of this study is the low relative validity of the FFQ for the assessment of fish consumption (r = 0.32 for men and 0.37 for women) [29]. It may have been hard for the participants to estimate their fish intake over the previous year, partly because fish is not regularly consumed by most Dutch people. However, any subject misclassification is expected to be non-differential, because all study participants were free of chronic diseases at baseline. Therefore, this may have attenuated the associations and may have contributed to the null associations we observed in our study [33]. A second limitation of the present study is that fish intake was assessed at baseline only, so any changes in fish consumption during the 15.1 years of follow-up are unknown and could not be taken into account.

In this study, the consumption of ≥1 portion of fatty and lean fish per week, as compared with no fish consumption, was associated with a 21% lower risk of ischaemic stroke. Although a direct comparison is difficult because of differences in the reference categories, this finding is essentially in line with two previous meta-analyses [8, 9] and with the previous analysis in the MORGEN-arm of the EPIC-NL cohort [17], but not with three previous studies in other European cohorts [22, 34, 35] in which no associations were observed. Our choice to include solely fish non-consumers in the reference category in our analyses could explain why our findings differ from those of the latter three European cohorts. It is conceivable that the participants who reported to never consume fish, dislike fish and have been less likely to become fish consumers during follow-up [36]. Therefore, our reference group may have been more stable during the long follow-up time in terms of fish intake changes as compared with the frequently used reference groups that also include participants who consume very low amounts of fish [22, 34, 35].

Our study supports, at least for ischaemic stroke, the recommendation of the Health Council of the Netherlands to consume ≥ portion of fish per week to reduce the risk of stroke [7]. From our study, we cannot confirm that besides the consumption frequency the type of fish matters with regard to ischaemic stroke risk. Although we observed no association between the consumers of only fatty fish and ischaemic stroke risk, the consumers of only fatty fish represented just 1.7% of the study population and their fish intake (median: 1.4 g/week) was far ≤1 portion per week. When we categorized participants according to the consumption frequencies of fatty fish and lean fish, for both fish types an intake of ≥1 portion, as

Table 4. Associations between fish consumption, categorized according to portions [a] of fatty fish intake and of lean fish intake and risks of incident total stroke, haemorrhagic stroke, ischaemic stroke, coronary heart disease, myocardial infarction, and of cardiovascular mortality.

	Categories of fatty fish consumption			Categories of lean fish consumption		
	Median (5^{th} – 95^{th} percentiles) fish intake (g/week)			Median (5^{th} – 95^{th} percentiles) fish intake (g/week)		
	No consumption	**<1 portion/week**	**≥ 1portion/week**	**No consumption**	**< 1 portion/week**	**≥ 1 portion/week**
Total fish	-	56.7 (7.0 – 180.8)	314.5 (133.1 – 765.5)	-	50.4 (7.7 – 125.5)	163.5 (122.6 – 414.3)
Fatty fish	-	10.5 (1.2 – 54.1)	131.1 (102.0 – 298.6)	-	9.8 (1.2 – 49.4)	8.2 (21.7 – 49.4)
Lean fish	-	32.4 (2.5 – 136.5)	153.0 (19.3 – 462.5)	-	28.0 (3.1 – 85.8)	133.0 (102.6 – 300.2)
		HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)
No. of participants	2593	30966	474	3188	29956	3889
Total stroke						
Cases	69	675	9	85	569	99
Model 1 [b]	Ref	0.93 (0.82 – 1.05)	**0.65 (0.45 – 0.92)**	Ref	0.96 (0.86 – 1.08)	0.91 (0.78 – 1.05)
Model 2 [c]	Ref	0.93 (0.82 – 1.06)	**0.64 (0.45 – 0.92)**	Ref	0.97 (0.86 – 1.09)	0.92 (0.79 – 1.07)
Haemorrhagic stroke						
Cases	19	197	4	23	165	32
Model 1	Ref	0.90 (0.71 – 1.15)	0.98 (0.57 – 1.71)	Ref	0.96 (0.77 – 1.21)	1.04 (0.79 – 1.37)
Model 2	Ref	0.90 (0.71 – 1.15)	0.96 (0.55 – 1.68)	Ref	0.96 (0.77 – 1.20)	1.04 (0.78 – 1.37)
Ischaemic stroke						
Cases	39	369	5	51	317	45
Model 1	Ref	0.90 (0.76 – 1.06)	0.62 (0.39 – 1.01)	Ref	0.91 (0.78 – 1.05)	**0.68 (0.55 – 0.84)**
Model 2	Ref	0.91 (0.77 – 1.08)	0.63 (0.39 – 1.02)	Ref	0.91 (0.78 – 1.07)	**0.70 (0.57 – 0.86)**
Coronary heart disease						
Cases	178	1920	36	210	1682	242
Model 1	Ref	1.03 (0.96 – 1.12)	1.09 (0.91 – 1.31)	Ref	1.09 (1.02 – 1.18)	0.98 (0.89 – 1.08)
Model 2	Ref	1.03 (0.95 – 1.12)	1.08 (0.90 – 1.30)	Ref	1.09 (1.01 – 1.17)	0.97 (0.88 – 1.07)

Myocardial infarction						
Cases	62	618	13	68	552	73
Model 1	Ref	1.00 (0.87 – 1.14)	1.11 (0.82 – 1.51)	Ref	1.12 (0.99 – 1.28)	0.96 (0.81 – 1.13)
Model 2	Ref	1.00 (0.88 – 1.15)	1.12 (0.82 – 1.52)	Ref	1.13 (0.99 – 1.28)	0.97 (0.82 – 1.15)
Cardiovascular mortality						
Cases	54	477	9	59	406	75
Model 1	Ref	0.95 (0.82 – 1.09)	0.88 (0.61 – 1.26)	Ref	1.08 (0.94 – 1.25)	1.09 (0.84 – 1.43)
Model 2	Ref	0.96 (0.83 – 1.11)	0.88 (0.61 – 1.27)	Ref	1.10 (0.95 – 1.26)	1.13 (0.94 – 1.35)

[a] 1 portion equals 100 grams.
[b] Model 1 is adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake and total energy intake.
[c] Model 2 is adjusted for model 1 and for intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

compared with no fish consumption, was related to lower ischaemic stroke risks. These lower risks were of similar magnitude, which suggests that the consumption frequency may be more important than the type of fish. On the other hand, most of the participants (62%) who consumed ≥1 portion of fatty fish per week also consumed ≥1 portion of lean fish per week, whereas of those consuming ≥1 portion of lean fish per week only 7.6% consumed ≥1 portion of fatty fish per week. This suggests that the lower ischaemic stroke risk may, at least in part, be attributable to lean fish. In line with that suggestion is the lower ischaemic stroke risk that was observed for the consumption of lean fish, but not (salted) fatty fish, in a Swedish cohort [23]. On the other hand, in a Spanish population with a higher fish intake than in the Swedish cohort as well as in our Dutch cohort, no associations were observed between lean fish or fatty fish consumption and ischaemic stroke risk [22]. To draw firmer conclusions about the association between the consumption of subtypes of fish and (ischaemic) stroke risk, more research is warranted in other populations with more distinct differences in types of consumed fish.

The null association between total fish consumption and incident CHD risk that we observed is in contrast to the findings of a meta-analysis of seven cohort studies [37], in which fish consumption of ≤2 portions per week compared with no to very low fish consumption, was associated with a lower risk of incident CHD. Fish intake in our population was potentially too low to detect an association. To illustrate, the cut-off for one portion size in that meta-analysis was higher (114 g) than in our present study (100 g). Nevertheless, with respect to incident MI, our findings are in line with previous studies in the MORGEN cohort [18] and in the EPIC-Germany cohort [35] and with a meta-analysis of five cohort studies [12]. In the latter, a lower risk of nonfatal MI was observed only for fish intakes of ≥5 portions per week, and not for a less frequent intake that is comparable to the intake in our present cohort [12].

Finally, the null association we observed between total fish consumption and the risk of CVD mortality is in line with the evidence from other recent cohort studies as well [38, 39]. In the small subgroup (1.7%) of our cohort that consumed only fatty fish and no lean fish at all, we observed significantly lower risks of incident CHD and MI, and of CVD mortality as compared with the fish non-consumers. For the associations of different types of fish with risk of incident MI, [40] the current evidence from observational studies is scarce, but an inverse association between fatty fish and incident MI has been observed before [40]. Still, the associations we observed need to be interpreted with caution. Firstly, because of the low number of fatty fish consumers. Secondly, as mentioned above, the intake of fish among these participants is very low. If the lower risks that we observed would indeed be attributable to the consumption of fatty fish, we would expect to find similar associations in the analyses in which we categorized the participants according to the consumption frequency of fatty fish. However, in these analyses we observed no associations with CHD, MI or CVD. Thirdly, a recent study in the total EPIC-cohort, thus including EPIC-NL, observed no associations between subtypes of fish and CHD mortality. In that study,

the overall range of fish consumption is much larger. Therefore, it is conceivable that the associations that we observed are simply due to chance. Nevertheless, we cannot exclude the possibility that a potential association between fatty fish and CHD, MI or CVD does exist.

In conclusion, in this Dutch cohort the baseline consumption of less than one portion of fish per week was unrelated to incident total stroke or subtypes of stroke during a follow-up time of 15.1 years. However, fish intake of one portion or more per week was associated with a lower risk of ischaemic stroke. Although this association was observed for the consumption of both fatty fish and lean fish, it is unclear whether the association only depends on the portion size or also on the type of fish. Fish consumption was unrelated to incident CHD and MI and to CVD mortality, although a potential protective relation with fatty fish cannot be ruled out by the present study. The association between fish subtypes and CVD outcomes needs further investigation in other populations with more distinct differences in types of consumed fish.

Disclosures

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Supplemental material

Supplemental table S1. Details of the multiple imputation procedure

Multiple imputation procedure	
Software used	SPSS 21 for Windows
Imputation method and key settings	Fully conditional specification (Markov chain Monte Carlo method); Maximum iterations: 25
Number of imputed data sets created	10
Variables included in the imputation procedure and used in main analyses	
Imputed and used as predictor	BMI, waist circumference, smoking status, education level, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol
Used as predictor only	Age, sex, physical activity, hypertension, anti-hypertensive medication, diabetes, length, weight, hip, circumference, TC/HDL ratio, alcohol intake (ethanol), total energy intake, intake of saturated fatty acids, intake of trans fatty acids, EPA intake, DHA intake, fruit consumption, vegetable consumption, lean fish consumption, fatty fish consumption, shellfish consumption, incident CVD
Variables not used in main analyses, but used as predictors of missing data to increase plausibility of missing at random assumption	–
Treatment of non-normally distributed variables	Linear regression; non-normally distributed variables were log-transformed before being imputed.
Treatment of binary/categorical variables	Logistic regression

Abbreviations: CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; TC/HDL ratio, ratio between total cholesterol and high-density lipoprotein cholesterol.

Supplemental table S2. Baseline characteristics of the fish consumers in EPIC-NL according to the type of fish

	Categories of fish consumption			
	Median (5th - 95th percentiles) of total fish consumption (g/week)			
	Consumers of only fatty fish		**Consumers of fatty and lean fish**	
	3.6	(0.7 – 16.1)	59.3	(8.3 –208.3)
No. of participants	595		30845	
Male, %	0		26	
Age, *y* [b]	56.8	(± 5.5) [a]	48.5	(± 11.8)
Physically active, %	48		42	
Current smoker, %	21		31	
High education level, %	17		22	
Body Mass Index, *kg/m*^{2}	25.9	(± 4.1)	25.5	(± 3.9)
Waist circumference, *cm*	83.1	(± 10.1)	84.9	(± 11.4)
Systolic blood pressure, *mmHg*	133.3	(± 20)	125.5	(± 18.5)
Ratio total- :HDL-cholesterol	4.1	(3.4 – 5.1) [b]	4	(3.2 – 5.0)
Dietary intake [c]				
Total energy, *kcal/d*	1799	(± 442)	2072	(± 608)
Fatty fish, *g/wk*	1.4	(0.7 – 2.8)	11	(4.9 – 24.6)
Lean fish, *g/wk*	–	–	34	(14.4 – 67.3)
Shellfish, *g/wk*	2.1	(1.1 – 4.3)	4.9	(2.2 – 11.4)
EPA, *mg/d*	3	(2 – 6)	36	(16 – 64)
DHA, *mg/d*	15	(11 – 21)	81	(43 – 134)
ALA, *mg/d*	849	(693 – 1111)	912	(742 – 1133)
Alcohol, *g/d*	2.9	(0.3 – 10.3)	5.8	(1.0 – 16.5)
Fruit, *g/d*	299	(± 175)	260	(± 165)
Vegetables, *g/d*	145	(± 53)	138	(± 54)
Saturated fatty acids, *g/d*	34	(± 5.9)	32.6	(± 5.8)
Trans fatty acids, *g/d*	2.9	(± 1.0)	2.9	(± 1.1)
Fibre, *g/d*	24.6	(± 4.9)	23.3	(± 4.7)

[a] Data are presented as mean ± standard deviation (all such values).

[b] Data are presented as median with 25th - 75th percentiles (all such values).

[c] All nutrients and foods were adjusted for total energy intake, except for alcohol intake.

Supplemental Table S3. Pearson's correlation coefficients between consumption of total and types of fish

	Pearson's correlation coefficients [a]			
	Total fish	Lean fish	Fatty fish	Shellfish
Total fish	1			
Lean fish	0.92	1		
Fatty fish	0.74	0.46	1	
Shellfish	0.67	0.48	0.54	1

[a] All $P < 0.01$

Supplemental Table S4. Associations between fish consumption, categorized according to portion [a] sizes of the sum of fatty fish and lean fish, and risk of incident CVD events.

	Categories of total fish consumption		
	Median (5th – 95th percentiles) intake (g/week)		
	Non-consumers	**<1 portion/week**	**≥1 portion/week**
Total fish intake	-	21.1 (6.0 – 105.4)	140.9 (112.0 – 338.7)
	HR	HR (95% CI)	HR (95% CI)
No. of participants	2593	24637	6803
Total stroke			
Cases	69	516	168
Model 1[b]	Ref	0.94 (0.82 – 1.07)	0.88 (0.76 – 1.02)
Model 2[c]	Ref	0.94 (0.83 – 1.07)	0.89 (0.77 – 1.03)
Haemorrhagic stroke			
Cases	19	151	50
Model 1	Ref	0.92 (0.72 – 1.17)	0.88 (0.67 – 1.16)
Model 2	Ref	0.91 (0.71 – 1.17)	0.87 (0.66 – 1.15)
Ischaemic stroke			
Cases	39	289	85
Model 1	Ref	0.93 (0.78 – 1.11)	**0.78 (0.64 – 0.95)**
Model 2	Ref	0.94 (0.79 – 1.12)	**0.79 (0.65 – 0.97)**
Coronary heart disease			
Cases	178	1505	451
Model 1	Ref	1.03 (0.95 – 1.12)	1.04 (0.95 – 1.14)
Model 2	Ref	1.03 (0.95 – 1.12)	1.03 (0.94 – 1.13)
Myocardial infarction			
Cases	62	491	140
Model 1	Ref	1.00 (0.88 – 1.15)	0.99 (0.85 – 1.16)
Model 2	Ref	1.00 (0.88 – 1.15)	1.00 (0.86 – 1.17)
Cardiovascular mortality			
Cases	54	364	122
Model 1	Ref	0.95 (0.82 – 1.10)	0.94 (0.80 – 1.10)
Model 2	Ref	0.92 (0.82 – 1.11)	0.96 (0.81 – 1.13)

[a] 1 portion equals 100 grams.

[b] Model 1 is adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake and total energy intake.

[c] Model 2 is model 1 and additionally adjusted for intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

Supplemental Table S5. Associations between fish consumption and risk of incident CVD events, after exclusion of the first two years of follow-up

	Categories of total fish consumption			
	Median (5th – 95th percentiles) intake (g/week)			
	Non-consumers	**All consumers** 57.8 (7.0 – 206.9)	**<1 portion[a] /week** 36.6 (5.6 – 92.8)	**≥1 portion/week** 130.1 (103.0 – 313.1)
No. of participants	2565	31059	22351	8708
Total stroke				
Cases	64	633	432	201
HR (95% CI) [b]	Ref	0.92 (0.80 – 1.05)	0.91 (0.80 – 1.05)	0.93 (0.80 – 1.07)
Haemorrhagic stroke				
Cases	16	181	128	53
HR (95% CI)	Ref	0.93 (0.72 – 1.22)	0.97 (0.74 – 1.27)	0.85 (0.63 – 1.13)
Ischaemic stroke				
Cases	37	344	237	107
HR (95% CI)	Ref	0.87 (0.73 – 1.04)	0.88 (0.74 – 1.05)	0.86 (0.71 – 1.04)
Coronary heart disease				
Cases	165	1763	1255	508
HR (95% CI)	Ref	1.00 (0.92 – 1.09)	1.00 (0.92 – 1.09)	1.00 (0.91 – 1.10)
Myocardial infarction				
Cases	53	550	396	154
HR (95% CI)	Ref	1.03 (0.89 – 1.20)	1.03 (0.89 – 1.19)	1.05 (0.89 – 1.23)
Cardiovascular mortality				
Cases	49	443	304	139
HR (95% CI)	Ref	0.95 (0.82 – 1.11)	0.95 (0.81 – 1.11)	0.97 (0.82 – 1.14)

[a] 1 portion equals 100 grams.

[b] HRs are adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake, total energy intake, and intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

Supplemental Table S6. Associations between consumption of types of fish and risk of incident CVD events after exclusion of the first two years of follow-up

	Categories of fish consumption		
	Median (5th – 95th percentiles) intake of total fish (g/week)		
	Non-consumers	**Only fatty fish**	**Fatty and lean fish**
		3.6 (0.7 - 16.2)	59.3 (8.3 – 208.0)
No. of participants	2565	589	30470
Total stroke			
Cases	64	13	620
HR (95% CI)[a]	Ref	0.74 (0.55 – 1.01)	0.92 (0.81 – 1.05)
Haemorrhagic stroke			
Cases	16	2	179
HR (95% CI)	Ref	0.41 (0.19 – 0.88)	0.95 (0.73 – 1.24)
Ischaemic stroke			
Cases	37	10	334
HR (95% CI)	Ref	0.95 (0.66 – 1.37)	0.87 (0.73 – 1.04)
Coronary heart disease			
Cases	165	28	1735
HR (95% CI)	Ref	0.77 (0.63 – 0.95)	1.01 (0.93 – 1.09)
Myocardial infarction			
Cases	53	5	545
HR (95% CI)	Ref	0.49 (0.31 – 0.79)	1.05 (0.90 – 1.21)
Cardiovascular mortality			
Cases	49	4	439
HR (95% CI)	Ref	0.33 (0.20 – 0.56)	0.97 (0.83 – 1.13)

[a] HRs are adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake, total energy intake and for intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

Supplemental table S7. Associations between portions (100 grams) of the sum of fatty fish and lean fish and incident CVD, after exclusion of the first two years of follow-up.

	Categories of total fish consumption		
	Median (5th – 95th percentiles) intake (g/wk)		
	Non-consumers	**< 1 portion/ week**	**≥ 1 portion/week**
		42.1 (6.0 – 105.4)	140.9 (112.0 – 336.7)
No. of participants	2565	24350	6709
Total stroke			
Cases	64	477	156
HR (95% CI)	Ref	0.93 (0.81 – 1.06)	0.89 (0.76 – 1.04)
Haemorrhagic stroke			
Cases	16	135	46
HR (95% CI)	Ref	0.93 (0.71 – 1.22)	0.82 (0.94 – 1.26)
Ischaemic stroke			
Cases	37	265	79
HR (95% CI)	Ref	0.91 (0.76 – 1.08)	**0.78 (0.64 – 0.95)**
Coronary heart disease			
Cases	165	1357	406
HR (95% CI)	Ref	1.00 (0.92 – 1.09)	1.01 (0.92 – 1.11)
Myocardial infarction			
Cases	53	426	124
HR (95% CI)	Ref	1.02 (0.88 – 1.19)	1.07 (0.91 – 1.27)
Cardiovascular mortality			
Cases	49	328	115
HR (95% CI)	Ref	0.94 (0.80 – 1.10)	0.99 (0.83 – 1.18)

[a] HRs are adjusted for age, sex, physical activity, smoking status, education level, BMI, alcohol intake, total energy intake and for intakes of saturated fatty acids, trans fatty acids, fruit, vegetables and dietary fibre.

Supplemental table S8. Associations of intake portions (100 grams) of fatty fish or lean fish, with risk of incident CVD, after exclusion of the first two years of follow-up

	Categories of fish consumption					
	Median (5th – 95th percentiles) fish intake (g/week)					
	No fatty fish	**< 1 portion/wk of fatty fish**	**≥ 1 portion/wk of fatty fish**	**No lean fish**	**< 1 portion/wk of lean fish**	**≥ 1 portion/wk of lean fish**
Total fish	-	56.7 (6.9 – 180.6)	314.5 (133.6 – 762.7)	-	50.4 (7.7 – 125.5)	163.6 (122.7 – 411.4)
Fatty fish	-	10.5 (1.2 – 54.1)	130.9 (101.9 – 300.7)	-	9.8 (1.2 – 49.4)	21.7 (8.2 – 118.3)
Lean fish	-	32.4 (2.5 – 136.5)	152.2 (19.3 – 456.8)	-	27.9 (3.1 – 85.8)	132.9 (102.5 – 298.0)
No. of participants	2565	30599	460	3154	26638	3832
Total stroke						
Cases	64	625	8	77	529	91
HR (95% CI)[a]	Ref	0.92 (0.81 – 1.05)	**0.63 (0.43 – 0.92)**	Ref	0.99 (0.87 – 1.12)	0.94 (0.80 – 1.09)
Haemorrhagic stroke						
Cases	16	177	4	18	150	29
HR (95% CI)	Ref	0.93 (0.71 – 1.21)	1.17 (0.66 – 2.05)	Ref	1.08 (0.84 – 1.40)	1.19 (0.88 – 1.61)
Ischaemic stroke						
Cases	37	340	4	47	293	41
HR (95% CI)	Ref	0.88 (0.74 – 1.05)	**0.55 (0.32 – 0.93)**	Ref	0.92 (0.78 – 1.08)	**0.69 (0.56 – 0.86)**
Coronary heart disease						
Cases	165	1732	31	193	1515	220
HR (95% CI)	Ref	1.00 (0.92 – 1.09)	1.02 (0.84 – 1.24)	Ref	1.06 (0.98 – 1.15)	0.97 (0.88 – 1.07)
Myocardial infarction						
Cases	53	538	12	58	479	66
HR (95% CI)	Ref	1.03 (0.89 – 1.19)	1.28 (0.92 – 1.77)	Ref	1.15 (1.00 – 1.33)	1.06 (0.88 – 1.27)
Cardiovascular mortality						
Cases	49	434	9	53	369	70
HR (95% CI)	Ref	0.95 (0.82 – 1.11)	0.97 (0.68 – 1.41)	Ref	1.11 (0.95 – 1.29)	1.17 (0.97 – 1.41)

[a] HRs are adjusted for age, sex, physical activity, smoking, education, BMI, intakes of alcohol, total energy, saturated fatty acids, trans fatty acids, fruit, vegetables and fibre.

Chapter 5.

Fluidity of the dietary fatty acid profile and risk of CHD and stroke in EPIC-NL

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Abstract

Background: Taking into account the fluidity of the dietary fatty acid profile may better capture biological effects of fatty acids on cardiovascular health. Lipophilic index (LI) represents overall fluidity of the dietary fatty acid profile. Lipophilic load (LL) represents a combination of overall fluidity and absolute intake of dietary fatty acids.

Objective: We investigated the relations of dietary LI and LL with risk of coronary heart disease (CHD) and ischemic stroke.

Design: We used data from the prospective EPIC-NL study, including 36520 participants aged 20-70 years. LI and LL were calculated using dietary intake data estimated with a validated food frequency questionnaire. Incident CHD (n = 2348) and ischemic stroke (n = 479) cases were obtained through linkage to national registers during 15 years follow-up.

Results: A high LI correlated with higher saturated (r = 0.47) and trans (r = 0.24) fatty acids, and lower polyunsaturated fatty acid (r = -0.66) intakes. High LL correlated with higher intakes of the sum of fatty acids (r = 0.87) and saturated fatty acids (r = 0.94). LI and LL were not associated with CHD risk (HRs comparing extreme quartiles: 0.93 [95% CI: 0.83, 1.04] and 0.92 [95% CI: 0.79, 1.07], respectively) and neither with ischemic stroke risk.

Conclusions: In this Dutch population, diets with high LI reflect an overall adverse fatty acid profile of the diet, whereas diets with high LL particularly reflect high saturated fatty acid intake. Neither the overall fluidity of the dietary fatty acid profile (LI), nor the combined fluidity and amount of fatty acids consumed (LL) were related to CHD or ischemic stroke risk.

Introduction

It is becoming more and more clear that not all fatty acids have similar effects on cardiovascular health, even within classes of fatty acids such as saturated fatty acids (SFAs) [1-3].This may be explained by differences in the fluidity of fatty acids, which is usually quantified by their melting points (transition temperature at which the fatty acid goes from solid/gel to liquid state). The longer and the more saturated fatty acids are, the higher are their melting point, and the lower is their fluidity. When there are many fatty acids with low melting points present in membrane phospholipids of cells or lipoproteins, the overall fluidity of the membrane will be high. The membrane fluidity will decrease with increasing presence of fatty acids with high melting points [4, 5]. Reduced membrane fluidity has been adversely associated with cardiovascular intermediates such as hypertension [6], endothelial function [7], and insulin resistance [8].

Recently, the lipophilic index (LI) was introduced by researchers from the United States of America as a way to summarize the fluidity of the fatty acid profile [9, 10]. It is a weighted average of the melting points of fatty acids consumed, regardless of the total amount of fatty acids consumed. A high dietary LI represents a diet with a relatively high fat melting point and thus a low fat fluidity. In a similar way, LI can be computed for the fatty acid profile in blood or adipose tissue. However, the interpretation of a dietary LI is different from a measure of LI in the blood cells or tissue. Given that membrane fatty acid profiles are not only determined by dietary fatty acids, but also by de novo synthesis of fatty acids, a dietary measure of LI will not directly reflect membrane fluidity. Rather, it will be a quantification of the fat quality of the diet. Evidence linking the LI of diet, blood or adipose tissue with cardiovascular diseases is limited to coronary heart diseases (CHD) and American populations, and shows inconsistent results [10, 11].

LI does not take into account the amount of fat consumed. Thus, individuals that consume very little fat with low fluidity will be assigned a high LI, whereas individuals that consume a large amount of fat, but with high fluidity, will be assigned a low LI. This may not fully capture the effects on cardiovascular health. Therefore, the lipophilic load (LL) was introduced [11, 12], which additionally takes the total amount of fat consumed into account. It is important to note that LL may be largely determined by total fatty acid intake, and it may be questionable whether one can actually tell apart the effects of total fatty acids from LL. As far as we know, such comparisons have not yet been made, and no articles have been published that report on the relation of LL with cardiovascular health.

With this study, we cross-sectionally investigated whether higher dietary LI, LL, and the sum of fatty acids associated with adverse concentrations of biochemical cardiovascular risk factors. In addition, we investigated prospectively whether higher dietary LI, LL, and the sum of fatty acids were associated with higher risk of CHD and ischemic stroke in a population of 36520 Dutch adults.

Subjects and Methods

Study population and design

EPIC-NL consists of the Prospect- and MORGEN-cohorts that cover the Dutch contribution to EPIC, as described in detail previously [13, 14]. In brief, Prospect is a prospective cohort study of 17,357 women aged 49-70, living in Utrecht and vicinity, who participated in the breast cancer screening between 1993 and 1997 [15]. The MORGEN-cohort consists of 22654 men and women aged 20-59 years, selected from random samples of the Dutch population in three Dutch towns (Amsterdam, Doetinchem, Maastricht) between 1993 and 1997 [16]. All participants signed informed consent prior to study inclusion. Both cohorts comply with the Declaration of Helsinki, and were approved by local medical ethics committees.

After exclusion of individuals without consent for linkage to disease- or municipal registries (n = 1760), with prevalent cardiovascular disease (n = 1224), missing dietary data (n = 164), or extremely low or high reported energy intakes (i.e., those in the top 0.5% and bottom 0.5% ratio of reported energy intake over estimated energy requirement [estimated with basal metabolic rate (BMR)] n = 343), 36520 participants were left for the main analysis. For the analysis involving biochemical cardiovascular risk factors, we used data of a random 6.5% sample of the total study population (n = 2604) representative of the full cohort at baseline. Similar exclusions were applied as described above, leaving 2085 (for high-sensitive C-reactive protein [hsCRP]) to 2252 (for total cholesterol) participants for the analysis.

Covariables

Data on demographic characteristics, presence of chronic diseases and cardiovascular risk factors were obtained at baseline with a self-administered questionnaire. Body height and weight were measured. Blood pressure (BP) was measured twice on the left arm while the participants were in supine position. In the Prospect-study the systolic and diastolic BP were measured using a Boso Oscillomat, whereas a Random Zero Sphygmomanometer was used in the MORGEN-cohort. The mean of the two BP measurements was used in the analysis. Hypertension was defined by either a self-reported physician diagnosis, measured hypertension (>140 mmHg systolic or >90 mmHg diastolic) or by use of anti-hypertensive medication. Diagnosis of hyperlipidemia was determined based on self-report. Physical activity, assessed by a validated questionnaire, was categorized using the Cambridge Physical Activity Score [17]. Smoking status was categorized into never, former or current. Education was categorized into low (primary education up to completing intermediate vocational education), intermediate (up to higher secondary education) or high (higher vocational education and university).

Dietary intake and calculation of LI and LL

Food intake at baseline was assessed using a semi-quantitative food frequency questionnaire (FFQ) covering consumption frequency of 178 foods during the year preceding enrollment [18]. Intakes of individual fatty acids (based on carbon atom chain length and positions of double bonds) were calculated based on the Dutch food composition database 1998. The FFQ was validated against 12 24-h recalls. Pearson correlations for the most abundantly consumed individual fatty acids varied from 0.62 for C16:0 (both men and women) to 0.38 (in women) for C18:2n-6c [19]. All nutrients were adjusted for energy intake using the regression residual method [20].

There were 55 individual fatty acids in the food composition database (accounting for 92.0% of total fatty acid intake in our study population). We included 44 of those (accounting for 91.4% of total fatty acid intake in our study population) in the calculation of dietary LI, LL and the sum of fatty acids (**Table 1**). The remaining fatty acids could not be included due to lack of melting point data.
Dietary LI was calculated by multiplying the intake of each individual fatty acid (g/d) by its melting point (°C), summing the products, and then dividing by the sum of the intake of the individual fatty acids (g/d). Melting points were derived from the LipidBank database (http://lipidbank.jp/, accessed on August 28th 2015), similar to previous reports [9, 10]. The calculation of dietary LL was similar to dietary LI but without dividing by the sum of fatty acid intake.

Biochemical measurements

Blood sampling, handling of blood samples and measurement of biochemical parameters have been described in detail elsewhere [13]. Plasma concentrations of total cholesterol were measured using enzymatic methods, and hsCRP was measured with a turbidimetric method. Plasma HDL and LDL cholesterol were measured using a homogeneous assay with enzymatic endpoint. HbA1c was measured in erythrocytes using an immunoturbidimetric latex test. All assays were performed on an autoanalyser (LX20, Beckman Coulter, Mijdrecht, the Netherlands).

Coronary heart disease and stroke events

Vital status of the participants was obtained through linkage with municipal population registries. Subsequently, causes of death for the deceased persons were obtained through linkages with Statistics Netherlands. Morbidity data were obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. In this register, admission files have been filed continuously from general and university hospitals in the Netherlands from 1990 onwards [13, 21]. Incidences of

Table 1. Most frequently consumed individual fatty acids: melting points, percent of total fatty acid intake, and Spearman correlations with the dietary lipophilic index (LI) and load (LL) and the sum of fatty acid intake

		Melting point (ºC)	Percent of total fatty acid intake* (%)	Correlations (r) with dietary LI	dietary LL	sum of fatty acid intake
SFA	4:0	-7.9	0.79	0.32	0.37	0.25
	6:0	-3.4	0.56	0.32	0.38	0.25
	8:0	16.7	0.36	0.27	0.34	0.24
	10:0	31.6	0.71	0.41	0.52	0.37
	12:0	44.2	2.27	0.29	0.46	0.36
	13:0	41.5	0.06	0.31	0.36	0.24
	14:0	53.9	5.38	0.53	0.71	0.50
	15:0	52.3	0.77	0.47	0.57	0.38
	16:0	63.1	24.46	0.38	0.97	0.85
	17:0	61.3	0.56	0.53	0.74	0.53
	18:0	69.6	12.03	0.40	0.91	0.78
	19:0	68.6	0.02	0.20	0.40	0.33
	20:0	76.75	0.49	-0.07	0.52	0.62
	22:0	81.5	0.37	-0.45	0.22	0.48
	24:0	87.75	0.12	-0.45	0.09	0.33
MUFA-cis	C16:1n-7c	0.0	0.88	0.46	0.77	0.60
	C18:1n-6c	18.55	0.13	0.16	0.43	0.38
	C18:1n-7c	15.0	0.72	0.05	0.50	0.52
	C18:1n-8c	22.5	0.03	0.14	0.30	0.26
	C18:1n-9c	16.0	23.83	-0.11	0.72	0.86
	C18:1n-12c	33.0	0.29	0.16	0.44	0.40
	C20:1-c	23.5	0.87	-0.04	0.41	0.48
	C24:1-c	42.75	0.02	0.18	0.26	0.19
PUFA	C18:2n-6c	-5.0	17.43	-0.79	0.07	0.47
	C18:3n-3c	-11.15	1.65	-0.52	0.18	0.46
	C18:3n-6c	-11.15	0.02	0.27	0.53	0.44
	C20:4n-6c	-49.5	0.05	-0.02	0.13	0.14
	C20:5n -3c	-54.1	0.08	-0.14	-0.11	-0.05
	C22:5n-3c	-54.1	0.02	-0.15	-0.11	-0.05
	C22:6n-3c	-44.15	0.17	-0.15	-0.11	-0.05
Trans	C16:1n-7t	31.0	0.25	0.37	0.49	0.34
	C18:1n-7t	44.0	0.36	0.25	0.51	0.43
	C18:1n-9t	45.5	3.32	0.13	0.44	0.41
	C18:1n-12t	56.5	0.26	0.16	0.43	0.39
	C18:2n-6t	28.5	0.13	0.27	0.55	0.47

n = 36,520

*the sum of 44 fatty acids used to calculate LI and LL.

fatal and nonfatal events were combined, taking only the first-occurring events into account. For the present analyses we used ICD-9-CM 410-414 for CHD, 433 and 434 for ischemic stroke, and 430-434 and 436 for total stroke. Follow-up was complete until 1 January 2011.

Data analysis

Baseline characteristics were presented as mean (SD) or median (IQR) for continuous variables and as percentages for categorical variables, according to quartiles of dietary LI and LL. Spearman correlations were calculated to determine correlations between dietary LI, LL, the sum of fatty acids, and individual fatty acids.
The relation of dietary LI, LL and the sum of fatty acids with biochemical cardiovascular risk factors (total, HDL, and LDL cholesterol, hsCRP, HbA1c) at baseline was assessed with linear regression. Dietary LI, LL and sum of fatty acids were expressed per SD increase. hsCRP was log transformed before the analysis due to non-normal distribution of the residuals, and back transformed after the analysis to allow interpretation of the Betas. Potential confounders were selected based on a priori knowledge, and whether they changed the Betas of LI and/or LL in the regression models by ~10% or more. In model 1, we adjusted for age (years; continuous) , sex (male, female), smoking status (never, former, current), physical activity (inactive, moderately inactive, moderately active, active), BMI (kg/m^2; continuous) and highest level of education (low, intermediate, high). In model 2, we additionally adjusted for systolic blood pressure (mmHg; continuous), presence of hypertension (yes, no) and diabetes (yes, no). In the final model, model 3, we additionally adjusted for dietary intakes of total energy (Kcal/d), cholesterol (mg/d), carbohydrates (energy%/d; all continuous) and alcohol (≤ 10g/d, 11-25 g/d, 26-50 g/d, >50 g/d).
Cox proportional hazards regression analysis was used to determine crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) for the associations of dietary LI, LL, and the sum of fatty acids (in quartiles) and risk of CHD and ischemic and total stroke. Follow-up time was calculated from date of inclusion until the date of diagnosis of CHD or ischemic stroke, death, loss to follow-up or censoring at the end of follow-up, whichever came first. *P* values for linear trend were estimated by using the median dietary LI, LL, or the sum of fatty acids per quartile as a continuous variable in the Cox regression model. We used the same adjustment models as described above.

In sensitivity analyses (all applied on model 3), we additionally added dietary intakes of SFA and PUFA to the analyses of LI with CHD and stroke. Previous studies added those intakes to the multivariable models in order to investigate effects of LI beyond original fat classifications. However, one could argue that such adjustments may be over-adjustments, because SFA and PUFA indirectly contribute to the LI. Secondly, we excluded potential energy misreporters, defined according to the Goldberg cut-offs [22] (*n* = 27218 left for the analysis). Thirdly, we excluded participants with baseline hyperlipidemia and/or hypertension (*n* = 21896 left for the analysis). Finally, we repeated the analysis with LI and LL that took additionally into account the 8% of fatty acids that could not be assigned a melting

Table 2. Baseline characteristics according to quartiles of energy adjusted dietary LI

	Q1	Q2	Q3	Q4	Correlation with continuous dietary LI (r)
Quartile cut-offs	18.15 – 32.82	32.83 – 34.75	34.76 – 36.53	36.54 – 44.71	
Subjects, n	9130	9130	9130	9130	
Male, %	27	28	25	21	0.05
(moderately) inactive, %	31	31	31	34	-0.03
Hypertension, %	34	36	37	40	0.05
Hyperlipidemia, %	9	8	7	7	-0.07
Diabetes, %	1.4	1.0	1.4	1.6	0.01
Current smoker,%	31	30	30	30	-0.03
Low level of education, %	30	35	41	50	0.16
Age, years, [a]	50 (39, 57)	50 (39, 57)	51 (42, 57)	54 (48, 60)	0.15
Systolic BP, mmHg	124 (19)	125 (18)	127 (19)	128 (19)	0.09
Diastolic BP, mmHg	77 (11)	78 (11)	78 (11)	78 (11)	0.06
BMI, kg/m^2	25.2 (3.9)	25.5 (3.8)	25.8 (4.0)	26.1 (4.1)	0.09
Total cholesterol, mmol/L [b]	4.7 (0.9)	4.8 (0.9)	4.8 (1.0)	4.9 (0.9)	0.10
HDL cholesterol, mmol/L [b]	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.1 (0.3)	-0.04
LDL cholesterol, mmol/L [b]	2.7 (0.8)	2.7 (0.8)	2.8 (0.9)	3.0 (0.8)	0.13
hsCRP, mg/l [a b]	1.0 (0.5, 0.3)	1.1 (0.5, 2.4)	1.1 (0.5, 2.4)	1.3 (0.6, 2.7)	0.06
HbA1c, g/dl [b]	0.6 (0.2)	0.6 (0.2)	0.6 (0.2)	0.6 (0.1)	0.04
Daily dietary intake					
Energy, kcal	2038 (630)	2093 (606)	2064 (596)	2017 (592)	-0.01
Total FA, en%	33 (5)	33 (5)	33 (5)	34 (5)	0.02
SFA, en%	12 (2)	14 (2)	14 (2)	16 (3)	0.47
MUFA-CIS, en%	10 (2)	10 (2)	10 (2)	9 (2)	-0.11
PUFA, en%	8 (2)	7 (1)	6 (1)	5 (1)	-0.66
Trans-fat, en%	1.2 (0.5)	1.4 (0.5)	1.4 (0.5)	1.5 (0.5)	0.24
Lipophilic load	1819 (322)	1966 (309)	2060 (324)	2210 (373)	0.41
Cholesterol, mg/d	197 (62)	215 (55)	225 (56)	233 (56)	0.27
Protein, en%	15 (2)	15 (2)	16 (2)	16 (3)	0.17
Carbohydrates, en%	45 (7)	45 (6)	45 (6)	45 (6)	-0.03
Glycemic index	0.53 (0.04)	0.53 (0.04)	0.53 (0.03)	0.52 (0.04)	-0.08
Alcohol, en%, [a]	2.2 (0.4, 6.0)	2.0 (0.4, 5.6)	1.7 (0.3, 5.1)	1.2 (0.1, 4.5)	-0.09
Vitamin C, mg/d	117 (50)	109 (44)	107 (43)	106 (44)	-0.09
Fiber, g/d	24 (5)	24 (5)	23 (5)	23 (5)	-0.16

[a] expressed as medians with 25th and 75th percentiles

[b] among subcohort members only. n = 2252 for total cholesterol, 2220 for HDL cholesterol, 2220 for LDL cholesterol, 2085 for hsCRP, and 2241 for HbA1c.

Table 3. Baseline characteristics according to quartiles of energy adjusted dietary LL

	Q1	**Q2**	**Q3**	**Q4**	Correlation with continuous dietary LL (r)
Quartile cut-offs	301.42 – 1775.51	1775.52 – 2005.20	2005.24 – 2239.02	2240.03 – 3928.67	
Subjects, n	9130	9130	9130	9130	
Male, %	31	30	23	17	0.13
(moderately) inactive, %	30	30	31	37	-0.05
Hypertension, %	37	35	36	39	0.01
Hyperlipidemia, %	12	8	6	5	-0.08
Diabetes, %	1.3	1.2	1.2	1.6	0.01
Current smoker,%	29	29	29	34	0.03
Low level of education, %	32	35	40	50	0.15
Age, years [a]	51 (40, 58)	50 (39, 57)	51 (42, 57)	51 (46, 59)	0.08
Systolic BP, mmHg	127 (19)	126 (18)	126 (19)	127 (19)	0.01
Diastolic BP, mmHg	78 (11)	78 (11)	78 (11)	78 (11)	0.01
BMI, kg/m2	25.3 (3.8)	25.5 (3.8)	25.7 (4.0)	26.2 (4.3)	0.08
Total cholesterol, mmol/L[b]	4.7 (0.9)	4.8 (0.9)	4.8 (0.9)	4.9 (0.9)	0.08
HDL cholesterol, mmol/L [b]	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.1 (0.3)	-0.02
LDL cholesterol, mmol/L [b]	2.7 (0.8)	2.8 (0.8)	2.8 (0.8)	2.9 (0.8)	0.12
hsCRP, mg/l [b]	1.0 (0.5, 2.1)	1.1 (0.5, 2.5)	1.1 (0.5, 2.5)	1.4 (0.6, 2.9)	0.09
HbA1c, g/dl [b]	0.6 (0.2)	0.6 (0.1)	0.6 (0.2)	0.6 (0.2)	0.08
Daily dietary intake					
Energy, kcal	2042 (640)	2110 (623)	2074 (587)	1986 (567)	-0.02
Total FA, en%	28 (4)	32 (3)	35 (3)	38 (4)	0.79
SFA, en%	11 (1)	13 (1)	15 (1)	17 (2)	0.94
MUFA-CIS, en%	8 (2)	9 (1)	10 (2)	11 (2)	0.66
PUFA, en%	6 (2)	6 (2)	6 (2)	6 (2)	<0.01
TFA, en%	1.0 (0.3)	1.3 (0.4)	1.5 (0.4)	1.7 (0.5)	0.58
Lipophilic index	33 (3)	34 (3)	35 (2)	36 (2)	0.49
Cholesterol, mg/d	183 (52)	208 (49)	225 (52)	253 (59)	0.41
Protein, en%	15 (3)	15 (2)	16 (2)	16 (2)	0.09
Carbohydrates, en%	49 (7)	46 (5)	44 (5)	41 (5)	-0.51
Glycemic index	0.52 (0.04)	0.53 (0.03)	0.53 (0.03)	0.53 (0.04)	0.02
Alcohol, en%, [b]	3.0 (0.4, 8.2)	2.1 (0.4, 5.7)	1.6 (0.3, 4.6)	1.0 (0.1, 3.5)	-0.18
Vitamin C, mg/d	129 (54)	113 (42)	105 (39)	92 (35)	-0.30
Fiber, g/d	25 (5)	24 (5)	23 (4)	22 (4)	-0.28

[a] expressed as medians with 25th and 75th percentiles

[b] among subcohort members only. N = 2252 for total cholesterol, 2220 for HDL cholesterol, 2220 for LDL cholesterol, 2085 for hsCRP, and 2241 for HbA1c.

point. We did this by assigning a weighted average of melting points of the most comparable fatty acids. Analyses were performed using SPSS version 20.0.

Results

The average fatty acid consumption in the study population was 33% of energy (73 g/d), with C16:0, C18:1n-9c and C18:2n-6c being the major contributors (**Table 1**). Spearman correlations were 0.41 between LI and LL, -0.04 between LI and the sum of fatty acids and 0.87 between LL and the sum of fatty acids.

The median age at enrolment was 51 years, and 25% was male. The percentage of males and individuals with hyperlipidemia decreased with higher dietary LI. The percentage of individuals with hypertension and with a low level of education increased with higher LI. There was a slight increase in BMI, total and LDL cholesterol, hsCRP and HbA1c over the quartiles of LI, and a slight decrease in HDL cholesterol and alcohol consumption (**Table 2**) For LL, a similar pattern was seen (**Table 3**).

Relations of LI and LL with dietary fatty acid intakes

A higher dietary LI correlated with higher intakes of SFA (r = 0.47), trans fatty acids (r = 0.24) and cholesterol (r = 0.27), and with lower intakes of PUFA (r = -0.66). MUFA intake did not change substantially with increasing dietary LI (r = -0.11; Table 2). Concerning the individual fatty acids, LI correlated highest with C18:2n-6c (r = -0.79; Table 1).
A higher dietary LL correlated strongly with higher intakes of SFA (r = 0.94) and total fatty acids (r = 0.79). Furthermore, higher dietary LL correlated with higher trans fatty acid (r = 0.58) and MUFA (r = 0.66) intake, but not with PUFA intake (r = 0.002) (Table 3). Concerning the individual fatty acids, LL correlated most strongly with C16:0 (r = 0.97) (Table 1).

Biochemical cardiovascular risk factors

In unadjusted analyses, a higher dietary LI was associated with an adverse profile of biochemical cardiovascular risk factors. After multivariable adjustment for other cardiovascular risk factors (model 3), associations remained present for total cholesterol (0.045 [95% CI: 0.006, 0.083] mmol/L per SD increase in dietary LI), and LDL cholesterol (0.061 [95% CI: 0.026, 0.096] mmol/L). Dietary LL associated with an adverse profile of biochemical cardiovascular risk factors as well. After multivariable adjustment (model 3), LL associated with higher concentrations of LDL cholesterol and HbA1c, and with lower concentrations of HDL cholesterol. We observed very similar results for the sum of fatty acids, except that the association with LDL cholesterol was weaker and not statistically significant in the multivariable model 3 (**Table 4**).

Table 4. Cross-sectional associations of energy adjusted dietary lipophilic index, lipophilic load and the sum of fatty acid intake with biochemical cardiovascular risk factors.

	Total cholesterol, mmol/L	HDL cholesterol, mmol/L	LDL cholesterol, mmol/L	hsCRP, mg/L	HbA1c, g/dL
	ß* (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)
Lipophilic index					
Crude	**0.097 (0.057, 0.136)**	-0.018 (-0.031, -0.004)	**0.110 (0.080, 0.150)**	**5.44 (0.40, 10.85)**	0.005 (-0.002, 0.012)
Model 1	**0.040 (0.003, 0.077)**	-0.009 (-0.021, 0.004)	**0.059 (0.025, 0.093)**	0.80 (-3.97, 5.76)	0.001 (-0.006, 0.008)
Model 2	**0.040 (0.003, 0.077)**	-0.009 (-0.022, 0.003)	**0.058 (0.025, 0.092)**	1.01 (-3.77, 5.97)	0.003 (-0.004, 0.009)
Model 3	**0.045 (0.006, 0.083)**	-0.006 (-0.019, 0.006)	**0.061 (0.026, 0.096)**	-0.90 (-5.87, 4.08)	0.001 (-0.005, 0.008)
Lipophilic load					
Crude	**0.072 (0.035, 0.110)**	-0.007 (-0.020, 0.006)	**0.093 (0.059, 0.126)**	**8.76 (3.87, 14.00)**	**0.012 (0.005, 0.018)**
Model 1	**0.038 (0.003, 0.073)**	-0.007 (-0.019, 0.004)	**0.061 (0.029, 0.093)**	2.43 (-2.12, 7.04)	**0.010 (0.004, 0.016)**
Model 2	**0.039 (0.004, 0.074)**	-0.007 (-0.019, 0.004)	**0.062 (0.031, 0.094)**	2.74 (-1.71, 7.47)	**0.009 (0.003, 0.015)**
Model 3	0.044 (-0.007, 0.096)	**-0.027 (-0.043, -0.011)**	**0.082 (0.036, 0.128)**	3.67 (-2.94, 10.52)	**0.011 (0.002, 0.019)**
Sum of fatty acids					
Crude	0.028 (-0.008, 0.065)	0.001 (-0.012, 0.014)	**0.042 (0.009, 0.075)**	**6.18 (1.51, 11.07)**	**0.010 (0.004, 0.016)**
Model 1	0.019 (-0.015, 0.053)	-0.003 (-0.015, 0.008)	**0.034 (0.003, 0.065)**	1.71 (-2.63, 6.18)	**0.010 (0.004, 0.017)**
Model 2	0.020 (-0.014, 0.054)	-0.003 (-0.015, 0.008)	**0.036 (0.005, 0.066)**	1.92 (-2.43, 6.40)	**0.009 (0.003, 0.015)**
Model 3	-0.001 (-0.054, 0.052)	**-0.026 (-0.043, -0.009)**	0.026 (-0.022, 0.073)	4.29 (-2.53, 11.40)	**0.012 (0.003, 0.021)**

N = 2252 for total cholesterol, 2220 for HDL cholesterol, 2220 for LDL cholesterol, 2085 for hsCRP, and 2241 for HbA1c. Estimates are derived from linear regression and expressed per SD increase in dietary lipophilic index (SD = 3), lipophilic load (SD = 351) or sum of fatty acids (SD = 9). hsCRP was log-transformed before the analysis due to non-normal distribution of the residuals of the regression of hsCRP on the exposures. Beta's and 95% CI for hsCRP were back-transformed after the analysis in order to obtain interpretable estimates. Values in bold are statistically significant at the $p<0.05$ level.

*Estimated beta-coefficients

Model 1: adjusted for age, sex, smoking, physical activity index, BMI, education.

Model 2: additionally adjusted for systolic blood pressure, hypertension, diabetes.

Model 3: additionally adjusted for dietary intakes of energy, cholesterol, carbohydrates and alcohol.

Table 5. Hazard ratios and their 95% CI for the association of quartiles of energy adjusted dietary lipophilic index, lipophilic load and the sum of fatty acid intake with incident risk of coronary heart disease and ischemic stroke

	Lipophilic index				
Quartile cut-offs	(18.15 – 32.8)	(32.83 – 34.75)	(34.76 – 36.53)	(36.54 – 44.71)	*P* for trend
CHD					
n total / cases	9130 / 549	9130 / 543	9130 / 576	9130 / 680	
Crude	Ref	0.98 (0.87, 1.10)	1.03 (0.92, 1.16)	1.19 (1.07, 1.34)	0.002
Model 1	Ref	0.96 (0.85, 1.08)	0.95 (0.84, 1.07)	0.94 (0.84, 1.05)	0.28
Model 2	Ref	0.96 (0.86, 1.09)	0.95 (0.84, 1.07)	0.95 (0.85, 1.07)	0.38
Model 3	Ref	0.96 (0.85, 1.08)	0.93 (0.83, 1.05)	0.93 (0.83, 1.04)	0.19
Ischemic stroke					
n total / cases	9130 / 101	9130 / 108	9130 / 106	9130 / 164	
Crude	Ref	1.04 (0.79, 1.37)	0.99 (0.75, 1.30)	1.41 (1.10, 1.81)	0.01
Model 1	Ref	1.02 (0.78, 1.34)	0.91 (0.69, 1.20)	1.10 (0.86, 1.42)	0.59
Model 2	Ref	1.03 (0.79, 1.35)	0.91 (0.69, 1.20)	1.13 (0.88, 1.45)	0.49
Model 3	Ref	1.04 (0.79, 1.37)	0.93 (0.70, 1.22)	1.15 (0.89, 1.48)	0.42
	Lipophilic load				
Quartile cut-offs	(301.42 – 1775.51)	(1775.52 – 2005.20)	(2005.24 – 2239.02)	(2240.03 – 3928.67)	*P* for trend
CHD					
n total / cases	9130 / 575	9130 / 554	9130 / 577	9130 / 642	
Crude	Ref	0.96 (0.85, 1.07)	0.98 (0.87, 1.10)	1.08 (0.96, 1.21)	0.14
Model 1	Ref	0.97 (0.87, 1.10)	0.99 (0.88, 1.11)	0.97 (0.86, 1.09)	0.65
Model 2	Ref	0.99 (0.88, 1.11)	1.02 (0.91, 1.14)	1.00 (0.89, 1.12)	0.93
Model 3	Ref	0.96 (0.85, 1.08)	0.96 (0.84, 1.10)	0.92 (0.79, 1.07)	0.31
Ischemic stroke					
n total / cases	9130 / 107	9130 / 120	9130 / 125	9130 / 127	
Crude	Ref	1.12 (0.86, 1.45)	1.11 (0.86, 1.43)	1.06 (0.82, 1.37)	0.72
Model 1	Ref	1.15 (0.88, 1.49)	1.12 (0.86, 1.45)	0.95 (0.73, 1.23)	0.58
Model 2	Ref	1.17 (0.90, 1.52)	1.15 (0.89, 1.49)	0.98 (0.76, 1.28)	0.79
Model 3	Ref	1.18 (0.90, 1.55)	1.16 (0.86, 1.55)	0.98 (0.70, 1.38)	0.81

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Table 5 continued

	Sum of fatty acid intake				
Quartile cut-offs	(9.06 – 52.04)	(52.05 – 58.24)	(58.25 – 64.36)	(64.37 – 110.75)	*P* for trend
CHD					
n total / cases	9130 / 608	9130 / 541	9130 / 564	9130 / 635	
Crude	Ref	0.88 (0.78, 0.99)	0.92 (0.82, 1.03)	1.02 (0.91, 1.14)	0.51
Model 1	Ref	0.94 (0.83, 1.05)	0.99 (0.88, 1.11)	1.03 (0.92, 1.15)	0.48
Model 2	Ref	0.95 (0.84, 1.06)	1.01 (0.90, 1.14)	1.06 (0.95, 1.19)	0.19
Model 3	Ref	0.93 (0.83, 1.06)	1.00 (0.88, 1.14)	1.04 (0.89, 1.22)	0.42
Ischemic stroke					
n total / cases	9130 / 124	9130 / 112	9130 / 129	9130 / 114	
Crude	Ref	1.16 (0.90, 1.49)	1.04 (0.80, 1.35)	1.18 (0.92, 1.52)	0.41
Model 1	Ref	0.95 (0.73, 1.22)	1.10 (0.86, 1.40)	0.86 (0.67, 1.11)	0.41
Model 2	Ref	0.96 (0.74, 1.23)	1.12 (0.87, 1.43)	0.89 (0.69, 1.16)	0.60
Model 3	Ref	0.94 (0.72, 1.24)	1.09 (0.82, 1.45)	0.84 (0.59, 1.20)	0.49

Model 1: adjusted for age, sex, smoking, physical activity index, BMI, education.
Model 2: additionally adjusted for systolic blood pressure, hypertension, diabetes.
Model 3: additionally adjusted for dietary intakes of energy, cholesterol, carbohydrates and alcohol.

Risk of CHD and stroke

During a mean follow-up of 15 (SD: 2) years, 2348 CHD events occurred and 849 stroke events of which 479 were ischemic. Dietary LI was associated with increased risk of CHD and ischemic stroke in unadjusted analysis. After multivariable adjustments (model 3), these associations attenuated (HRQ4-Q1: 0.93 [95% CI: 0.83, 1.04] and 1.15 [95% CI: 0.89, 1.48], respectively). Age and level of education mainly caused this attenuation (**Table 5**). For total stroke, results were in line with those for ischemic stroke (**Supplemental Table S1**). Dietary LL was not related to risk of CHD in unadjusted analysis, and neither in multivariable adjusted analysis (HR_{Q4-Q1}, model 3: 0.92 [95% CI: 0.79, 1.07]) (Table 5). Dietary LL was not related to ischemic stroke in unadjusted analysis or adjusted analysis (HR_{Q4-Q1}, model 3: 0.98 [95% CI: 0.70, 1.38]) (Table 5). For total stroke, results were very similar to those for ischemic stroke (Supplemental Table S1). The sum of fatty acids was not related to risk of CHD, ischemic stroke (Table 5), or total stroke (Supplemental Table S1).

Sensitivity analyses

In sensitivity analyses (adjustment model 3), no meaningful changes in HRs were observed, except for the following. Addition of SFA and PUFA intake to model 3 changed the HR_{Q4-Q1} of LI to 1.05 (95% CI: 0.88, 1.24) for CHD and to 1.72 (95% CI: 1.19, 2.50) for ischemic stroke (**Supplemental Table S2**).

Discussion

In this study, we found that diets with higher LI correlated with high intakes of SFA and *trans* fatty acids, and with low PUFA intakes. Diets with high LL correlated strongly with intakes of SFA and the sum of fatty acids, and not with PUFA intake. Diets with high LI, LL and sum of fatty acids were associated with an adverse baseline cholesterol profile. In prospective analyses in 36520 individuals followed up for 15 years, we found no associations with risk of CHD and ischemic stroke.

Strengths of our study are its prospective design, long follow-up, and a large number of incident CHD and stroke cases. A limitation is that we were unable to assign melting points to 8% of total fatty acid intake. Even though sensitivity analyses showed that additionally taking into account those fatty acids did not change our findings, we cannot rule out that we missed effects of dietary LI and LL due to our inability of including these fatty acids in the scores. Another limitation is that our study population has a relatively high SFA and low PUFA intake, with relatively little variation in intake between individuals. This led to a low level of variation in dietary LI and LL and may have limited us to detect associations with CHD or stroke. Our findings will not be directly applicable to populations with wider variations in the amount and types of fat intake.

Only few studies have investigated whether LI relates to cardiovascular outcomes [9, 10]. We found that diets with high LI were associated with an adverse cholesterol profile. This is in line with what would be expected and is also in line with a previous report on LI [9]. However, in prospective analyses we found no association of LI with CHD. We are aware of only one comparable study on the relation of dietary LI and myocardial infarction risk among 1627 case-control pairs from Costa-Rica [9]. In contrast to our study, the study from Costa-Rica reported increased risk of myocardial infarction with higher LI. The Costa-Rican study had lower LI scores (average of 30 among cases and 29 among controls) and much more variation in LI compared with our study. This may have allowed them to pick up an association. Moreover, the contribution of individual fatty acids to the LI differed. For example, in our study population the contribution of C18:1n-9c was lower, and the contribution of dairy derived fatty acids, such as C15:0, was higher. In addition, several conference abstracts presented the relation of LI with CHD, with reports of increased and neutral risks [11, 12]. Due to the very brief reports and preliminary results in these abstracts, we cannot discuss our results in comparison with those studies.

Regarding LL, we found no relation with CHD. To our knowledge, no other articles have been published on this topic, except two conference abstracts that reported neutral and harmful associations with CHD [11, 12]. The lack of association of LL, and also of the sum of fatty acids, with CHD in our study is in line with literature that suggests no association of total fat and saturated fat intake with CHD [1, 23, 24].

For ischemic stroke, we are the first to investigate its relation with dietary LI and LL, and we found no associations in the main analysis. In general, intakes of total fatty acids, saturated fatty acids and total PUFA and MUFA are not related with ischemic stroke in current literature [25, 26]. This is in line with our finding of no relation between the sum of fatty acid intake and ischemic stroke. In literature, there are suggestions for a protective association of fish fatty acids (long-chain *n*-3 PUFAs) and ischemic stroke [27]. Indeed, n-3 PUFAs have relatively low melting points and will thus contribute to relatively low LI and LL scores. However, the *n*-3 PUFA intake in our study population is very low (<1.5% of fatty acid intake), and thus contributed very little to the LI and LL, and likely did not drive the associations of LI and LL with ischemic stroke.

LI and LL are relatively new concepts, and we should critically evaluate their interpretation and value.

First of all, it should be noted that we calculated LI and LL based on fluidity of dietary fatty acids consumed. Most dietary fatty acids do not directly relate to blood or adipose tissue concentrations of fatty acids, including fatty acids in membrane phospholipids [28]. This is because fatty acid concentrations in cell membranes are not only determined by diet, but also by de novo synthesis of fatty acids. Dietary LI and LL are therefore not by definition related to cell membrane fluidity, which is hypothesized to affect cardiovascular health [6-8]. A previous study indeed showed low correlations between LI of diet with plasma or erythrocytes (0.18 and -0.10, respectively) [9]. Rather than reflecting membrane fluidity, the added value of the dietary LI and LL lies in that they provide an alternative way to quantify the overall fat quality of diet in only one variable, and take into account differential effects of fatty acids within original classifications of fatty acids.

We showed that higher dietary LI correlated with higher SFA and trans fatty acid intake, and lower PUFA intake. This suggests dietary LI provides a good summary of fat quality of the diet. Given this interpretation of dietary LI, adjustments for fatty acid classifications such as SFA in the statistical analyses would be over-adjustments, and such adjustments will not provide insight in whether LI has added value beyond original fatty acid classifications. For LL we found (extremely) high correlations with SFA intake ($r = 0.95$; particularly C16:0 [$r = 0.97$]), and the sum of fatty acid intake ($r = 0.87$), whereas PUFA intake remained stable over the quartiles of LL. From this, we conclude that LL does not represent a typical unfavorable fat profile of the diet. Rather, we must note that we were unable to separate effects of LL from SFA, and perhaps also from total fatty acid intake. This is also supported by our previous finding of no relation of C16:0 intake with CHD incidence in this study population [29]. The high correlation of LL with SFA and the sum of fatty acid intake may in part be due to the little variation in (classes of) fat intake in our population. The concept of LL might be more valuable in populations with larger variations in fatty acid intakes.

Finally, it should be noted that, even though the LI and LL are meant to be considered universal measures independent of the type of fatty acids consumed, we cannot rule out

that the foods or food pattern from which the fatty acids are derived affect the associations of the fatty acids and LI with disease. For instance, previous studies suggested that SFAs derived from meat may be harmful, whereas SFAs derived from dairy products may be protective [30]. This could be due to differences in types of SFAs in meat and dairy products, but also due to the food matrix they are derived from and potential interactions of nutrients within such foods.

In conclusion, in this Dutch population that typically consumes diets high in SFA and low in PUFA, high LI diets represent an overall low fat quality of the diet. High LL diets particularly reflect high total fat and SFA intake, and may not provide additional information beyond just SFA intake. In our population, the overall fluidity of the dietary fatty acids consumed (LI), the amount of fatty acids consumed, and the combination of the two (LL), did not relate to risk of CHD or ischemic stroke. It remains to be seen in populations with more variation in SFA and PUFA intake whether dietary LI and LL are useful indicators of fat quality of the diet with regard to cardiovascular health.

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Conflicts of interests
IS and YTvdS report grants from Unilever Research and Development, Vlaardingen, the Netherlands, outside the submitted work. JP is financially supported by a restricted research grant from Unilever Research and Development, Vlaardingen, Netherlands. None of the other authors reported a conflict of interest related to this study.

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Chapter 6.

Substitution modelling in baseline dietary data versus observed substitution modelling over time

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Abstract

Background: The association between saturated fat (SFA) and coronary heart disease (CHD) may depend on the substituting macronutrient. However, a substantial amount of evidence for this comes from observational cohort studies in which substitution of SFA with other macronutrients during follow-up was not actually observed, but instead inferred from comparisons between subjects.

Objective: to investigate *1*) whether substitution of dietary SFA occurs within subjects over time and if so; *2*) whether within-subject substitution over time is associated with changes in cholesterol concentrations, and *3*) if the results of within-subject substitution are similar to those of between-subject substitution based on baseline data.

Design**:** 277 postmenopausal women (55–70 years) from Prospect-EPIC were included. Diet and serum cholesterol concentrations were measured at baseline and after a median follow-up time of 3.8 years. With multivariable linear regression analysis, we estimated the association of within-subject substitution and between-subject substitution of SFA for carbohydrates and changes in cholesterol.

Results**:** Mean baseline concentrations of total-, HDL- and non-HDL cholesterol were 5.9 (± 1.0), 1.5 (± 0.4), and 4.3 (± 1.1) mmol/L, respectively. Median baseline SFA intake was 14.0 en%/day. During follow-up, the mean (±SD) SFA intake decreased by 0.6 (± 2.5) en%, which was primarily compensated for by an increase in carbohydrate intake. Subject ranking according to SFA intake changed during follow-up ($\kappa_w = 0.54$). Within-subject substitution of SFA for carbohydrates over time was associated with a -0.023 mmol/L (95%CI: -0.047, 0.001) difference in HDL cholesterol per 1 en%. Between-subject substitution of SFA for carbohydrates using baseline data was associated with a 0.033 mmol/L (95%CI: 0.008, 0.057) difference in HDL cholesterol. Both within-subject and between-subject substitution were not associated with differences in total-cholesterol and non-HDL cholesterol.

Conclusions: In this study, we observed opposite associations of within-subject substitution as compared with between-subject substitution of SFA for carbohydrates with HDL cholesterol differences over time. This suggests that inference from between-subject substitution in baseline data only may be unreliable if substitution actually occurs within subjects during follow-up.

Introduction

The current ongoing debate [1] about the link between dietary saturated fatty acids (SFA) and coronary heart disease (CHD) is largely caused by the discrepancy between the low density lipoprotein (LDL)-cholesterol raising effect of SFA in randomized controlled trials [2] and the lack of significant associations between SFA and clinically manifest outcomes in observational studies [3-5]. One of the possible explanations for this discrepancy is the fact that these observational cohort studies did not take into account the type of macronutrient that SFA was substituted for. Since intervention studies have shown that the effect of SFA on the ratio of total cholesterol : HDL cholesterol depends on whether SFA replaces either unsaturated fat or carbohydrates [2], this may also influence the observed association between SFA and CHD in observational studies. Indeed, a pooled analyses of 11 cohort studies [6] observed that statistically modelled substitution of SFA for polyunsaturated fat (PUFA) was related to a higher CHD risk, whereas substitution of SFA for carbohydrates or monounsaturated fat (MUFA) was associated with a lower or equivalent CHD risk. However, two recent observational studies in two Dutch cohorts [7, 8] did not confirm that the association between SFA and CHD depended on the substituting macronutrient.

Usually, the substitution of macronutrients in prospective observational studies is modelled rather than observed, with use of, for instance, the nutrient density model[9]. With such a model the association between SFA and CHD is adjusted for total energy intake and all energy contributing macronutrients, except for the macronutrient that SFA is substituted for. The estimated regression coefficient for SFA is then interpreted as the CHD risk per unit increase in intake of energy from SFA at the expense of an equal amount of energy from the substituted macronutrient. The dietary data used in these studies to model macronutrient substitution is usually collected at a single moment in time. Therefore, in fact, a between-subject comparison is made in which subjects with a relatively high intake of SFA and a relatively low intake of another macronutrient, such as polyunsaturated fat (PUFA), are compared to subjects with a relatively high intake of PUFA and a relatively low intake of SFA. The results of these models are interpreted as within-person substitution effects of SFA for PUFA. However, such a model does not investigate actual macronutrient substitution within a person over time but relies on between-person comparisons. Moreover, most substitution analyses [6] were done using only a single baseline dietary measure. And in the studies [10-12] that used repeated measures of dietary intake, the macronutrient substitution was modelled in the same way, with the only addition that the substitution modelling was repeated for each time point at which the data was collected.

It is unknown to what extent macronutrient substitution actually occurs within subjects over time in an observational setting. Also, to our knowledge, no study examined whether an association between substitution and CHD risk factors, based on between-subject comparisons, is representative for the association between actually observed substitution within subjects and CHD risk factors.

Therefore, in the present study, we investigated to what extent SFA intake changed within subjects over time, and whether these changes in SFA were substituted with other macronutrients. Secondly, we investigated whether within-subject substitution over time was associated with changes in serum cholesterol concentrations and if the results of within-subject substitution over time yielded similar results as those of between-subject substitution.

Methods

Study population

For the present study, we used data from a previously described cohort [13] comprising 402 women, aged 49–70 years old. In short, women were recruited from the Prospect-EPIC study [14]. Women were selected when they had experienced a natural menopause, had an intact uterus and at least one intact ovary, and they should not have used sex steroids after the reported date of last menstruation. The study complied with the declaration of Helsinki, and was approved by the Institutional Review Board of the University Medical Center Utrecht. All participants provided written informed consent.
Of the 402 women, we excluded all women with missing dietary data (n = 100), and women with missing data on blood cholesterol concentrations (n = 25), leaving 277 women for the present study.

Dietary measurements

Dietary information was obtained with a validated self-administered FFQ between 1993 and 1997 (baseline) and between 1999 and 2000. The FFQ gathered information on the average consumption frequency of 79 main food items, which allowed estimation of the habitual consumption of 178 food items for each individual in grams per day [15] The FFQ was validated before against twelve 24 hour recalls [16, 17]. The correlation coefficients for the relative validity of SFA, PUFA, MUFA and carbohydrates were, respectively, 0.55, 0.52, 0.66 and 0.71 in men and 0.50, 0.22, 0.58 and 0.72 in women. To calculate the intake of nutrients and total energy, an updated version of the computerized Dutch food composition table 1996 was used. For the present analyses, intakes in g/d of SFA, MUFA, PUFA, carbohydrates, protein and *trans*-fat were converted to % of total energy intake (en%). Total energy intake was the sum of energy from all these macronutrients, and excluded energy from alcohol.

Measurement of cholesterol concentrations

At baseline, participants donated 30-ml non-fasting blood samples, which within 24 hours were stored at -80 °C, and later at -196 °C under liquid nitrogen. Total cholesterol and HDL cholesterol were measured in serum and/ or citrate plasma. Total cholesterol was determined with an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson). HDL

cholesterol was measured with a colorimetric assay on a Hitachi 904 (Johnson & Johnson) [18]. In participants of whom both serum and citrate plasma values were measured, these values were highly correlated (r_p = 0.93 for both total and HDL cholesterol) but the corrected citrate plasma values were systematically lower than the serum values. Therefore, we standardized the two measurements into one serum measurement by single imputation with non-Bayesian linear regression (MICE package in R[19]), in which we predicted the serum cholesterol values based on all available (uncorrected) citrate cholesterol values.
At the second data assessment between 1999 and 2000, fasting blood samples were donated before 1100 h. Serum total cholesterol and HDL cholesterol were reflectometrically measured with use of commercial enzymatic kits with a Vitros 250 (Johnson & Johnson).

Measurement of covariables

From the general questionnaires and the medical history at both visits, information was obtained on smoking status and education level. Smoking status was defined as current, former and never. Education level was defined as low (primary education up to completing intermediate vocational education), intermediate (up to higher secondary education) or high (higher vocational education and university). Physical activity was measured with a validated questionnaire on three types of activities (household, sporting and other leisure time activities), which was adapted to an elderly population [20]. The total score was used in the analyses. For 22 participants in whom physical activity data was missing at baseline or at follow-up, the values were imputed using predictive mean matching (MICE package in R[19]). Use of lipid lowering medication was assessed during the second assessment. Anthropometric measures included the measurement of waist circumference, height and weight. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

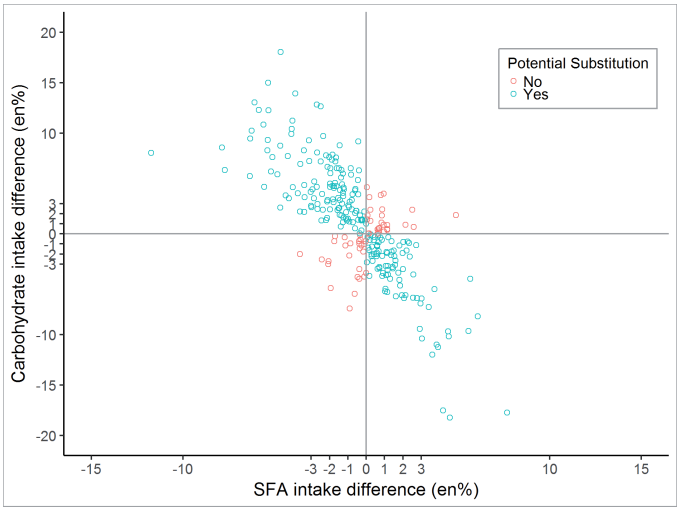

Figure 1. Differences between measurements after ~ 4 years of follow-up and at baseline of the reported saturated fat consumption against the carbohydrates consumption in 277 women.

Data analysis

For all 277 women, we calculated the intake differences (in en%) of SFA, MUFA, PUFA, total carbohydrates, vegetable protein, animal protein and *trans*-fat by subtracting the baseline intakes from the intakes of the second measurement. Differences in total-, HDL- and non-HDL cholesterol concentrations and covariables were calculated in the same way. Baseline characteristics were determined across quintiles of the observed substitution of SFA during follow-up and across quintiles of the baseline SFA intake distribution (in en%). To examine whether subject ranking according to SFA intake at baseline changed after follow-up, we compared quintiles of the SFA intake distributions as measured at baseline with those measured after follow-up. In addition, we calculated the weighted kappa (κ_w) to quantify the agreement between the two measurements.

Within subject substitution versus cholesterol concentrations

To examine the association of within-subject substitution with change in serum cholesterol, we included 223 participants who actually substituted SFA with or for another macronutrient, i.e., those in whom an increase in SFA went together with a decrease in the other macronutrient or vice versa. Among these participants, we calculated a substitution score. This score indicated how much of the energy intake of SFA was substituted for the other macronutrient. To illustrate, the substitution score for the substitution of SFA for carbohydrates was equal to the intake difference in energy from SFA (in en%) for which the opposite difference in energy from carbohydrates was observed (**Figure 1)**. A participant who reported intake differences of respectively +1.5 en% SFA and -3 en% carbohydrates was assigned the score +1.5 en%. A participant who reported intake differences of -3 en% SFA and +1.5 en% carbohydrates was assigned -1.5 en% (**Figure 2**).

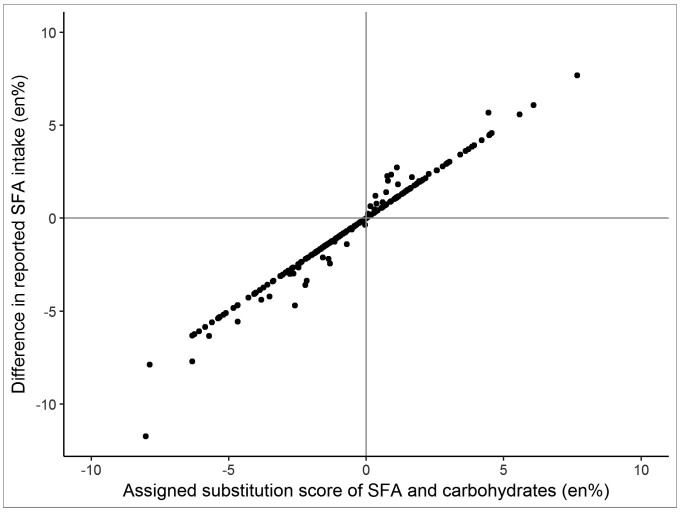

Figure 2. The assigned substitution score against the differences between measurements after ~ 4 years of follow-up and at baseline in 223 women.

We used multivariable linear regression modelling, including the difference in cholesterol concentrations as the dependent variable and the substitution score as the independent variable. The substitution score was modelled continuously per 1 en% increment. In addition, we modelled the score categorically. To illustrate, for the substitution score of SFA and carbohydrates the following categories were used: ≥3 en% from carbohydrates for SFA; 2-3 en% from carbohydrates for SFA; 1-2 en% from carbohydrates for SFA; 0-1 en% from carbohydrates for SFA or 0-1 en% from SFA for carbohydrates; 1-2 en% from SFA for carbohydrates; ≥2 en% from SFA for carbohydrates (**Figure 3**). Substitution of SFA and carbohydrates between -1 en% and 1 en% served as the reference category. All models were adjusted for the following potential confounders: age, education level and smoking status at baseline, baseline intakes of SFA and the substituting macronutrient, differences between follow-up and baseline intakes of total energy, alcohol, dietary cholesterol and fibre, differences in BMI and physical activity score, follow-up time and the use of lipid lowering medication during follow-up.

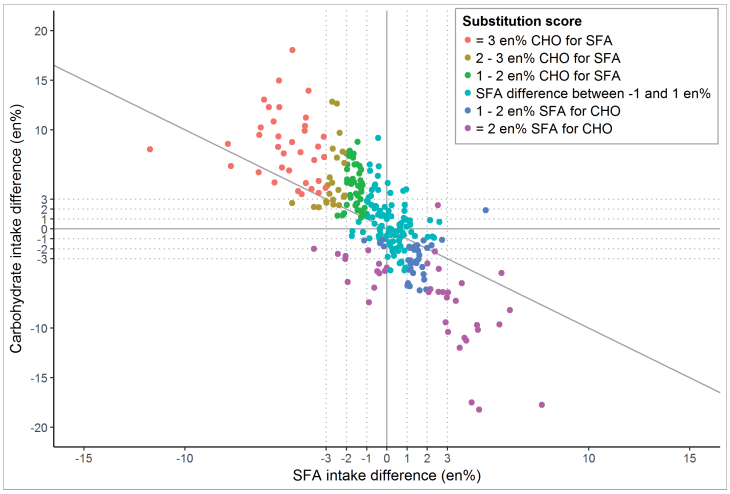

Figure 3. Assignment of substitution score categories in a plot of intake (in en%) differences in saturated fat (SFA) intake against the difference in carbohydrate intakes (CHO) in 223 women

Between subject substitution versus cholesterol concentrations

In the same participants in whom we examined the within-subject substitution, we investigated the association of between-subject substitution with cholesterol concentration differences with use of to the multivariable nutrient density model [9]. This model was also a linear regression model and included the difference in cholesterol concentrations as outcome variable. The independent variables were the baseline intakes of SFA, MUFA, PUFA, *trans*-fat, vegetable protein and animal protein (all in en%), as well as the sum of energy (kcal) from all the latter macronutrients and from carbohydrates in the model. The estimated regression coefficient of SFA is then to be interpreted as the difference in cholesterol concentration per 1 en% increase in SFA intake at the expense of 1 en% reduction of carbohydrates intake. These models were adjusted for the baseline values of the same potential

Table 1. Population characteristics across quintiles of the observed substitution of SFA and carbohydrates within 223 postmenopausal women of Prospect-EPIC

SFA for carbohydrates, en%	Q1	Q2	Q3	Q4	Q5
(min, max)	(-8.02, -2.64)	(-2.60, -1.35)	(-1.32, -0.04)	(-0.01, 1.14)	(1.15, 7.68)
Subjects, *n*	44	45	45	45	44
Baseline measures					
Age, *y*	63.1 (± 3.6)	63.1 (± 4.3)	62.3 (± 3.4)	62.6 (± 4.0)	63.5 (± 3.9)
BMI, kg/m^2	27.0 (± 4.9)	27.0 (± 4.6)	25.4 (± 2.9)	25.8 (± 5.1)	25.3 (± 3.4)
HDL cholesterol,	6.0 (± 1.2)	6.3 (± 1.1)	5.8 (± 0.8)	6.2 (± 1.0)	5.8 (± 0.9)
Total cholesterol, *mmol/L*	1.5 (± 0.5)	1.6 (± 0.5)	1.6 (± 0.4)	1.6 (± 0.4)	1.6 (± 0.5)
Non-HDL cholesterol, *mmol/L*	4.5 (± 1.2)	4.7 (± 1.2)	4.2 (± 0.9)	4.6 (± 1.1)	4.2 (± 1.0)
High education level, *%*	15.4	22.0	6.6	19.8	13.2
Current smoker, *%*	13.2	13.2	15.4	4.4	15.4
Physical activity score	8.4 (± 5.2)	6.3 (± 4.4)	5.8 (± 3.9)	7.3 (± 6.1)	7.2 (±4.4)
Energy, *kcal*	1861 (± 370)	1741 (± 377)	1733 (± 350)	1716 (± 325)	1734 (± 414)
Saturated fat, *en%*	17.8 (± 2.5)	16.5 (± 2.8)	15.9 (± 2.3)	14.5 (± 2.7)	13.8 (± 2.5)
Carbohydrates, *en%*	43.3 (± 4.9)	44.1 (± 5.2)	45.1 (± 5.0)	49 (± 6.5)	50.5 (± 5.9)
Monounsaturated fat, *en%*	14.1 (± 2.2)	14.3 (± 2.7)	13.9 (± 2.1)	12.4 (± 2.7)	11.9 (± 2.3)
Polyunsaturated fat, *en%*	7.0 (± 2.2)	6.9 (± 1.7)	7.4 (± 2.1)	6.5 (± 1.8)	6.9 (± 1.9)
Trans-fat, *en%*	1.3 (± 0.3)	1.3 (± 0.3)	1.3 (± 0.4)	1.2 (± 0.4)	1.1 (± 0.3)
Vegetable protein, *en%*	5.4 (± 1.0)	5.3 (± 0.7)	5.6 (± 0.9)	6.0 (± 1.0)	5.8 (± 0.8)
Animal protein, *en%*	11.0 (± 2.7)	11.5 (± 2.2)	10.8 (± 1.9)	10.3 (± 2.6)	10.0 (± 2.2)
Vitamin C, *mg*	108 (± 42)	114 (± 36)	107 (± 40)	124 (± 38)	143 (± 72)
Cholesterol, *mg*	215 (± 57)	216 (± 58)	206 (± 67)	186 (± 74)	176 (± 61)
Fibre, *g*	23.1 (± 6.5)	21.9 (± 5.1)	22.3 (± 6.1)	24.2 (± 4.9)	23.9 (± 5.1)
Alcohol, *g*	2.9 (0.2-9.2)	3.1 (0.6-13.0)	3.5 (0.3-13.4)	1.8 (0.0-10.2)	4.1 (0.8-15.6)

Differences after follow-up					
Δ Follow-up time, *y*	3.7 (± 0.7)	3.8 (± 0.7)	3.7 (± 0.7)	3.7 (± 0.8)	3.9 (± 0.7)
Δ BMI, kg/m^2	-0.2 (± 1.6)	0.4 (± 1.3)	0.3 (± 1.2)	0.2 (± 1.3)	0.3 (± 1.6)
Δ Physical activity score	5.1 (± 8.3)	7.7 (± 6.8)	7.2 (± 6.6)	6.6 (± 8.1)	7.8 (± 7.8)
Δ Saturated fat, *en%*	-4.6 (± 1.8)	-2.0 (± 0.6)	-0.8 (± 0.5)	0.8 (± 0.6)	2.8 (± 1.5)
Δ Carbohydrates, *en%*	7.7 (± 3.7)	4.8 (± 2.5)	2.9 (± 2.0)	-1.9 (± 1.6)	-6.3 (± 4.3)
Δ Vegetable protein, *en%*	0.7 (± 0.9)	0.7 (± 0.6)	0.3 (± 0.8)	-0.2 (± 0.8)	-0.3 (± 0.8)
Δ Animal protein, *en%*	-0.7 (± 2.6)	-1.2 (± 1.9)	-1.0 (± 1.7)	0.3 (± 1.7)	1.3 (± 2.1)
Δ Monounsaturated fat, *en%*	-2.7 (± 1.6)	-2.1 (± 1.4)	-1.1 (± 1.2)	0.3 (± 1.1)	1.8 (± 1.7)
Δ Polyunsaturated fat, *en%*	-0.3 (± 2.5)	-0.1 (± 1.3)	-0.2 (± 1.8)	0.7 (± 1.3)	0.5 (± 1.9)
Δ *Trans*-fat, *en%*	-0.3 (± 0.3)	-0.2 (± 0.2)	0.0 (± 0.3)	0.1 (± 0.2)	0.3 (± 0.3)
Δ Alcohol, *g*	2.0 (± 16.3)	1.5 (± 7.4)	0.4 (± 6.0)	-0.4 (± 5.5)	-1.0 (± 6.4)
Δ Fibre, *g*	-0.5 (± 4.4)	0.9 (± 4.6)	0.7 (± 4.6)	-0.6 (± 4.3)	-2.1 (± 4.3)
Lipid lowering medication use, *%*	8.8	8.8	15.4	15.4	18.7

En% represents the percentage of the sum of energy from all macronutrients, except for energy from alcohol.

confounders as used the within-subject substitution model, for follow-up time, and for the use of cholesterol lowering medication during follow-up.

Because of the differences between the methods used to measure cholesterol concentrations at baseline and after follow-up, the estimated cholesterol concentration differences in our analysis cannot be interpreted as an absolute increase or decrease in cholesterol. Instead, it indicates the difference in the change of the cholesterol concentrations between participants who substitute SFA for carbohydrates compared with those who substitute carbohydrates for SFA. More precisely, the represents the difference in cholesterol concentration change per 1 en% of SFA substituted for carbohydrates. However, for ease of reading, we will refer to the as an increase or decrease in cholesterol concentration in the remaining part of this article.

Sensitivity analyses

We repeated the analyses in all 277 participants, thereby also including those participants in whom no within-subject substitution was observed. Also, we repeated all analyses excluding participants who reported the use of cholesterol lowering medication at follow-up (n = 30) and excluding the participants with missing physical activity scores (n = 22).
Analyses were done in R for Windows, version 3.3.0 (R Development Core Team. Released 2016. Vienna, Austria: R Foundation for Statistical Computing).

Results

The mean (± SD) difference in SFA intake after follow-up was -0.6 (± 2.5) en%. The maximum increase in SFA intake was 7.7 en% and the maximum decrease was 11.7 en%. In 80.5% of the participants the change in SFA intake went together with an opposite change in carbohydrates intake **(**Figure 1**)**. In the remaining participants, SFA and carbohydrates both increased or decreased. Exchange of SFA with any of the other macronutrients was only observed in a few participants **(Supplemental Figure S1).** Population characteristics are therefore shown for the 223 participants in whom we observed substitution of SFA and carbohydrates over time.

In **Table 1** the population characteristics for the selected 223 participants are presented across quintiles of the within-subject substitution of SFA and carbohydrates (i.e., the substitution score). Compared with subjects who substituted carbohydrates for SFA (i.e., lower quintiles), the subjects who substituted SFA for carbohydrates (i.e., higher quintiles) on average had a lower baseline intake of SFA and a higher baseline intake of carbohydrates, and lower baseline values of HDL cholesterol, BMI and blood pressure. In addition, they were less educated and slightly less physically active. The mean (± SD) follow-up time between the baseline and second measurement was 3.8 (± 0.7) years. In the 223 participants, the mean (± SD) difference between the reported intakes of SFA was -0.8 (± 2.7) en%, and the

amount of SFA that was substituted for carbohydrates ranged from -8.0 to 7.7 en%. Within-subject substitution of SFA for carbohydrates was associated with an increase in BMI, with the use of lipid lowering medication and with slightly higher intakes of MUFA, PUFA, *trans-fat* and animal protein, and with lower intakes of vegetable protein and fibre after follow-up. **Supplemental Table S1** shows the population characteristics across quintiles of the baseline SFA intake, which ranged from 8.2 to 24.0 en%.

Supplemental Table S2 shows a cross table of the quintiles of SFA intake measured after ~4 years of follow-up versus at baseline. After follow-up, 40% of the 277 participants remained in the same quintile. Of the other participants, 36.2% shifted to an adjacent quintile and 23.8% to a non-adjacent quintile. The κ_w was 0.54 (0.44-0.63).

Within-subject substitution of SFA and carbohydrates in relation to serum cholesterol

The estimated associations of within-subject substitution of SFA for carbohydrates with total-, HDL-, and non-HDL cholesterol differences over time are shown in **Figure 4** (continuously) and **Figure 5** (in categories).
The within-subject substitution of energy from SFA for energy from carbohydrates was not associated with total cholesterol (per 1 en%: 0.030 mmol/L, 95%CI: -0.043, 0.104) or non-HDL cholesterol (per 1 en%: 0.053 mmol/L, 95%CI: -0.020, 0.127) **(Figure 4)**. Each additional within-subject substitution of 1 en% SFA for carbohydrates was borderline significantly associated with a -0.023 mmol/L (95% CI: -0.047, 0.001) difference in HDL cholesterol. The latter finding was confirmed across categories of the substitution (**Figure 5)**.

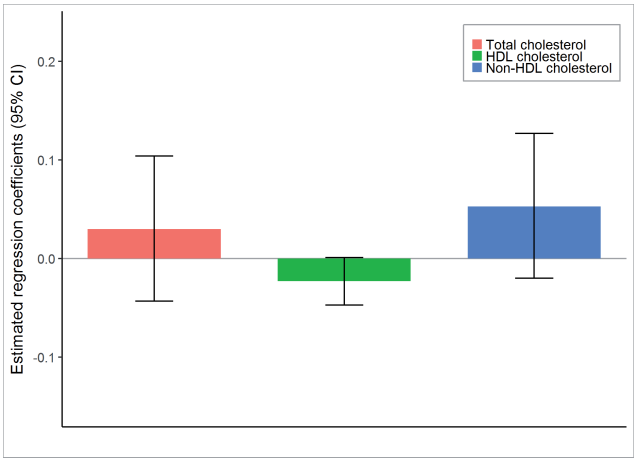

Figure 4.Estimated regression coefficients* with 95% CI for the association of within-subject substitution of SFA for carbohydrates over time (per 1 en%) and differences in cholesterol concentrations after 4 years of follow-up.

*Coefficients are adjusted for age at baseline, lipid lowering medication use during follow-up, intake differences in total energy, alcohol, fibre, and dietary cholesterol, differences in BMI and physical activity score, baseline intakes of SFA and carbohydrates (en%), baseline smoking status, education level, and follow-up time.

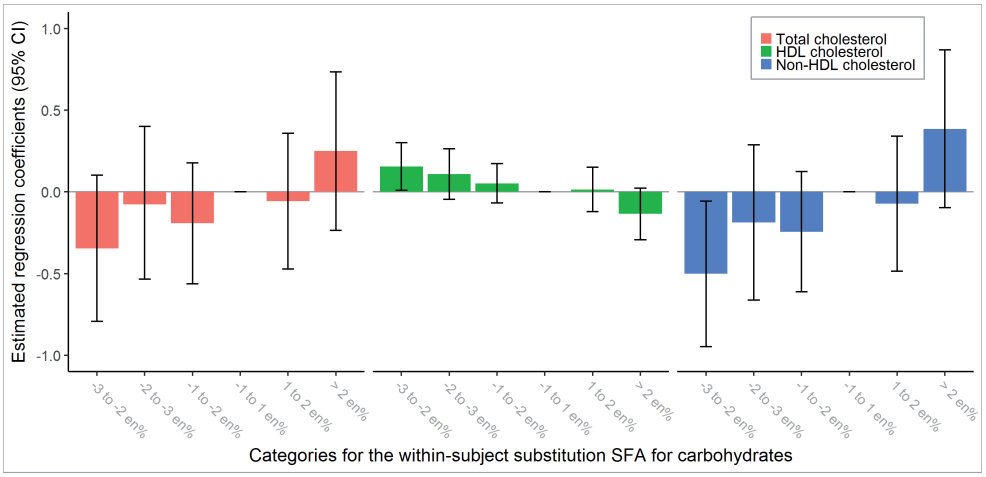

Figure 5. Estimated regression coefficients* with 95% CI for the association of within-subject substitution of SFA for carbohydrates over time (in categories) and differences in cholesterol concentrations after 4 years of follow-up.

6

Between-subject substitution of SFA and carbohydrates in relation to serum cholesterol

Figure 6 shows the multivariable adjusted beta-coefficients for the association of between-subject substitution of SFA and carbohydrates in baseline data with cholesterol concentration differences after follow-up. No significant associations were observed with total cholesterol (per 1 en%: 0.016 mmol/L, 95% CI: -0.059, 0.092) and with non-HDL cholesterol (per 1 en%: -0.016 mmol/L, 95% CI: -0.093, 0.061). Each increment of 1 en% SFA at the expense of 1 en% carbohydrates was significantly associated with a 0.033 mmol/L (95%
CI: 0.008, 0.057) difference in HDL cholesterol. Analysis across quintiles also showed an increase in HDL cholesterol differences in all quintiles compared to the first **(Figure 7)**.

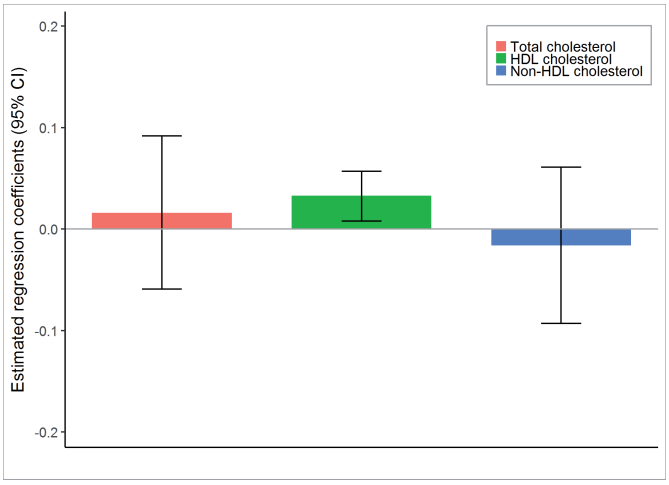

Figure 6. Estimated regression coefficients* for the association of between-subject substitution of SFA for carbohydrates in baseline data (per 1 en%) and differences in cholesterol concentrations after 4 years of follow-up.

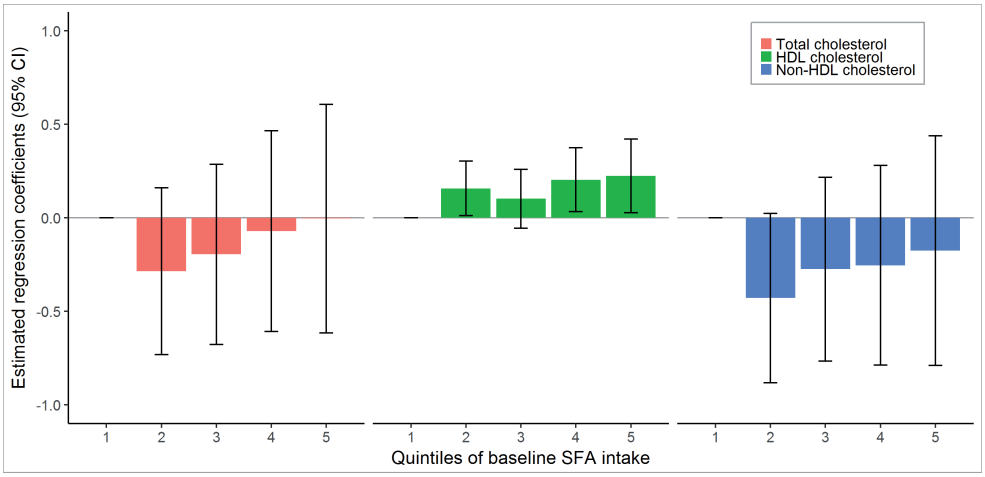

Figure 7. Estimated regression coefficients* with 95% CI for the association of between-subject substitution of SFA for carbohydrates in baseline data (in quintiles) and differences in cholesterol concentrations after 4 years of follow-up.

*Coefficients are adjusted for age at baseline, baseline measures of total energy, PUFA, MUFA, trans-fat, vegetable protein, animal protein, fibre, alcohol, and dietary cholesterol, BMI, physical activity, smoking status, education level, lipid lowering drug use during follow-up, and follow-up time.

Sensitivity analyses

The associations of within-subject substitution and between-subject substitution of SFA for carbohydrates and cholesterol concentrations in all 277 participants were slightly attenuated but similar to those in the selected 223 and still with opposite results (**Supplemental Figure S2**). The estimated difference in HDL cholesterol was -0.012 mmol/L (95% CI: -0.033, 0.010) for each additional within-subject substitution of 1 en% SFA for carbohydrates and 0.022 mmol/L (95%CI: -0.001, 0.044) for each additional between-subject substitution of 1 en% SFA for carbohydrates. The associations for within-subject versus between-subject substitution of SFA for carbohydrates remained opposite to each other after exclusion of participants who reported the use of cholesterol lowering medication (**Supplemental Figure S3**) and after excluding the participants with missing physical activity scores (**Supplemental Figure S4**).

Discussion

In this subgroup of 277 Dutch postmenopausal women of the Prospect-EPIC cohort, we observed modest changes in the reported SFA intake during the first 4 years of follow-up, which led to changes in subject ranking over time. A change in SFA intake was generally compensated by an opposite change in intake of carbohydrates. Within-subject substitution of SFA for carbohydrates over time was associated with a decrease in HDL cholesterol concentrations, as compared with within-subject substitution of carbohydrates for SFA. In con-

trast, between-subject substitution of SFA for carbohydrates in baseline data was associated with an increase in HDL cholesterol concentrations, when compared with between-subject substitution of carbohydrates for SFA.

The availability of repeated measurements in this study can be considered a strength. Furthermore, because the same FFQ was used for both dietary assessments the reported dietary changes were not the result of a change in the FFQ. Limitations of this study include the differences in the blood sampling procedure and cholesterol concentration measurements at baseline versus 4 years later and the fact that the study population was not a random sample of the total PROSPECT cohort. Because of the differences in the blood sampling procedures and cholesterol concentration measurements, the associations we present in this study should be interpreted only as relative cholesterol changes and are not suitable to draw any conclusions about absolute changes in cholesterol concentrations. As a consequence, comparisons with trials or other studies that present absolute changes cannot be made. In addition, the dietary changes and associations we observed may not be representative for the total Prospect cohort. However, for our methodological study objective to compare the associations of between-subject versus within-subject macronutrient substitution with differences in cholesterol concentrations, none of these limitations affect our conclusions.

In 34% of the 277 women in our study, the reported SFA intake could be considered stable, because the intake change was 1 en% or less. On the other hand, 20% of the women changed their intake with 3 en% or more, and 7% even with 5 en% or more. These findings are in line with the changes in SFA intake that were observed on population level in a US cohort study [10], where the SFA intake change during an average follow-up time of 2 to 4 years ranged from approximately -6 en% through +4 en%. In our study, a change in SFA was mainly compensated for by carbohydrates and not by PUFA. To our knowledge, no previous studies have reported on the within-subject substitution in an observational setting, but on population level this phenomenon was observed before in other cohorts [10, 21]. Part of the intake change over time in the present study will be due to (random) measurement error. The size of this measurement error is unknown. However, the variation of the SFA intake difference in the present study (-0.4 ± 2.5 % of total energy including energy from alcohol) is larger than the variation observed in a previous study on the reproducibility of our FFQ over a six month interval (-0.14 ± 1.6 en%) [17]. Therefore, it is very unlikely that the total difference is solely the result of measurement error, all the more because we observed a significant association with HDL cholesterol concentrations, which makes it safe to assume that the result is to a large extent caused by an actual change.

Our study shows that if substitution actually occurs within subjects during follow-up, the interpretation of the associations between macronutrients and (clinical) outcomes after follow-up found in between-subject substitution modelling may be unreliable. The results of between-subject substitution modelling are based on between-subject comparisons. Essentially, subjects with a high intake of SFA and a low intake of carbohydrates are

compared with subjects with a low SFA intake and a high carbohydrates intake. By comparing their risks on for instance CHD, or an intermediate risk marker such as the change in cholesterol concentrations, inferences are made about what will happen if individuals remain on the existing intake concentrations of SFA and carbohydrates during follow-up. Inferences are based on the assumption that subjects actually do not change their intake (i.e., that the relative contribution of different macronutrients to the total energy intake of a person is constant during follow-up). If that assumption holds, one can derive from such a between-subject comparison an expected within-subject substitution effect. However, if subjects do not remain on the same intake level, i.e., there is within-subject substitution of macronutrients over time, that assumption does not hold and between-subjects comparisons lack the interpretation that we often attribute to them.

Since the majority of cohort studies only use baseline data for substitution analyses [6], this may explain why in earlier observational studies substitution of SFA for other macronutrients was unrelated to, or inversely related to, CHD risk after follow-up. Exceptions are the Nurses' Health Study and the Health Professional Follow-up Study, in which repeated measurements were used for substitution analyses [10-12]. Nevertheless, these substitution analyses were conducted in a similar way as in the observational studies that used only baseline data, that is by between-subject comparisons. The only difference is that these analyses were repeated for each dietary measurement or were done using an average intake measure. The use of repeated measurements will provide a more precise measure of intake compared to just one (baseline) measurement [22], which may explain why an expected lowered CHD risk for modelled substitution of PUFA for SFA was found in those US cohorts. Nevertheless, again it is not clear how this between-subject substitution modelling of repeated measurement relates to observed substitution within subjects over time and whether between-subject comparisons can be directly translated to within-subject recommendations. The observed associations of the between-subject substitution of SFA for carbohydrates with differences in HDL cholesterol concentrations slightly attenuated in our sensitivity analyses in all 277 participants. Nevertheless, the association was still opposite to the association that we observed for the within-subject substitution. This shows that inference from between-subject substitution may be unreliable, even if not all participants actually substitute SFA and carbohydrates over time.

Our study shows that intakes of certain dietary determinants, such as SFA and carbohydrates, may change during follow-up, which leads to changes in subject ranking over time. Changes in diet over time have been shown in previous studies [23, 24]. It is, however, largely unknown to what extent such changes are present in other cohort studies that investigate diet disease relationships. Therefore, our findings should be replicated in other cohorts with two or more repeated dietary measurements, to further investigate to what extent ranking of individuals according to baseline intake, changes during follow-up, and also whether and how this affects relationships with disease endpoints. It would be of interest to explore this not only in dietary studies with unexpected results, but also in studies where results are in

line with what one would expect based on evidence form earlier trials. In case changes in ranking over time are observed, it would be valuable to further study how many repeated measurements of diet, and at which time interval, would be needed to account for the within-subject changes. Furthermore, this phenomenon may also apply to other time-varying determinants that are studied in longitudinal settings, and this deserves further studies as well.

To conclude, in this study in 277 Dutch postmenopausal women, we observed changes in SFA intake during four 4 of follow-up. A lowering in SFA generally went together with an increased intake of carbohydrates, and vice versa. The associations with change in serum HDL cholesterol were opposite to each other when comparing within-subject substitution with between-subject substitution of SFA and carbohydrates.
Our study shows that, ironically, inference from substitution modelling in only baseline data, may be unreliable when substitution over time actually occurs. This is not a problem of the substitution model but the result of subject misclassification due to use of single time-points measurements of time-varying variables. Whether, and if so, how many repeated measurements of diet are necessary to account for the within-subject changes in observational studies requires further investigation.

Disclosures
JP is financially supported by a restricted grant from Unilever R&D, Vlaardingen, the Netherlands. Unilever R&D had no role in design and conduct of this study; collection, management, analysis and interpretation of the data; and preparation, review or approval of the manuscript. The other authors declare no conflict of interest.

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Supplemental Material

Supplemental table S1. Population characteristics across quintiles of the baseline saturated fat (SFA) intake distribution (en%)

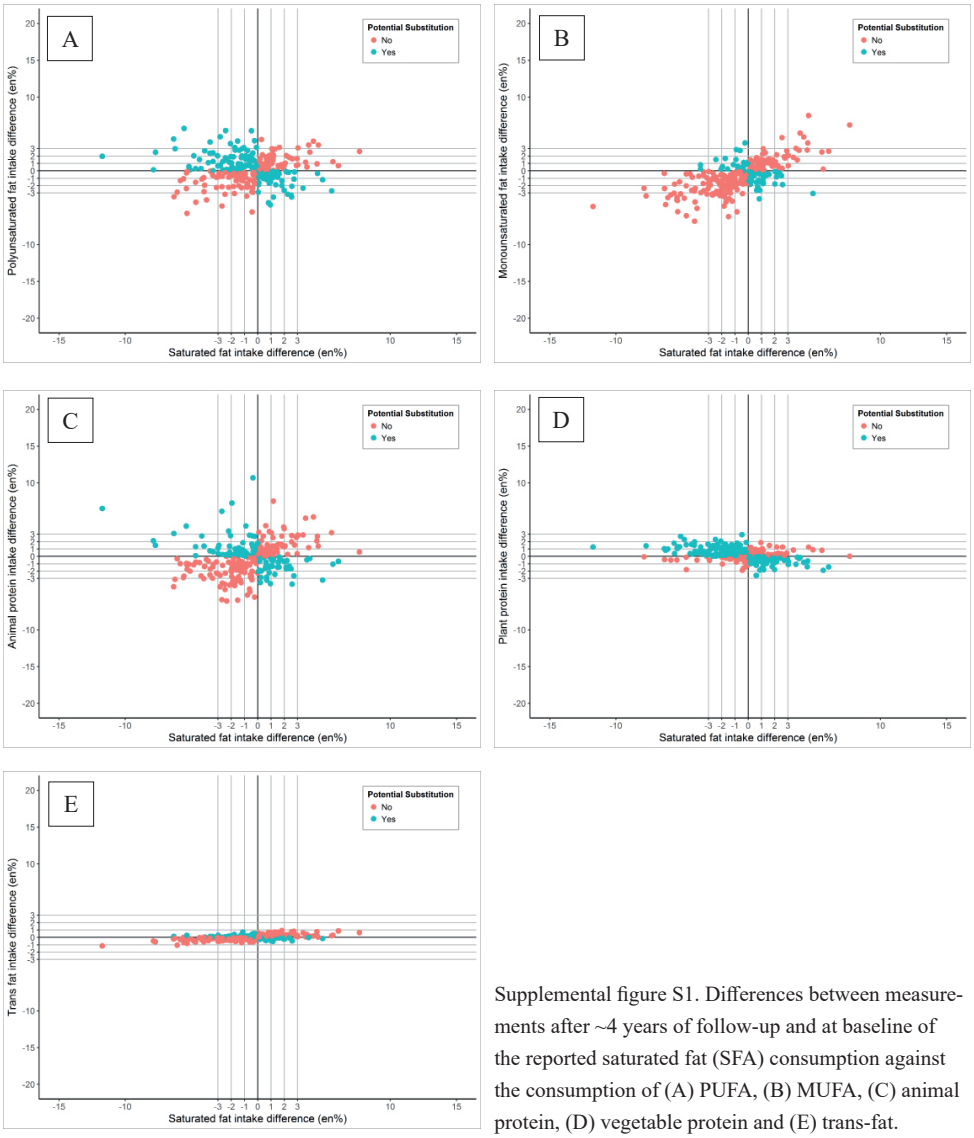
	Q1	Q2	Q3	Q4	Q5
SFA intake, en% (*min, max*)	(8.2, 13.3)	(13.4, 14.9)	(15.0, 16.2)	(16.3, 18.3)	(18.3, 24.0)
Subjects, *n*	44	45	45	45	44
Baseline values					
Age, *y*	63.2 (± 3.7)	61.9 (± 3.0)	62.8 (± 4.5)	64.3 (± 3.7)	62.4 (± 4)
BMI, *kg/m²*	25.3 (± 3.8)	25.4 (± 3.7)	27.2 (± 5.2)	26.4 (± 4.0)	26.0 (± 4.4)
High education level, *%*	23.1	17.6	11.0	13.2	13.2
Current smoker, *%*	6.6	6.6	8.8	13.2	27.5
Physically active, *%*	49.5	30.8	26.4	26.4	49.5
Saturated fat, *en%*	11.8 (± 1.4)	14.2 (± 0.5)	15.5 (± 0.3)	17.2 (± 0.6)	19.9 (± 1.4)
Carbohydrates, *en%*	53.7 (± 5.3)	48.2 (± 3.9)	46.5 (± 3.7)	43.5 (± 3.6)	40.1 (± 4.4)
Monounsaturated fat, *en%*	10.6 (± 1.8)	12.7 (± 1.8)	13.3 (± 1.8)	14.3 (± 2.2)	15.7 (± 2.0)
Polyunsaturated fat, *en%*	6.5 (± 1.9)	7.4 (± 1.7)	6.8 (± 1.9)	7.3 (± 2.3)	6.7 (± 1.9)
Trans-fat, *en%*	0.9 (± 0.2)	1.1 (± 0.4)	1.2 (± 0.2)	1.3 (± 0.3)	1.5 (± 0.3)
Vegetable protein, en*%*	6.4 (± 0.9)	5.8 (± 0.8)	5.6 (± 0.7)	5.2 (± 0.7)	5.2 (± 0.8)
Animal protein, *en%*	10.2 (± 2.8)	10.4 (± 2.7)	11.0 (± 2.3)	11.1 (± 2.1)	10.9 (± 1.9)
Vitamin C, *mg*	149 (± 69)	127 (± 40)	119 (± 37)	110 (± 39)	89 (± 30)
Cholesterol, *mg*	144 (± 48)	181 (± 53)	216 (± 69)	209 (± 44)	249 (± 59)
Fibre, *g*	25.5 (± 5.2)	23.7 (± 5.2)	23.6 (± 5.8)	21.3 (± 5.6)	21.3 (± 5.3)
Energy, *kcal*	1615 (± 361)	1762 (± 398)	1803 (± 345)	1737 (± 344)	1867 (± 362)
Alcohol, *g*	3.4 (0.3-15.3)	3.5 (0.7-11.1)	2.7 (0.0-3.4)	3.9 (0.1-9.3)	3.2 (0.2-18.7)
Differences after follow up					
Follow up time, *y*	3.7 (± 0.7)	3.8 (± 0.7)	3.9 (± 0.7)	3.7 (± 0.8)	3.7 (± 0.8)

Δ BMI, *kg/m²*	0.5 (± 1.8)	0.1 (± 1.3)	0.4 (± 1.2)	0.1 (± 1.3)	-0.1 (± 1.5)
Lipid lowering medication use, *%*	20.9	13.2	17.6	8.8	6.6
Δ Saturated fat, *en%*	1.0 (± 2.4)	0.0 (± 2.2)	-0.5 (± 2.1)	-1.5 (± 2.3)	-2.9 (± 2.9)
Δ Carbohydrates, *en%*	-2.6 (± 6.9)	0.3 (± 4.9)	1.0 (± 4.3)	4.0 (± 5.6)	4.5 (± 4.0)
Δ Vegetable protein, *en%*	0 (± 0.8)	0.1 (± 0.9)	0.2 (± 0.9)	0.2 (± 0.9)	0.5 (± 0.8)
Δ Animal protein, *en%*	0.2 (± 2.4)	-0.3 (± 2.0)	-0.2 (± 1.9)	-0.4 (± 2.4)	-0.5 (± 2.2)
Δ Monounsaturated fat, *en%*	0.6 (± 2.5)	-0.2 (± 1.8)	-0.6 (± 1.7)	-1.6 (± 2.2)	-1.8 (± 1.6)
Δ Polyunsaturated fat, *en%*	0.7 (± 2.0)	0.1 (± 1.4)	0.1 (± 1.6)	-0.6 (± 2.2)	0.4 (± 1.9)
Δ *Trans*-fat, *en%*	0.0 (± 0.3)	0.0 (± 0.3)	0.0 (± 0.3)	-0.1 (± 0.3)	-0.1 (± 0.4)
Δ Alcohol, *g*	-0.7 (± 7.2)	-1.4 (± 6.2)	3.0 (± 15.3)	1.5 (± 4.8)	0.2 (± 8.5)
Δ Fibre, *g*	-1.1 (± 5.6)	-0.5 (± 4.9)	-0.5 (± 3.5)	0.8 (± 4.0)	-0.4 (± 4.2)
Δ Substitution score (*min, max*)	(-4.7, 7.7)	(-5.4, 4.5)	(-4.3, 4.4)	(-6.1, 3.6)	(-8.0, 1.5)

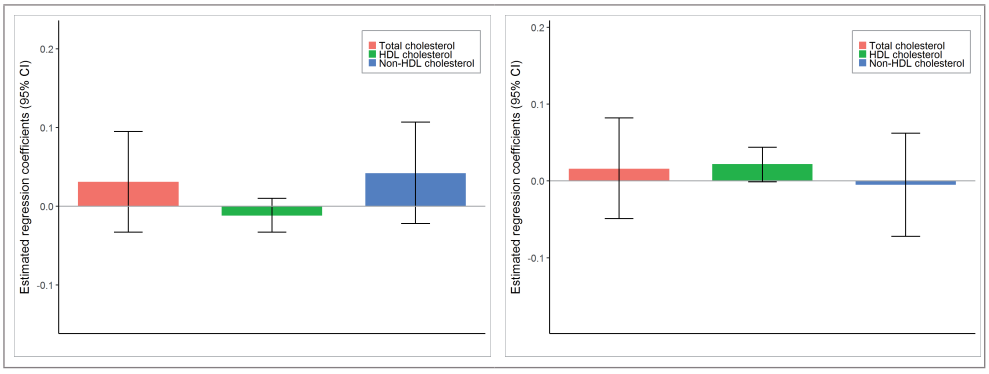
En% represents the percentage of the sum of energy from all macronutrients, except for energy from alcohol.

Supplemental table S2. Cross table of quintiles of saturated fat (SFA) intake measured after ~4 years of follow- up against quintiles of baseline saturated fat intake

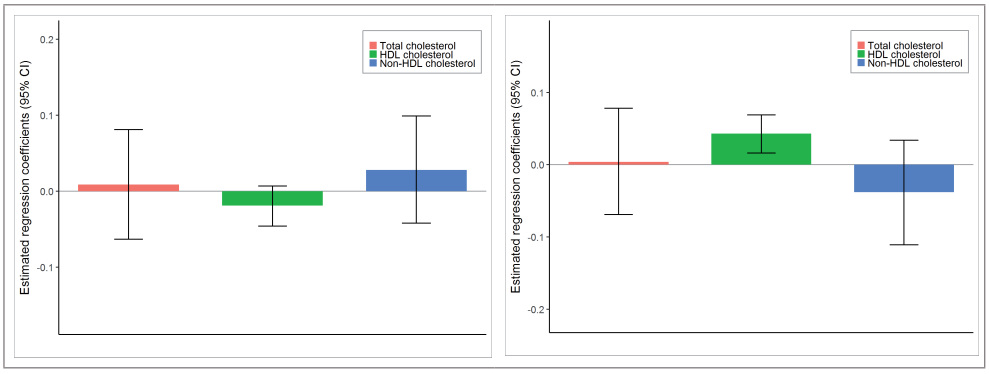
	Quintiles of SFA intake after follow-up					κ_w (95%CI)
Quintiles of SFA intake at baseline	Q1	Q2	Q3	Q4	Q5	
In 223 participants						
Q1	**21**	10	8	4	2	
Q2	10	**12**	9	10	3	0.46 (0.34-0.57)
Q3	5	12	**10**	12	6	
Q4	5	4	15	**11**	9	
Q5	4	6	3	7	**25**	
In 277 participants						
Q1	**29**	13	10	2	2	
Q2	10	**18**	13	11	3	0.54 (0.44-0.63)
Q3	8	12	**14**	16	5	
Q4	5	6	14	**17**	13	
Q5	4	6	4	9	**33**	



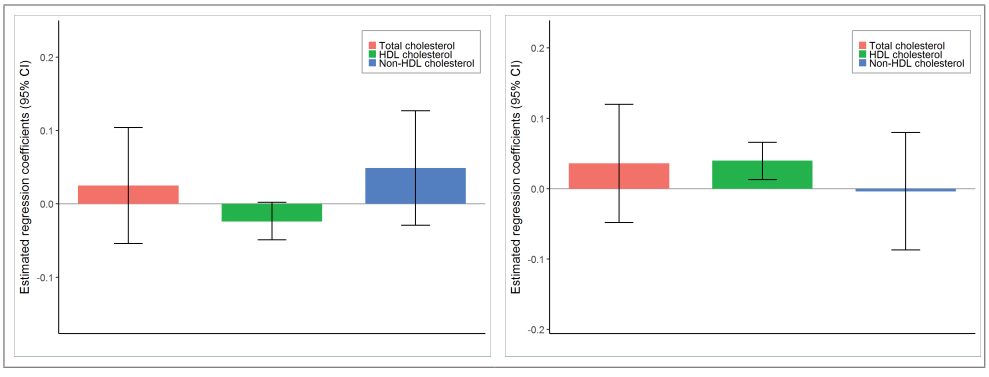
Supplemental figure S1. Differences between measurements after ~4 years of follow-up and at baseline of the reported saturated fat (SFA) consumption against the consumption of (A) PUFA, (B) MUFA, (C) animal protein, (D) vegetable protein and (E) trans-fat.



Supplemental figure S2. Estimated regression coefficients for the association of (left figure) within-subject substitution and (right figure) between-subject substitution of SFA for carbohydrates over time (per 1 en%) and differences in cholesterol concentrations after 4 years of follow-up in 277 women.



Supplemental figure S3. Estimated regression coefficients for the association of (left figure) within-subject substitution and (right figure) between-subject substitution of SFA for carbohydrates per 1 en% and differences in cholesterol concentrations after 4 years of follow-up in 193 women who did not report the use of lipid-lowering medication during follow-up.



Supplemental figure S4. Estimated regression coefficients for the association of (A) within-subject substitution and (B) between-subject substitution of SFA for carbohydrates per 1 en% and differences in cholesterol concentrations after 4 years of follow-up in 193 women who did not report the use of lipid-lowering medication during follow-up.

6

Chapter 7.

General discussion

General discussion

This thesis mainly focuses on the further study of the association between dietary fat and cardiovascular disease (CVD) in observational cohort studies. The main motivation for this topic is the discrepancy between the serum cholesterol raising effects of dietary saturated fat (SFA) in controlled trials on the one hand, and the absence of a consistent association between SFA and coronary heart disease (CHD) in observational cohort studies on the other.

In line with previous observational studies [1-3], we did not observe adverse associations between dietary SFA and CHD in two Dutch cohorts. However, contrary to our expectations, substitution of SFA for PUFA, MUFA, or carbohydrates did not yield evident differences either in the association between SFA and CHD. Distinction of SFAs based on their carbon chain-length resulted in a crude distinction of the short- to medium-chained SFAs and the long-chain SFAs with respect to their associations with the risk of CHD or myocardial infarction (MI). In three EPIC cohorts we observed a lower risk of CHD or MI for higher intakes of the short- to medium chain SFAs, whereas in the Rotterdam Study a higher intake of the long-chain SFA palmitic acid was related to a higher CHD risk.

The results we observed after distinction of SFA based on their food source in the two Dutch cohorts corresponded to the results for the carbon-chain lengths. To illustrate, in EPIC-NL we observed a lower CHD risk for a higher intake of SFA from dairy, which is rich in short- to medium chain SFAs, whereas in the Rotterdam Study we observed a higher CHD risk for higher intake of SFA from meat, which is rich in palmitic acid. So, although some associations were found, none of the three abovementioned factors appear to provide sufficient explanation for the discrepancy between controlled trials and observational studies.

The striking finding that the substituting macronutrient did not matter triggered questions about substitution modelling. Our study in data from a subset of the Prospect-EPIC cohort with repeated dietary intake measurements revealed that SFA intake changed during the observed period of four years, and that these changes were mainly compensated by changes in carbohydrates. In further analyses, when we compared model based between-subject substitution in baseline data with observed within-subject substitution of SFA and carbohydrates, we observed opposite associations with changes in serum HDL-cholesterol levels. In EPIC-NL, baseline intake of at least 1 portion of fish per week as compared with no fish consumption was related to a lower risk of ischaemic stroke, and the dietary lipophilic index was unrelated to the risk of CVD.

In this chapter we discuss the limitations of observational studies in answering remaining questions about the association between saturated fat and CHD risk. In addition, we provide practical implications of the results in this thesis, and suggestions for future research.

Limitations of observational cohort studies for the study of dietary SFA and CHD

In nutritional research both randomized controlled trials (RCTs) and observational studies are widely used [4]. RCTs are essential to prove causal relations and have a number of clear advantages such as leaving the researcher the choice of the levels of the factors under study and the possibility to control for other factors. On the other hand, it is clear that deliberate exposure of subjects to food patterns that are expected to be harmful is unethical, and that dietary factors cannot easily be controlled in large populations and/or over long time intervals. Therefore, for the study of clinically manifest disease such as CHD we have to rely on observational studies too. For several nutrients, foods and dietary patterns, such as *trans*-fat [1, 5], fibre [6-8], fruits and vegetables [9-12], nuts [13, 14] and the DASH diet [15, 16], the observed associations with chronic disease risk in both RCTs and observational studies are consistent and support each other. However, for SFA and CHD risk the evidence from RCTs is not supported by findings in observational studies.

In our studies on the relation between SFA and CHD, where the main objective was to clarify the inconsistent associations in observational studies, we encountered a number of limitations of observational cohort studies which might in fact be one of the causes for these inconsistent findings. Since these limitations may apply to other observational studies as well, we discuss three of them in more detail.

1. *The use of only baseline measurements of diet.*

In the cohort studies included in this thesis dietary intake was assessed using a food frequency questionnaire (FFQ). Besides the standard limitations of the use of FFQ [4], the dietary assessments had the limitation that they were single measures at baseline, whereas the follow-up time in all the studies is over 10 years. The assumption in these studies was that the dietary intakes as measured at baseline were representative for the intakes during follow-up.

As we show in chapter 6, and as was shown in the Nurses' Health Study (NHS) and Health Professionals Follow up Study (HPFS) [17], diet is not stable over time. Changes in the diet do not automatically lead to concerns in observational studies, because in these studies the disease risk in subjects is compared after ranking them according to their dietary intake. In other words, the conclusion that a higher intake of a certain nutrient or food is related to a higher disease risk, is based on a comparison of subjects who have a relatively high intake with subjects who have a relatively low intake. The observed association is valid, as long as the ranking of the subjects is unaffected (and considering that no other bias is present). Whether this is the case, is usually unknown. The general assumption is that potential changes did not affect subject ranking, and that in the case of any change this will be random and thereby leads to non-differential misclassification [18]. The assumed consequence of non-differential misclassification is in general attenuation of the risk estimates, but could

just as well be biased estimates [18-20] .
We illustrated a problem of misclassification in a subset of the EPIC-Prospect cohort, and its consequences for subject ranking in chapter 6. We used two approaches to calculate the association between the substitution of SFA for carbohydrates and serum cholesterol changes over time. On the one hand, we used a between-subject comparison in which we compared subjects with a relatively high baseline intake of saturated fat and a low intake of carbohydrates with subjects with a low baseline intake of saturated fat and high intake of carbohydrates. On the other hand, we used actual substitution of SFA for carbohydrates within-subjects over time. We observed that these two substitution approaches yielded opposite associations with serum cholesterol changes. Because of the within-subject substitution in the diet over time, the subject ranking according to the baseline dietary assessment was not representative for the subject ranking after follow-up. This phenomenon could be a potential explanation for the inconsistent findings in several other cohort studies on the association between SFA and CHD, because they also relied on baseline dietary measures only [1-3]. At the same time, it is uncertain whether dietary changes over time may also have occurred in studies that did observe an association between SFA and CHD in line with what could be expected [21], and to what extent this may have affected the observed associations. An example of a nutrient of which we know that the intake definitely changed over time, is *trans*-fat. Since 1990, the *trans*-fat content of foods was drastically lowered by the food industry [22, 23]. In all EPIC-cohorts and the Rotterdam Study, diet was assessed in the period during which this reduction was carried through. The fact that the measured *trans*-fat intake at baseline is not representative for the intake during follow-up is therefore a given. As a result, residual confounding by *trans*-fat could be present in the observed associations. Other CVD risk factors including physical activity, smoking, drinking behaviour and medication use are generally also correlated with specific dietary behaviours and specifically with SFA intake. To avoid confounding by those factors, we corrected for them in the analyses. Nevertheless, again these factors were measured at baseline, and potential changes during follow-up were not taken into account and could have confounded or biased the observed associations. The use of lipid lowering medication, for instance, became more common during the 1990s and thereafter. The effects of their use in the cohort studies in this thesis were not accounted for, because these data was lacking.

2. *The study of individual nutrients*

The inconsistent results of the different cohort studies on SFA and CHD so far indicate that individual nutrients, like saturated fatty acids, have small effects on CHD risk, especially in comparison to other factors such as smoking or medication use. Moreover, many nutrients in observational studies are correlated, sometimes making it impossible to analyse them separately. The latter particularly pertains to the individual SFAs, which have many shared food sources, as illustrated in the studies included in this thesis as well as in the US cohorts [24] .We made an attempt to disentangle the SFAs by correcting them for each other, which led to varying changes in their associations with CHD. Nevertheless, as we mentioned in

chapter 3.3, this could also lead to over adjustment. Another issue in observational studies is that it is difficult to distinguish between the intake of nutrients and their food sources. For instance, as we saw in the EPIC-NL study, an inverse association with CHD was observed for dairy products, as well as for the SFAs that mainly come from dairy products.

3. *Limited dietary variation within the cohorts.*

To detect an association between diet and a clinical endpoint, such as CVD, the intake variation of the nutrient or food should be sufficiently large. After all, subjects with a relatively high intake have to be compared with those with a lower intake, to be able to draw a conclusion about the risk that comes with a higher intake. Also, the absolute intakes in the population should range from low to high as well. Even though the intake range of SFA in most cohorts is rather large, it does not cover much of the low intakes that are recommended by the guidelines. The maximum SFA intake in the dietary guidelines is set at 10 en%, with the aim to reduce the risk of CVD. Nevertheless, most subjects in the cohort studies in this thesis had a consumption over 10 en%. The comparisons between a high and low intake of SFA is thus actually a comparison between a very high and a less high intake. This does not necessarily have to cause a problem, under the condition that the association is linear and the effect size is large enough to detect in the smaller range. In other cases, for instance when a high consumption is only associated with CHD when compared with a much lower intake (below 10 en%), it will be difficult, if not impossible, to detect an association in a population that only covers the higher intake range of SFA.
A lack of intake variation in the population may potentially also present a problem in substitution analyses. Statistically, the substitution of macronutrients can be modelled. However, this can easily lead to wrong conclusions if the variation in one of the macronutrients is too small to get an accurate estimate of its association with disease risk, while the substitution actually implies extrapolation outside the observed range. The intake range of PUFA in the EPIC-NL cohort for instance was small and constant over the intake range of SFA. This may have limited the ability to detect an association for PUFA, and thereby may explain why modelling its substitution with SFA did not affect the association between SFA and CHD.

Practical implications

A recurring question throughout the decennia-long debate over SFA and CHD, is whether the recommendation to limit SFA consumption should be removed from the nutritional guidelines [25], seeing that so many cohort studies did not confirm its association with CHD outcomes. This conclusion is tempting, but also short-sighted considering that the above-mentioned limitations, including the use of only baseline measurements without information about dietary change during follow-up as well as the high correlations between saturated fat and other nutrients, pertain to the majority of the observational studies in which SFA was unrelated to CHD. Therefore, we cannot reject the hypothesis that the null-results may

be due to misclassification or over-adjustment. On top of that, we know from controlled trials that SFA has serum LDL-cholesterol raising effects [26] ,the recommendation to limit SFA consumption is still justified.

A related question is whether distinction should be made based on the carbon-chain length of SFA in the dietary guidelines, because the even-chained SFAs with chain lengths of 12 through 18 carbons have varying effects on serum cholesterol levels in controlled trials [28]. However, knowing that individual SFAs have different effects on cholesterol levels does not necessarily mean that they affect hard CHD outcomes differently as well [29] . It appears so, based on the cohort studies in this thesis and two studies from the US [24, 30], but considering the high correlations between individual SFAs this could be the result of overcorrection or confounding. Also, the fact that palmitic acid represents ~50% of the total SFA intake whereas lauric acid represents less than 5% of the intake, one may question whether the differences in their effects may also be a matter of quantity. Furthermore, it would be very impractical to implement such a guideline, because of the shared food sources of the individual SFAs. Therefore, we see no reason for such a distinction in the dietary guidelines. For the food industry on the other hand, distinction between individual SFAs can be of great interest. Recommendations for food industry on which type of SFA they should use or avoid in their products, should for now be based on the results from RCTs [26], rather than from observational studies, for the same reasons as described above.

Regardless of the remaining questions about SFA, cholesterol levels and CHD, for the dietary guidelines it may be a better option to focus on the intake of specific foods instead of nutrients. Such a food based approach has already been implemented in several nutritional guidelines nowadays [31-33] , and makes more sense considering that people do not eat isolated nutrients, but combined in whole foods. The interaction of all nutrients in whole foods may be more important than the effects of each individual nutrient when isolated in an experimental setting.

Suggestions for future research

In light of the limitations that we encountered in this thesis, it is safe to conclude that additional observational studies using only a baseline dietary measurement will not sufficiently help us in figuring out whether or not dietary fatty acids are related to CVD outcomes, and will definitely not end the debate on SFA and CHD. Of course this does not mean that observational studies are useless and should all be discarded, but to answer the question whether dietary saturated fat is related to CHD risk, improvements are needed to cope with the limitations.

One rather obvious improvement would be the use of repeated measurements, that will most likely yield a more reliable measure of diet, compared with one baseline measurement only. In, for example, the Nurses' Health Study (NHS) and Health Professionals' Follow Up

study (HPFS) repeated dietary measurements at 2 to 4 year intervals are used for analysis. Relevant questions are how to choose the best time interval, and how many measurements are needed to capture the dietary behaviour of cohort members during follow-up. To answer these questions, we suggest to examine dietary changes during follow-up in cohort studies with repeated measurements, similarly to what we have done in Prospect-EPIC (chapter 6). In case of within-subject changes, we suggest to examine how these changes relate to the risk of clinical endpoints, and whether this relationship is captured with between-subject comparisons using repeated measurements.
Next, observational studies with repeated measurements in different populations are needed. Currently the only observational studies that reported on the association between repeated measurements of SFA and CHD risk during follow-up are the NHS and HPFS, which are both American and very specific, rather healthy, populations. Therefore it is uncertain how representative their findings are for other populations and countries, where dietary intake and lifestyles are different.
On top of that, future studies should include populations with different SFA intake ranges than previous studies, to ensure a broadening of the intake range. Up to now, in most cohort studies that examined the relation between dietary SFA and CHD, the SFA intake is relatively high in all participants. The associations in these studies are based on a comparison of participants with a high intake as compared with participants with an even higher intake. Therefore, future studies should include cohorts in which the intake is below 10en% in a considerable part of the population. Parts of the world where the consumption of SFA were observed to be lower are South Asia, East Asia, and Latin America [34, 35]. This would make it more likely to detect a potential association.

With respect to individual SFAs, observational cohort studies appear to be unsuitable to separate their effects. Repeated measures and different populations will not solve the issue of their high correlations. Therefore, to further study their individual associations with CHD risk, we should rely on RCTs. For some SFAs, including the short-to medium chain SFAs butyric through capric acid, and the odd-chain SFAs pentadecylic acid and margaric acid, evidence from RCTs is lacking, and is therefore interesting for future research. Besides RCTs, Mendelian Randomisation could be valuable in the study of individual SFAs (and maybe total SFA) and their association with CHD risk. In such analysis the variation in a particular gene is used as a proxy for a disease risk factor [36].This technique limits the potential of confounding and reverse causation, that are limitations in classical observational cohort studies. To our knowledge up to now no proxies are identified yet for individual SFAs, except for the very long even-chain SFAs with 20 through 24 carbons [37], which are barely present in the diet. Therefore, it would be interesting to investigate with future GWA-studies whether there are common genetic variants that associate with circulating SFAs with chain up to 18 carbons. However, the majority of circulating SFAs are not representative for dietary SFAs, but also represent endogenously derived SFAs [38], and are therefore not suitable to make inferences on dietary intake of SFAs. Nevertheless, Mendelian Randomisation could help in the future investigation of the causal association between

individual SFAs and CHD risk.

The use of the dietary lipophilic index, which is suggested as a measure for the total fat quality of the diet [39], may overcome the issues of high correlations between the individual fatty acids. Nevertheless, the index is based on the assumption that the fluidity of cell membranes is related to CHD risk [40]. Because of endogenous production of fatty acids, the lipophilic index (i.e. overall fat fluidity) of the diet only partly contributes to cell membrane fluidity. Therefore, the interpretation of the dietary lipophilic index is difficult, and it does not appear to add new information on the relationship between dietary fat intake and CHD risk.

For the support of dietary guidelines, and to answer the question what one should eat to lower the risk of CHD, it may be more valuable for future studies to shift from dietary SFA to dietary patterns in observational studies. The correlation between nutrients and single foods is less of an issue in the investigation of dietary patterns [41]. Also, these types of studies are more comprehensible for laymen, and directly translatable into dietary guidelines. Of course, in the study of dietary patterns, potential changes over time should also be considered [42].

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7

Summary

Summary

Dietary fat is a valuable component of the diet and essential for the human body. Over time it has become clear that certain types of fat(ty acids) are also potentially harmful for our health. For example, we know from intervention studies that saturated fat and trans-fat have unfavourable effects on serum cholesterol concentrations, which in turn is related to a higher risk of coronary heart disease (CHD). In general, there is scientific consensus on the harmful effects of trans-fat, but the link between saturated fat and CHD has been debated for decennia now. This debate is mainly caused by the discrepancy between the results of randomized controlled trials and the results of observational studies. Whereas the first show that a higher intake of saturated fat raises LDL cholesterol concentrations, most observational studies found no relation between dietary saturated fat and CHD risk.

In this thesis, I present the results of a number of observational studies on the association between dietary fat and cardiovascular disease risk. The main focus of the thesis is on the association between saturated fat and CHD. We started by investigating whether this association depends on a number of predefined factors that were ignored in most previous observational studies, and that could potentially explain the disagreement with the results of intervention studies. Before we discuss the results of those studies in chapter 3, we discuss in chapter 2 the validity and reproducibility of a crucial assessment in nutrition research; the dietary assessment.

In the EPIC-NL cohort, data on dietary intake were assessed with a food frequency questionnaire at baseline. For the interpretation of the results of a study on the consumption of individual fatty acids and a clinical manifest outcome, it is important to know the questionnaire's ability to rank subjects according to their consumption of these individual fatty acids. This is the topic of Chapter 2. To assess the relative validity, the fatty acid intakes measured with the food frequency questionnaire were compared against the measurements of 12 non-consecutive 24 hour recalls. For most fatty acids, the validity was moderate to good, except for the less-abundant fatty acids, including the short-chain saturated fatty acids and the n-3 polyunsaturated fatty acids, for which it was fair. The reproducibility was evaluated by comparing the fatty acid intake of three repeated measurements with the food frequency questionnaire at 6-month intervals. The reproducibility was good for all individual fatty acids.

As mentioned before, most observational studies do not confirm the assumed association between a higher intake of saturated fat and an increased risk of CHD, despite the LDL cholesterol raising effects of saturated fat in intervention studies. We investigated in observational data whether the following three factors affect the association between saturated fat and CHD: a) the substituting macronutrient, b) the type of saturated fat based on its carbon chain length, and c) the food source of saturated fat.

We started with the data from the Dutch EPIC-NL cohort. The results of this study can be

found in chapter 3.1. Unexpectedly, we observed that a higher intake of total saturated fat was associated with a lower CHD risk. Modelled substitution of saturated fat for polyunsaturated fat, monounsaturated fat, carbohydrates or animal protein barely affected this association. When looking at individual SFAs based on the carbon chain length of SFA, we observed lower CHD risks for higher intakes of the sum of butyric acid (4:0), caproic acid (6:0), caprylic acid (8:0) and capric acid (10:0), of myristic acid (14:0) and of the sum of pentadecylic acid (15:0) and margaric acid (17:0). Regarding the dietary source of SFA, we observed lower CHD risks for higher intakes of saturated fat from dairy products, and no association for saturated fat from other food sources such as meat. Because of the unexpected results, we repeated our study in the Rotterdam Study, which is an independent but comparable Dutch population. The results are presented in chapter 3.2. In the Rotterdam Study, we observed no association between the intake of total saturated fat and CHD risk, irrespective of whether saturated fat was substituted for polyunsaturated fat, monounsaturated fat, carbohydrates or vegetable protein. We did observe a higher CHD risk for substitution of saturated fat for animal protein, for higher intakes of palmitic acid (16:0) and for a higher intake of saturated fat from meat, although the latter association was not statistically significant.

Because of the diverging results of these two studies, we investigated two more cohorts. In a cohort from the United Kingdom and a cohort from Denmark, we examined the association between the consumption of individual saturated fatty acids and the risk of a myocardial infarction (MI). The results are presented in chapter 3.3. In both cohorts, higher intakes of the short- to medium-chain saturated fatty acids (4:0 through 10:0) as well as myristic acid (14:0) were related to a lower risk of MI. A sensitivity analysis shows that none of the associations in these two cohorts were affected when the saturated fatty acids were substituted for polyunsaturated fat, monounsaturated fat, carbohydrates or protein.

Opposite to the assumed harmful effects ascribed to saturated fat, polyunsaturated fat is linked to beneficial effects on heart health. The results for polyunsaturated fat from intervention studies and observational studies are generally more consistent than the results for saturated fat. Especially the omega-3 (n-3) polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid, have been related to beneficial effects on cardiovascular disease risk. Fish is the main food source of these n-3 polyunsaturated fatty acids. In observational studies, fish consumption of at least one portion (≈ 100 g) per week is associated with lower risks of incident stroke and coronary heart disease mortality. The fish consumption in the Netherlands is much lower, and most people consume less than one portion of fish per week. Observational studies on associations between such low fish consumption and cardiovascular disease risk are scarce. In addition, it is unclear whether the type of fish, i.e., fatty fish or lean fish, matters. Therefore, we investigated whether fish consumption of less than one portion per week (<100 g/week) was associated with a lower risk of cardiovascular disease in the EPIC-NL cohort. The results of this study, in chapter 4, do not indicate that subjects who consume less than a portion of fish per week have a lower risk

of cardiovascular disease, regardless of the type of fish. However, we observed a lower risk of ischemic stroke in subjects who consumed more than one portion of fish per week, as compared to the fish non-consumers. This is in agreement with the current evidence, and found for fatty fish as well as for lean fish. Nevertheless, because it was not possible to fully separate the lean fish consumption from the fatty fish consumption, we cannot exclude the possibility that the two types differ with respect to their associations with cardiovascular disease.

Because fatty acids are never consumed in isolation, it would be useful if we could capture the quality of the total fatty acid profile in the diet in one measure. Recent studies suggested that the lipophilic index (LI) might be a suitable measure for this. This index is calculated based on the fluidity (melting points) of the fatty acids; the higher the melting point of the fatty acid, the higher is the LI. Previous studies showed that cell membranes with low fluidity are associated with harmful changes in for instance blood pressure and endothelial function. In chapter 5, we present the results of a study in which we calculated the LI of the fatty acid profile of the diets consumed by the subjects of the EPIC-NL cohort. Because the LI does not depend on the amount of fat that is consumed, we additionally calculated the lipophilic load (LL). The LL is measure of both the fluidity of the fatty acid profile and the amount of fat in the diet. We then investigated whether the LI and LL were related to the risk of stroke and coronary heart disease. This was not the case.

None of our studies confirmed that the association between saturated fat and coronary heart disease depends on the substituting macronutrient. We used the same approach to model the substitution as previous studies, that is with use of between-subject comparisons in dietary data obtained at one single time point (usually baseline). In such analysis, the substitution is not actually observed but modelled. Therefore, in chapter 6, we investigated whether substitution of saturated fat for other macronutrients actually occurs within subjects during follow-up. We used data of a subgroup from the Prospect-EPIC cohort for which repeated measurements of diet and serum cholesterol concentrations were available. In this subgroup we observed that on average there was a modest decrease in saturated fat intake during a follow-up period of ~4 years. In over 80% of the subjects these differences in saturated fat intake were compensated for by carbohydrates. We then investigated whether the observed within-subject substitution was related to differences in serum cholesterol concentrations, and whether these associations were similar to the associations of between-subject substitution with use of only baseline data. These two approaches yielded opposite conclusions. The association of within-subject substitution of saturated fat for carbohydrates and the difference in HDL cholesterol was opposite to the association of between-subject substitution of saturated fat for carbohydrates and the difference in HDL cholesterol. In further analysis, we observed that the subject ranking based on the saturated fat intake at baseline was significantly different from the ranking based on the second measurement ~ 4 years later. From these results, we can therefore conclude that the interpretation of an association of between-subject substitution modelled in only baseline data and disease risk during follow-up could be wrong if within-subject substitution

actually occurs during follow-up.
In chapter 7, I discuss the main findings of this thesis, their implications and suggestions for future research. I discuss that observational studies are necessary and valuable in nutrition research, particularly in situations where randomized controlled trials are not feasible. Nevertheless, we should always bear in mind their limitations. In the studies in this thesis and in most previous observational studies, the dietary data from one dietary (baseline) assessment were used in the analysis. We showed that the intake from a single measure might not be representative for the intake during follow-up, which could lead to misleading associations with disease risk during follow-up. Furthermore, the study of individual nutrients, such as saturated fat, in observational cohorts can be problematic because of the correlations between the nutrients as a result of shared food sources, which may make it impossible to disentangle them. Moreover, the dietary intake in cohorts may have too little variation or cover absolute intake levels that that limit the possibility to detect the association under study. I suggest that, in general, these limitations by themselves may explain the inconsistent results in observational cohort studies on the association between dietary saturated fat and coronary heart disease. Therefore, based on observational studies alone, we cannot exclude the possibility that an association between saturated fat and coronary heart disease exists. In addition, I discuss the practical implications of our findings, with respect to the dietary guidelines. And finally, I provide suggestions for future research with the main focus on saturated fat.

Nederlandse Samenvatting (Summary in Dutch)

Nederlandse Samenvatting

Vet uit onze voeding is waardevol en essentieel voor ons lichaam. In de loop van de tijd is duidelijk geworden dat bepaalde typen vet(zuren) ook negatieve effecten op onze gezondheid kunnen hebben. Zo weten we bijvoorbeeld uit interventiestudies dat verzadigd vet en trans-vet ongunstige effecten hebben op de serum cholesterolspiegel, wat weer gerelateerd is aan een hoger risico op coronaire hartziekte. Terwijl er consensus bestaat over de ongunstige effecten van trans-vet, is er een langdurige discussie gaande over de relatie tussen verzadigd vet en coronaire hartziekte. Deze discussie wordt veroorzaakt door de discrepantie tussen de uitkomsten van interventiestudies enerzijds en observationele studies anderzijds. Waar de eerste laten zien dat verzadigd vet consumptie het LDL cholesterol verhoogt, wordt in veel observationele studies geen verband gevonden tussen de inname van verzadigd vet en het risico op coronaire hartziekten.

In dit proefschrift, presenteer ik de resultaten van een aantal observationele onderzoeken naar de relatie tussen vet consumptie en het risico op hart- en vaatziekten. Centraal staat de relatie tussen verzadigd vet en coronaire hartziekte, waarbij we in eerste instantie hebben onderzocht of die relatie afhankelijk is van een aantal factoren waar in veel eerdere studies geen rekening mee is gehouden. Deze factoren zouden een mogelijke verklaring kunnen zijn voor de verschillen met de uitkomsten van de interventiestudies. Voordat we in hoofdstuk drie de resultaten hiervan bespreken, gaan we in hoofdstuk 2 in op de reproduceerbaarheid en validiteit van één van de meest bepalende metingen in observationeel voedingsonderzoek, namelijk de meting van de voedingsinname.

In het EPIC-NL cohort is de voedingsinname bij start gemeten met behulp van een voedselfrequentievragenlijst. Voor de interpretatie van de resultaten van onderzoek naar de relatie tussen de consumptie van individuele vetzuren en klinische eindpunten, is het noodzakelijk om te weten hoe goed de vragenlijst individuen rangschikt op grond van hun innames van de verschillende vetzuren. Dat is het onderwerp van hoofdstuk 2. De relatieve validiteit is beoordeeld door de vetzuur inname metingen van de voedselfrequentievragenlijst te vergelijken met de metingen van twaalf niet-achtereenvolgende 24-uurs navraag methoden. Voor de meeste vetzuren bleek de validiteit voldoende tot goed te zijn, maar deze was minder goed voor de vetzuren met een heel lage inname, waaronder de verzadigde vetten met korte ketens, en de n-3 meervoudig onverzadigde vetzuren. De reproduceerbaarheid is onderzocht door vergelijking van drie verschillende afnames van de voedselfrequentievragenlijst met tussenpozen van zes maanden. De reproduceerbaarheid bleek goed voor alle individuele vetzuren.

Zoals gezegd, is er in veel observationele studies geen bevestiging gevonden van de aanname dat een hogere inname van verzadigd vet gerelateerd is aan een verhoogd risico op coronaire hartziekte, ondanks dat verzadigd vet het LDL-cholesterol in interventiestudies doet stijgen. In onze studie kijken we naar een drietal mogelijke factoren, die in observationele studies die relatie tussen verzadigd vet en hart- en vaatziekten zouden kunnen

beïnvloeden en daarmee een mogelijke verklaring vormt voor de gevonden discrepantie. Namelijk, a) het vervangende macronutriënt, b) het type verzadigd vet op grond van de ketenlengte, of c) de voedingsbron van het verzadigd vet.

Dit hebben we als eerste onderzocht in EPIC-NL, een Nederlands cohort, waarvan de resultaten staan beschreven in hoofdstuk 3.1. We vonden tot onze verrassing dat in dit cohort juist een hogere inname van totaal verzadigd vet samen gaat met een lager risico op coronaire hartziekte. Gemodelleerde vervanging van meervoudig onverzadigde vetten, enkelvoudig onverzadigde vetten, koolhydraten of dierlijk eiwit door verzadigd vet veranderde deze relatie vrijwel niet. Nadat we onderscheid maakten tussen typen verzadigde vetten op grond van ketenlengte, zagen we deze verlaagde risico's voor een hogere inname van de som van boterzuur (4:0), capronzuur (6:0), caprylzuur (8:0) en caprinezuur (10:0), van myristinezuur (14:0), en van de som van pentadecaanzuur (15:0) en margarinezuur (17:0). Ook vonden we de verlaagde risico's voor verzadigde vetten uit melk- en melkproducten, en niet uit ander voedingsbronnen zoals vlees. Vanwege deze onverwachte bevinding, herhaalden we het onderzoek in een andere, maar vergelijkbare, Nederlandse populatie: de Rotterdam Studie (hoofdstuk 3.2). In die populatie vonden we geen relatie tussen totaal verzadigd vet inname en coronaire hartziekte, ook niet bij vervanging van meervoudig onverzadigde vetten, enkelvoudig onverzadigde vetten, koolhydraten of plantaardig eiwit door verzadigd vet. Wel zagen we een verhoogd risico op coronaire hartziekte bij vervanging van dierlijk eiwit door verzadigd vet, voor een hogere inname van palmitinezuur (16:0), en voor een hogere inname van verzadigd vet uit vlees, al was de laatste relatie niet statistisch significant.

Dit alles was aanleiding om nog twee andere cohorten in ons onderzoek te betrekken (hoofdstuk 3.3). In een Engels en een Deens cohort is de relatie tussen de inname van individuele vetzuren en het risico op myocard infarct, een coronaire hartziekte, onderzocht. In beide cohorten blijkt dat een hogere inname van de korte en middelkorte ketenvetzuren (4:0 t/m 10:0) en van myristinezuur (14:0) gerelateerd is aan een lager risico op een myocard infarct. Een sensitiviteitsanalyse laat zien dat het niet uitmaakt of deze vetzuren meervoudig onverzadigde vetten, enkelvoudig onverzadigde vetten, koolhydraten dan wel eiwit vervangen.

In tegenstelling tot verzadigd vet, wordt van meervoudig onverzadigd vet aangenomen dat dit het risico op hart- en vaatziekten verlaagt. Interventiestudies en observationele studies laten voor dit macronutriënt een eenduidiger beeld zien dan voor verzadigd vet. In het bijzonder worden veel gunstige effecten op hart- en vaatziekten risico toegeschreven aan de omega-3 (n-3) meervoudig onverzadigde vetzuren eicosapentaeenzuur en docosahexaeenzuur. Vis is de belangrijkste bron van deze n-3 vetzuren. Vis inname van minimaal één portie (≈ 100 gram) per week is in observationele cohort studies gerelateerd aan een lager risico op beroerte en fatale coronaire hartziekte. In Nederland is de visinname veel lager en eten de meeste mensen nog geen portie per week. Er zijn weinig observationele studies waarin is onderzocht of zo'n lage vis inname (minder dan één portie per week) ook al risico

verlagend is. Ook is het niet duidelijk of het uitmaakt of er vette dan wel magere vis wordt gegeten. Daarom hebben wij in het EPIC-NL cohort onderzocht of ook een vis inname van minder dan een portie per week (<100 gram per week) gerelateerd is aan een lager risico op hart- en vaatziekten (hoofdstuk 4). Dat bleek, ongeacht het type vis, niet het geval. Wel vonden we, in lijn met bestaande studieresultaten, dat de inname van ten minste 1 portie per week gerelateerd is aan een lager risico op ischemische beroerte. Dat gold zowel voor vette als magere vis. Daarbij moet worden opgemerkt dat de innames van de verschillende typen vis in dit cohort dusdanig sterk met elkaar verstrengeld zijn, dat we niet met zekerheid kunnen uitsluiten dat magere en vette vis verschillen in hun relatie met hart- en vaatziekten.

Omdat vetzuren niet afzonderlijk worden gegeten, is het mogelijk zinvol om te kijken naar een maat die de kwaliteit van de totale combinatie van alle soorten vetzuren in de voeding weergeeft. Recent is gesuggereerd dat de lipofilische index (LI) hiervoor gebruikt kan worden. Deze index wordt berekend op grond van de vloeibaarheid (smeltpunten) van de vetzuren; hoe hoger het smeltpunt, des te hoger is de LI.
Er is in eerdere studies aangetoond dat een lage vloeibaarheid van celmembranen gerelateerd is aan ongunstige veranderingen in bijvoorbeeld bloeddruk en endotheel functie. Zoals beschreven in hoofdstuk 5, hebben wij voor de deelnemers van het eerder genoemde EPIC-NL cohort de LI van het vetzuurprofiel van hun voedingsinname berekend. Omdat de LI geen rekening houdt met de hoeveelheid vet die iemand eet, is ook de lipofilische last (LL) berekend. De LL is een maat voor zowel de vloeibaarheid van het vetzuurprofiel als de hoeveelheid vet in de voeding. We hebben vervolgens onderzocht of de LI en de LL gerelateerd zijn aan het risico op beroerte en coronaire hartziekte. Dat bleek niet het geval. Hoewel algemeen wordt aangenomen dat de relatie tussen verzadigd vet en coronaire hartziekte afhankelijk is van het vervangende macronutriënt, zagen we dit in geen van de studies in dit proefschrift duidelijk terug. Daarbij hebben wij dezelfde statistische methode gebruikt om die substitutie te modelleren als eerdere studies, namelijk met behulp van een tussenpersoonsvergelijking van voedingsdata die op één moment -meestal baseline- is verzameld. De substitutie vindt dus niet daadwerkelijk plaats. In hoofdstuk 6 hebben we daarom in een subgroep van het Prospect-EPIC cohort onderzocht of substitutie van verzadigd vet voor andere macronutriënten daadwerkelijk plaatsvindt binnen personen gedurende een follow-up periode van ~4 jaar. Voor deze subgroep zijn namelijk herhaalde metingen van voeding en serum cholesterol beschikbaar. Gemiddeld nam de verzadigd vet inname af in de populatie. De verschillen in verzadigd vet tussen de twee metingen werden door ruim 80% van de deelnemers gecompenseerd met koolhydraten.
Vervolgens hebben we onderzocht of de geobserveerde binnen-persoons substitutie gerelateerd is aan verschillen in de serum cholesterol waarden, en hoe deze relatie zich verhoudt tot de relatie die wordt gevonden dus door een tussen-persoons vergelijking op basis van baseline data. De twee methoden resulteerden in tegenover gestelde conclusies. De relatie van binnen-persoons substitutie van verzadigd vet voor koolhydraten met het verschil in HDL-cholesterol was tegenovergesteld aan de relatie van tussen-persoons substitutie van verzadigd vet voor koolhydraten met het verschil in HDL-cholesterol.

Nadere analyse liet zien dat de ranking van de deelnemers op grond van hun verzadigd vet inname op baseline significant verschilde van de ranking op grond van de tweede meting. Hieruit kan worden geconcludeerd dat de interpretatie van tussen-persoons substitutie op basis van baseline data mogelijk onjuist is als er daadwerkelijk substitutie plaatsvindt gedurende follow-up.

In hoofdstuk 7 bespreek ik de belangrijkste bevindingen uit dit proefschrift, hun betekenis, en suggesties voor toekomstig onderzoek. Ik bediscussieer dat observationele studies noodzakelijk en nuttig zijn in het onderzoek naar de relatie tussen voeding en ziekte, zeker omdat interventiestudies in veel gevallen niet mogelijk zijn, maar dat we ook rekening moeten houden met hun beperkingen. Zowel in onze eigen studies, als in de meeste bestaande observationele studies is de voedingsinname gebaseerd op een enkele baseline meting. Zoals we hebben laten zien, is deze inname mogelijk niet representatief voor de inname gedurende follow-up, waardoor de gevonden relaties met ziekte tijdens follow-up mogelijk onjuist zijn. Ook wordt het onderzoek naar relaties op nutriënt niveau, zoals verzadigd vet, bemoeilijkt door de onderlinge verstrengeling van de verschillende voedingscomponenten, en is het maar de vraag of we die voldoende uit elkaar kunnen trekken. Bovendien is het mogelijk dat er niet voldoende variatie in de voedingsinname is binnen de bestudeerde cohorten is om een verband op te pikken. Ik suggereer dat deze beperkingen op zichzelf een mogelijke verklaring kunnen zijn voor de inconsistente bevindingen van observationele studies naar verzadigd vet inname en coronaire hartziekte in het algemeen. En dat we daarom op grond van observationele studies alleen niet kunnen uitsluiten dat een dergelijke relatie bestaat. Verder bediscussieer ik de praktische implicaties van onze bevindingen, met het oog op de voedingsrichtlijnen. Als laatste bespreek ik suggesties voor toekomstig onderzoek, waarbij verzadigd vet centraal staat.

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About the author

Jaike was born on December 22nd 1982 in Geldrop and was raised in Nuenen. After graduation from the Augustinianum in Eindhoven (Atheneum) in 2001, she attended the one year study program at the School of Liberal Arts in Driebergen. In 2006, she decided that Architecture and Teacher Education were both not the right studies for her and she moved to The Hague to study Nutrition and Dietetics. During that study, she got the opportunity to attend six months of the Master study Clinical Epidemiology in Amsterdam and she was immediately hooked. Therefore, after graduation in 2010, she moved to Wageningen to study Nutrition and Health, with specialization Epidemiology and Public Health. She received her diploma in 2012. After working as a research assistant at the department of Human Nutrition of the Wageningen University, she started as a PhD student at the Julius Center of Health Sciences and Primary Care in Utrecht in 2013. At the department of cardiovascular epidemiology, she studied the relationship between saturated fat intake and cardiovascular disease of which the results are outlined in this thesis. In 2016, she received the Foppe ten Hoor Award for her presentation and the defense of the study covered in chapter 6 in this thesis. During the three year period as a PhD student, she also obtained her postgraduate Master degree in Clinical Epidemiology at the University of Utrecht. Currently, Jaike works as a clinical programmer at the Life Science Solutions Unit of OCS Consulting B.V. in ‘s Hertogenbosch.