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DAIRY PRODUCTS AND CARDIOMETABOLIC HEALTH OUTCOMES

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Dairy Products and Cardiometabolic Health Outcomes

ABSTRACT

The association between dairy fat intake and type 2 diabetes (T2D) risk remains unclear despite evidence from several studies examining the relationship between dairy product consumption stratified by fat content and risk of T2D. In chapter 1, we investigated the association between dairy fat intake and risk of T2D in the Nurses' Health Study (NHS), NHSII, and the Health Professionals Follow-Up Study (HPFS) with emphasis on modeling isocaloric replacements of dairy fat with other macronutrients. Dairy fat was not associated with risk of T2D when compared with calories from carbohydrate. However, replacing 5% of calories from dairy fat with the equivalent energy from other sources of animal fat or carbohydrate from refined grains was associated with a 14% and a 4% increased risk of T2D, respectively. Conversely, a 5% calorie substitution of carbohydrate from whole grains was associated with 7% lower risk of T2D.

T2D is an established risk factor of cardiovascular disease (CVD) but there is sparse evidence to support dietary recommendations for T2D patients. In chapter 2, we examined the association between total dairy and individual dairy product consumption and risk of CVD among participants with T2D in the NHS and HPFS. Intake of total dairy product or individual dairy products were not associated with CVD risk, with the exception of an inverse association between

ice-cream intake and CVD health outcomes. In substitution analyses, replacing one serving of dairy products with red meat or processed red meat was associated with a 6% and 13 increase in CVD risk, respectively. Conversely, replacing one serving of total dairy products with one serving of nuts was associated with a 12% risk reduction of CVD.

In chapter 3, we examined the association between very-long chain saturated fatty acids (VLCSFAs), such as 20:0, 22:0, and 24:0 and risk of T2D in participants from the NHS and HPFS who previously provided blood samples. Comparing extreme quartiles of plasma levels, 20:0, 22:0, 24:0, and their sum were associated with 50%, 58%, 60%, and 60% lower T2D risk, respectively. Only erythrocyte biomarkers of 20:0 and 22:0 fatty acids were inversely associated with T2D risk.

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INTRODUCTION

Dairy products occupy an important place in the U.S. diet accounting for almost 10% of total energy intake.[1] Current dietary guidelines recommend consuming low-fat dairy products as part of a healthy eating pattern while keeping overall saturated fat under 10% of total calories.[2] Recent meta-analyses have reported that the association between total dairy product intake and cardiovascular disease (CVD) is inconclusive even when stratifying by fat content[3,4] and low-fat dairy products were modestly inversely associated with risk of T2D whereas high-fat dairy products were not associated with risk of T2D.[5] It is important to consider that many products contribute dairy fat to the diet given its availability in the U.S. food supply. In 2017, 3.6 million metric tons of dairy fat were produced from cow's milk alone, which corresponds to about 274 kcal per capita per day.[6] Most of it is consumed through dairy foods, butter spreads, and as an ingredient in processed foods such as pastries, pizzas, sauces, etc. Given dairy fat's relevance in modern diet, studying its role in the development to cardiometabolic health is of importance. A recent analysis from our cohorts reported that dairy fat intake was not associated with CVD compared with an equivalent amount of energy from carbohydrates.[7] However, specific isocaloric substitutions showed that replacing dairy fat with vegetable fat, polyunsaturated fatty acids, and carbohydrates from whole grains was associated with reduced CVD risk.[7] Whether dairy fat intake is associated with T2D risk remains unclear given the divergent findings between studies that use dietary questionnaires to derive dairy intake[5] and those that use biomarkers of fatty acids found in dairy fat such as pentadecanoic (C15:0), heptadecanoic (C17:0) and *trans*-palmitoleic acids (*trans* 16:1 n-7) as the exposure which have been consistently inversely associated with T2D risk.[8–14] Thus, in Chapter 1, we used data from the Health Professionals

Follow-up Study (HPFS), the Nurses' Health Study (NHS), and NHSII to prospectively examine the association between dairy fat intake and T2D. We conducted specific substitutions of dairy fat with other macronutrients to study their association with T2D risk.

Complications from T2D such as diabetes dyslipidemia place this patient population at an increased risk of cardiovascular disease compared to the general population.[15] Few dietary recommendations related to dairy products have been issued for this patient population other than restricting intakes of saturated fatty acids in favor of promoting the intake of mono-unsaturated and poly-unsaturated fatty acids.[16,17] Thus, in Chapter 2, we used a cohort of T2D patients from the HPFS and NHS and assessed whether total dairy consumption or specific dairy products were associated with CVD risk. We additionally examined the association stratified by fat content and between dairy fat intake and CVD risk and we modeled replacements of dairy products with fish, poultry, and red and processed red meat, nuts, and beans and their association with CVD risk. Our findings can help inform dietary guidelines for patients living with T2D.

In Chapter 3, we examined the association between plasma and erythrocyte membrane biomarkers of very-long chain saturated fatty acids (VLCSFAs), such as 20:0, 22:0, and 24:0 and T2D risk. Recent studies have shown that VLCSFAs are inversely associated with T2D in other cohorts.[8,18,19] The dietary and lifestyle determinants of VLCSFA circulating levels are unclear. Thus, we also evaluated the correlation between multiple dietary variables and their plasma and erythrocyte levels.

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CHAPTER 1:

Dairy fat intake and risk of type 2 diabetes in three cohorts of U.S. men and women

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ABSTRACT

Background: Previous studies have examined dairy products with various fat contents in relation to type 2 diabetes (T2D) risk, although data regarding dairy fat intake *per se* and incident T2D risk are sparse.

Objective: The aim of this study is to evaluate the association between dairy fat intake and risk of T2D. We also examined associations for isocalorically replacing dairy fat with other sources of macronutrients.

Methods: We prospectively followed up 41,569 men in the Health Professionals Follow-Up Study (HPFS; 1986–2012), 65,964 women in the Nurses' Health Study (NHS; 1984–2012), and 90,144 women in the NHSII (1991–2013). Diet was assessed every 4 years with the use of validated food-frequency questionnaires. Fat intake from dairy products and other relevant food items was summed to calculate total intake, which was expressed as percent of total energy. Self-reported incident T2D cases were confirmed using a validated supplementary questionnaire. Time-dependent Cox proportional hazards regression was used to estimate the hazard ratio (HR) for dairy fat intake and T2D risk.

Results: During 4,178,418 years of follow-up, we documented 16,346 incident T2D cases. In multivariate models, compared with calories from carbohydrates, dairy fat was not associated with risk of T2D [RR for extreme quintiles: 0.98; 95% CI: 0.94, 1.01]. In isocaloric substitution models, the replacement of 5% of calories from dairy fat with the equivalent energy from other sources of animal fat or carbohydrate from refined grains was associated with a 14% [RR: 1.14; 95% CI: 1.10, 1.19] and a 4% [RR: 1.04; 95% CI: 1.00, 1.07] increased risk of T2D, respectively. Conversely, a 5% calorie substitution with carbohydrate from whole grains was associated with

7% lower risk of T2D [RR: 0.93; 95% CI: 0.88, 0.97]. Replacing dairy fat with PUFAs, MUFAs from plant sources, and vegetable fat was not associated with T2D risk.

Conclusions: In conclusion, dairy fat intake was not associated with T2D risk. However, replacement of dairy fat with carbohydrates from whole grains was associated with lower T2D risk, whereas replacement of dairy fat with other animal fats or refined carbohydrates was associated with higher risk of T2D.

INTRODUCTION

The prevalence of type 2 diabetes (T2D) has reached an epidemic level. According to estimates from the International Diabetes Federation, 425 million adults worldwide had T2D in 2017 (8.8% of adult population).[1] The CDC estimated that 23.1 million people were living with diagnosed diabetes in the U.S. in 2015.[2] Genetic risk factors may determine T2D risk to certain extent, and the critical role of lifestyle and diet, as well as their interactions with genes has been well-recognized.[3,4] In the US diet, dairy products are one important food group and in general accounted for 9.4% of total energy intake.[5] The current dietary guidelines recommend limiting the intake of high-fat dairy products due to the concerns regarding high contents of saturated fatty acids (SFAs) in dairy fat. Saturated fat intake is a risk factor in the development of CHD, particularly long-chain SFAs (C14:0 - C18:0) when substituting for PUFAs.[6,7] However, evidence regarding associations between dairy fat and T2D risk remain sparse and inconclusive. The associations between dairy product intake and incident T2D by dairy fat content have been examined in several meta-analyses of cohort studies, and the results showed that high-fat dairy products, on average, were not associated with risk of T2D,[8] whereas an inverse association has

been mostly observed for low-fat dairy intake.[8] However, in meta-analysis of high-fat or low-fat dairy products, some important sources of dairy fat such as butter either consumed directly or as an ingredient of other food products were usually excluded, thus findings from these studies may not directly pertain to dairy fat. To date, no studies have directly examined the association between dairy fat intake and T2D risk. Studies of biomarkers of fatty acids found in dairy fat such as pentadecanoic (C15:0), heptadecanoic (C17:0) and *trans*-palmitoleic acids (*trans* 16:1 n-7) have consistently reported inverse associations with T2D risk.[9–15] However, these fatty acids are, in general, weakly correlated with dairy fat intake and may represent effects of metabolism or intake of other foods such as fish.[16]

To elucidate the association between dairy fat and diabetes risk, in the current study we aimed to evaluate dairy fat intake derived from various dairy products, as well as other food items that may contain dairy, in relation to T2D risk in three prospective cohorts of U.S. men and women. We also examined isocaloric replacement of dairy fat with other macronutrients with regards to T2D risk. We hypothesize that higher consumption of dairy fat intake is associated with increased T2D risk.

METHODS

Study population: We used data from participants in three prospective cohorts: The Health Professionals Follow-Up Study (HPFS), the Nurses' Health Study (NHS), and the NHSII. The HPFS started in 1986, when 51,529 male health professionals, who were 40 – 75 years of age at recruitment.[17] The NHS was established in 1976 and recruited 121,700 female nurses aged 30 to

55, and the NHSII was established in 1989 with a recruitment of 116,671 nurses who were 24 to 44 years of age.[18] In all three cohorts, questionnaires were administered at baseline to collect information on lifestyle, medical history, and other characteristics, which was updated biennially. The cumulative follow-up of the participants in these cohorts was >90%.[19]

In the current analysis, we excluded participants who had diagnoses of diabetes, cancer, or CVD at baseline (1986 for HPFS, 1984 for NHS, and 1991 for NHSII). Additionally, participants were excluded if they left more than 70 food items blank on the baseline food frequency questionnaire (FFQ) and those who reported unusual total energy intake levels (i.e., daily energy intake <800 or >4200 kcal/day for men, and <500 or >3500 kcal/day for women). We also excluded participants without baseline information on dairy consumption or T2D diagnosis date, leaving a sample of 41,569 HPFS participants, 65,964 NHS participants, and 90,144 NHSII participants for the present analysis. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. Return of a completed questionnaire was considered as informed consent.

Assessment of diet: A FFQ with 61 items was administered to participants in 1980 to collect information about habitual dietary intake. An expanded questionnaire with 131 items was sent to participants in 1984, 1986, and quadrennially thereafter to update their diet information. In the HPFS, the expanded, quadrennial FFQ has been administered since 1986, and in the NHSII diet has been assessed since 1991 quadrennially. For each food item in the FFQ, we inquired about the consumption frequencies ranging from 'never or less than once per month' to '6 or

more times per day'. Dairy fat intake was calculated by multiplying the eating frequency of a food item with a pre-specified portion size with the dairy fat content of that food item and then summing the dairy fat intake across all relevant food items. The dairy products considered in the current analysis included whole milk, skim/low fat milk, whole milk, sherbet, yogurt, cheese, cream cheese, cream, ice cream, cottage/ricotta cheese, butter, and other cheese. Other food items included in the computation included cakes and pastries and mashed potatoes because they may contain dairy fat. Intake of other nutrients was calculated using the same algorithm. Nutrient contents of food items were extracted from the Harvard University Food Composition Database. From 1994 in the NHS and HPFS and 1995 in the NHSII, yogurt consumption was separated into plain yogurt (plain or with NutraSweet) and flavored yogurt (without NutraSweet). The standard serving size was 8 oz. glass (240 ml) for skim, low fat milk, and whole milk, 1 cup (8 oz.) for yogurt until the 2006 (NHS and HPFS) or 2007 questionnaire (NHSII) or 4-6 oz. starting in the 2010 and 2011 questionnaires,1 tablespoon (20g) for cream and sour cream, ½ cup (122.5g) for sherbet or frozen yogurt, ice cream, cottage and ricotta cheese, 1 oz. (30g) for cream cheese and other cheese. We also included butter added to food or bread and butter used for sautéing or frying, baking and cooking at home. The overall reproducibility and validity of the FFQ have been demonstrated in prior studies using dietary records and biomarkers as reference measurements of diet.[20–22] Regarding dairy products, the deattenuated correlations between FFQ assessments and those by diet records were 0.62 for both high-fat and low-fat dairy products.[23]

Ascertainment of T2D cases: Incident cases of T2D were identified by self-reports on the follow-up questionnaires and confirmed by a validated supplementary questionnaire enquiring symptoms, use of medications, and blood glucose levels at diagnosis. This supplementary questionnaire has been validated in previous studies: 97% of questionnaire-confirmed T2D cases were reconfirmed through medical record review by a blinded physician.[24,25] We used the National Diabetes Data Group (NDDG) criteria [26] to confirm T2D diagnosis prior to 1998: (1) manifestation of classic symptoms such as excessive thirst, polyuria, weight loss and hunger, in conjunction with elevated fasting glucose ≥140 mg/dL (7.8 mmol/L) or non-fasting glucose levels ≥200 mg/dL (11.1 mmol/L) (2) asymptomatic but elevated plasma glucose in two separate occasions or abnormal glucose tolerance test results and (3) receiving any hypoglycemic treatment for diabetes. After 1998, a fasting glucose concentration ≥126 mg/dL (7.0 mmol/L) was adopted per the American Diabetes Association (ADA) diagnostic criteria.

Assessments of covariates. In the biennial follow-up questionnaires, we inquired about and updated information of body height and weight, cigarette smoking, physical activity, medication use, as well as history of chronic diseases, including diabetes, hypertension and hypercholesterolemia. Among the NHS and NHSII participants, we ascertained menopausal status, postmenopausal hormone use, and oral contraceptive use in the questionnaires. To quantify diet quality, we calculated a 2010 Alternative Healthy Eating Index (AHEI) score, which summarizes the consumption levels of 10 dietary components that are most predictive of chronic disease risk: higher intakes of fruits, vegetables, whole grains, nuts, long-chain omega-3 fatty acids, and PUFAs, and lower intakes of sugar sweetened beverages, red and processed meats,

TFAs, and sodium.[27] Each component was scored from 0 to 10 where 10 indicated best adherence to recommended servings per day, and 0 for the poorest adherence.

Statistical analyses

Person-years were calculated from the return date of the baseline questionnaire to the date when participants developed T2D, death, loss to follow-up, or the end of follow-up (NHS: June 30, 2012; NHSII: June 30, 2013; HPFS: January 31, 2012), whichever came first. Dairy fat intake, as percent of total energy, was categorized into quintiles. In order to represent long-term diet and minimize random within-person variability, we used the cumulative averages of dairy fat intake from the baseline questionnaire to the censoring events.[28] We stopped updating diet when participants developed coronary heart disease, coronary artery bypass surgery, stroke, or cancer because diagnoses of these outcomes may lead to dietary changes.[29]

We used a time-dependent Cox proportional hazards regression to estimate the hazard ratios (HRs) for dairy fat intake in relation to T2D risk. All analyses were conducted separately in each cohort, and HRs were combined using an inverse variance-weighted approach. The basic model was stratified jointly by age and calendar time to control for impact of these variables as well as their interactions on the associations of interest. In multivariate analyses, we controlled for BMI, race/ethnicity, physical activity (METs per week), smoking, alcohol use, family history of diabetes, history of hypertension or hypercholesterolemia at baseline, AHEI, and intakes of vegetable fat, animal fat other than dairy fat, protein, and total energy. In the NHS and NHSII, we further adjusted for use of contraceptive, menopausal status and, postmenopausal hormone use.

For the test for trend, we assigned median values of each quintile and model this variable in the Cox regression analyses. The coefficients from the final model were interpreted as the estimated effect of a specific percentage of energy from dairy fat replacing an equivalent percent of energy from carbohydrates, adjusted for dietary quality and other potentially confounding variables.

The associations with risk of T2D for isocaloric substitutions of other macronutrients for dairy fat were evaluated using a multivariable Cox proportional hazards model where dairy fat intake and other macronutrients were modeled as continuous variables in the same model. We then computed the difference in coefficients from this model to estimate the HRs for the replacing calories from dairy fat with the equivalent calories from other macronutrients, including vegetable fat, PUFAs, other animal fat, total carbohydrate, carbohydrate from whole grains, and carbohydrate from refined grains.[30]

We tested for potential effect modifications on the associations of interest by age (<65 y and ≥ 65 y), sex, BMI (< 25 kg/m^2, 25 – 29.9 kg/m^2, and ≥ 30 kg/m^2), physical activity (below median, above median), smoking (current, other), baseline hypertension (yes, no), and baseline hypercholesterolemia (yes, no) by including a cross-product term between these binary variables and dairy fat intake (% of energy) in quintiles in the fully-adjusted models and evaluated the significance of interaction terms using a log likelihood ratio test.

We conducted additional sensitivity analyses to examine the robustness of our findings. We continued updating diets after diagnoses of cancer and CVD outcomes, used the most recent

dietary assessments in the analyses, used the average of the two most recent dietary assessments, or censored participants who developed cancer or CVD outcomes during follow-up. We also placed 0-4, 4-8, 8-12, or 12-16-year lags (simple update) between dietary assessments and T2D incidence by modeling dairy fat in relation to T2D incidence 0-16 years later. For example, in the HPFS, for 0-4 year lag, we used the 1986 questionnaire for the 1986-1990 follow-up, the 1990 questionnaire for the 1990-1994 follow-up, the 1994 questionnaire for the 1994-1998 follow-up, and so forth. For a 4-8 year lag we used the 1986 questionnaire for the 1990-1994 follow-up and the 1990 questionnaire for the 1994 to 1998 follow-up and we followed a similar lagged protocol for the 8-12 and 12-16 year lagged analyses. We followed the same approach to place similar lags in the intake of macronutrients used for isocaloric substitution analyses and T2D incidence.

All P values were 2-sided, and 95% confidence intervals (CIs) were calculated for HRs. Data were analyzed with the Statistical Analysis Systems software package, version 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

During 4,178,418 years of follow-up, we documented 16,346 incident T2D cases in the three cohorts. Dairy fat intake was positively associated with white ethnicity, current smoking, *trans* fatty acid (TFA) intake, and total calorie intake. Dairy fat intake was also associated with a higher BMI in the HPFS and the NHS. Dairy fat consumption was inversely correlated with baseline hypertension and hypercholesterolemia, postmenopausal hormone use in women,

physical activity, the AHEI, glycemic load, and intakes of alcohol, whole grains, fruits and vegetables, SSB, and vegetable fat (**Table 1.1**).

In the age-adjusted model, a 5% increase in energy from dairy fat was not associated with T2D risk (RR: 1.00; 95% CI: 0.97, 1.03) as shown in **Table 1.2**. Further adjustment for lifestyle, demographic, and dietary variables did not substantially change this null association: the pooled HR (95% CI) was 0.98 (0.94, 1.01) comparing 5% calories from dairy fat with equivalent calories from carbohydrates. Stratified analysis did not show any significant interactions (All P-values > 0.05) in **Table 1.3**.

In isocaloric substitution models, the replacement of 5% of calories from dairy fat with the equivalent energy from other sources of animal fat was associated with a 14% higher T2D risk [RR: 1.14; 95% CI: 1.10, 1.19], and replacement by carbohydrates from refined grains was associated with a 4% higher T2D risk [RR: 1.04; 95% CI: 1.00, 1.07] (**Table 1.4**). Conversely, a 5% calorie substitution by carbohydrates from whole grains was associated with 7% lower risk of T2D [RR: 0.93; 95% CI: 0.88, 0.97]. We did not observe a significant association when replacing 5% calories from dairy fat with the same percent of energy intake from vegetable fat, poly-unsaturated fatty acids (PUFAs), or total carbohydrates (P > 0.05). Replacing dairy fat with the same energy intake from omega-6 fatty acids, linoleic acid, monounsaturated fatty acids (MUFAs)

Table 1.1 Baseline age-adjusted characteristics of participants in the 3 cohorts according to quintiles of dairy fat (% of energy intake)*

*Data were age standardized except for age. HPFS, Health Professionals Follow-Up Study; METs, metabolic equivalent; NA, not available; NHS, Nurses' Health Study; Q, quintile.

Characteristics	HPFS (1986)			NHS (1984)			NHSII (1991)		
	Q1 (n=8313)	Q3 (n=8314)	Q5 (n=8314)	Q1 (n=13192)	Q3 (n=13193)	Q5 (n=13193)	Q1 (n=18028)	Q3 (n=18029)	Q5 (n=18029)
Dairy fat (% of energy)[1]	2.8 (0.8)	5.9 (0.4)	11.4 (3.0)	3.5 (0.7)	6.0 (0.3)	10.5 (2.3)	3.5 (0.9)	6.5 (0.4)	11.5 (2.6)
Age (y)[2]	53.9(9.4)	52.5(9.5)	53.3(9.7)	50.1(7.2)	50.1(7.2)	50.1(7.2)	36.9(4.6)	36.0(4.7)	35.4(4.7)
Physical. activity (MET-h/wk)	23.0(32.7)	21.6(28.7)	19.6(27.9)	14.7(20.4)	14.2(20.4)	13.2(21.3)	20.8(28.6)	21.0(27.0)	20.7(27.3)
BMI (kg/m^2)	24.5(4.9)	24.9(4.9)	25.2(5.0)	24.5(4.4)	25.0(4.6)	24.9(4.8)	24.6(5.4)	24.7(5.3)	24.4(5.2)
BMI 25 to <30 kg/m^2 (%)	39.8	44.9	45.7	24.0	25.5	24.4	20.0	21.3	19.8
BMI ≥ 30 kg/m^2 (%)	6.2	7.7	9.2	9.9	12.1	12.3	13.9	13.5	12.2
Race, white (%)	91.4	95.9	96.6	95.1	98.6	99.0	92.2	97.5	97.8
Current smoker (%)	9.0	8.3	12.0	23.6	21.5	29.4	13.8	11.1	13.4
Hypertension (%)	22.2	19.0	17.6	21.8	20.1	18.8	6.7	6.0	5.5
High cholesterol (%)	15.3	9.6	6.6	11.1	7.0	5.9	17.2	13.9	12.1
Family history of diabetes (%)	23.4	23.8	24.2	28.6	28.7	27.5	35.7	34.7	33.1
Postmenopausal (%)	NA	NA	NA	46.2	45.1	45.4	4.0	2.8	2.6
Current menopausal hormone use (%)[3]	NA	NA	NA	11.8	11.2	9.9	5.5	4.2	3.8
Curr. oral contraceptive (%)	NA	NA	NA	NA	NA	NA	10.8	11.3	10.7
Total energy (Kcal/d)	1911(617)	2021(606)	2051(659)	1676(429)	1768(428)	1792(467)	1734(565)	1822(541)	1784(556)
AHEI	50.1(11.6)	46.7(10.3)	43.5(10.0)	48.0(9.7)	46.1(8.8)	43.2(8.5)	49.8(12.0)	48.4(10.7)	47.1(10.3)
Alcohol (g/d)	13.9(18.7)	11.1(14.6)	9.5(13.3)	6.8(10.9)	5.6(8.6)	5.8(8.9)	3.2(6.9)	3.1(5.8)	3.1(5.9)
Glycemic load	131.1(31.5)	125.1(23.7)	115.9(22.8)	107.2(19.8)	103.0(15.7)	95.5(16.8)	130.4(25.7)	121.4(19.3)	112.7(18.7)
Carbohydrate from whole grains (% of total energy)	3.8(4.0)	3.1(2.7)	2.4(2.2)	3.5(2.5)	3.2(2.0)	2.5(1.8)	3.3(3.1)	3.1(2.4)	2.7(2.1)
Vegetable fat (% of total energy)	13.8 (5.3)	13.8 (4.3)	12.5 (4.1)	14.9(3.8)	14.5(3.3)	13.7(3.5)	14.7(4.6)	14.2(3.8)	13.2(3.9)
Polyunsaturated to saturated fat ratio	0.74(0.3)	0.56(0.2)	0.42(0.1)	0.68(0.16)	0.57(0.11)	0.45(0.10)	0.62(0.20)	0.51(0.13)	0.40(0.11)
Trans fat (% of total energy)	1.1(0.6)	1.3(0.5)	1.4(0.5)	1.6(0.5)	1.6(0.5)	1.7(0.4)	1.6(0.7)	1.6(0.6)	1.6(0.6)
Fruit and vegetables (servings/d)	6.0(3.3)	5.5(2.7)	4.7(2.4)	5.5(2.2)	5.3(1.9)	4.7(1.9)	5.3(3.3)	5.2(2.8)	4.6(2.6)
Red and processed meat intake (servings/d)	1.0(0.9)	1.2(0.8)	1.2(0.8)	0.93(0.56)	0.95(0.50)	0.98(0.54)	1.2(0.8)	1.2(0.7)	1.0(0.7)
Nut intake (servings/d)	0.5(0.7)	0.5(0.6)	0.4(0.6)	0.1(0.2)	0.1(0.2)	0.1(0.2)	0.1(0.3)	0.1(0.2)	0.1(0.2)
SSB intake (servings/d)	0.4(0.7)	0.4(0.6)	0.3(0.6)	1.1(0.9)	1.1(0.8)	1.0(0.80)	0.7(1.2)	0.4(0.8)	0.3(0.6)

1 Median; interquartile range in parentheses (all such values)

2 Mean SD (all such values)

3 Current menopausal hormone users among postmenopausal women.

Table 1.2 HRs (95% CI) of type-2 diabetes risk according to quintiles of dairy fat in HPFS, NHS, and NHSII*

	Quintile of dairy fat consumption						
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for 5% of energy
HPFS							
Daily intake (% of energy)§	2.9	4.2	5.3	6.7	9.2		
Cases/person-years	609/158,691	677/158,611	666/158,519	695/158,551	753/158,347		
Age-adjusted[1]	1.00	1.13 (1.02, 1.27)	1.13 (1.01, 1.26)	1.18 (1.06, 1.31)	1.26 (1.13, 1.40)	<0.0001	1.13 (1.06, 1.19)
Adjusted for lifestyle variables[2]	1.00	1.10 (0.98, 1.22)	1.05 (0.94, 1.18)	1.06 (0.95, 1.19)	1.12 (1.00, 1.24)	0.12	1.04 (0.98, 1.11)
Adjusted for dietary factors[3]	1.00	1.08 (0.97, 1.21)	1.04 (0.93, 1.16)	1.05 (0.93, 1.17)	1.09 (0.98, 1.23)	0.25	1.04 (0.97, 1.11)
NHS							
Daily intake (% of energy)§	3.7	5.0	6.0	7.3	9.7		
Cases/person-years	1,355/315,406	1,429/315,189	1,353/315,307	1,444/315,299	1,421/315,332		
Age-adjusted[1]	1.00	1.06 (0.98, 1.14)	1.01 (0.93, 1.09)	1.08 (1.00, 1.16)	1.05 (0.98, 1.14)	0.17	1.03 (0.99, 1.08)
Adjusted for lifestyle variables[2]	1.00	1.01 (0.94, 1.09)	0.94 (0.87, 1.01)	0.98 (0.91, 1.06)	0.96 (0.89, 1.04)	0.28	0.98 (0.93, 1.03)
Adjusted for dietary factors[3]	1.00	1.00 (0.93, 1.08)	0.92 (0.86, 1.00)	0.97 (0.90, 1.04)	0.96 (0.88, 1.03)	0.21	0.98 (0.93, 1.03)
NHSII							
Daily intake (% of energy)§	3.5	4.8	5.9	7.2	9.5		
Cases/person-years	1,377 /361,419	1,225/362,361	1,119/362,403	1,129/361,774	1,014/361,211		
Age-adjusted[1]	1.00	0.93 (0.86, 1.00)	0.93 (0.86, 1.00)	0.89 (0.82, 0.97)	0.82 (0.75, 0.89)	<0.0001	0.87 (0.83, 0.92)
Adjusted for lifestyle variables[2]	1.00	0.93 (0.86, 1.00)	0.96 (0.89, 1.04)	0.95 (0.88, 1.03)	0.89 (0.82, 0.97)	0.02	0.93 (0.88, 0.98)
Adjusted for dietary factors[3]	1.00	0.93 (0.86, 1.01)	0.97 (0.89, 1.05)	0.96 (0.88, 1.04)	0.91 (0.83, 0.98)	0.06	0.93 (0.88, 0.99)
Pooled analysis							
Age-adjusted[1]	1.00	1.02 (0.97, 1.07)†	1.00 (0.95, 1.05)†	1.02 (0.97, 1.07)†	1.00 (0.95, 1.05)†	0.93	1.00 (0.97, 1.03)†
Adjusted for lifestyle variables[2]	1.00	0.99 (0.95, 1.04)†	0.97 (0.92, 1.02)	0.99 (0.94, 1.03)	0.97 (0.92, 1.02)†	0.19	0.98 (0.95, 1.01)†
Adjusted for dietary factors[3]	1.00	0.99 (0.94, 1.04)	0.96 (0.92, 1.01)	0.98 (0.93, 1.03)	0.96 (0.91, 1.01)†	0.17	0.98 (0.94, 1.01)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

‡ *P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

§ Median values

1 Model adjusted for age (continuous)

2 Model was additionally adjusted for BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS I and II participants only), oral contraceptive use (NHS II participants only), baseline hypertension, baseline hypercholesterolemia, and family history of diabetes

3 Model was additionally adjusted for AHEI and dietary intakes of protein, vegetable fat, and animal fat without dairy fat

†*P* for heterogeneity <0.05

Table 1.3 HRs (95% CIs) of type 2 diabetes risk according to dairy fat intake stratified by age, sex, BMI, physical activities, Smoking status, Hypertension, and High cholesterol based on pooled data from HPFS, NHS, and NHSII

		Quintile of dairy fat consumption							
		Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend[1]	HR (95% CI) for 5% of energy	*P*-interaction[2]
Age	Cases								0.33
< 65 yrs	11,368	1.00	0.95 (0.90, 1.01)	0.95 (0.90, 1.01)	0.93 (0.88, 0.99)	0.93 (0.87, 0.98)	0.02	0.95 (0.92, 0.99)	
≥ 65 yrs	4,853	1.00	1.03 (0.94, 1.13)	0.95 (0.86, 1.04)	1.01 (0.92, 1.11)	0.99 (0.90, 1.08)	0.69	0.99 (0.93, 1.05)	
Sex									0.23
Male	3,400	1.00	1.08 (0.96, 1.20)	1.04 (0.93, 1.16)	1.04 (0.93, 1.16)	1.08 (0.96, 1.21)	0.37	1.03 (0.96, 1.10)	
Female	12,821	1.00	0.95 (0.90, 1.00)	0.93 (0.88, 0.98)	0.93 (0.88, 0.99)	0.91 (0.86, 0.96)	0.002	0.94 (0.91, 0.98)	
BMI									0.68
<25 kg/m^2	2,327	1.00	0.96 (0.85, 1.09)	0.94 (0.83, 1.07)	0.99 (0.87, 1.12)	0.90 (0.79, 1.02)	0.17	0.93 (0.85, 1.01)	
25-29.9 kg/m^2	4,693	1.00	0.97 (0.89, 1.06)	0.94 (0.86, 1.03)	0.94 (0.86, 1.04)	1.03 (0.94, 1.13)	0.53	1.02 (0.96, 1.08)	
≥30 kg/m^2	9,201	1.00	1.00 (0.93, 1.06)	0.98 (0.92, 1.05)	0.98 (0.92, 1.05)	0.96 (0.90, 1.03)	0.23	0.98 (0.93, 1.02)	
Physical Activity									0.27
< median level	9,529	1.00	1.00 (0.93, 1.06)	0.97 (0.91, 1.04)	0.95 (0.89, 1.01)	0.96 (0.90, 1.02)	0.14	0.96 (0.92, 1.00)	
≥ median level	5,295	1.00	0.95 (0.87, 1.03)	0.92 (0.84, 1.00)	0.94 (0.86, 1.03)	0.89 (0.82, 0.98)	0.02	0.95 (0.89, 1.01)	
Smoking status									0.35
Current	1,594	1.00	1.04 (0.88, 1.22)	0.92 (0.78, 1.08)	0.92 (0.78, 1.08)	0.82 (0.70, 0.96)	0.003	0.88 (0.80, 0.96)	
Other	14,627	1.00	0.97 (0.92, 1.02)	0.95 (0.90, 1.00)	0.95 (0.90, 1.00)	0.96 (0.91, 1.01)	0.13	0.97 (0.94, 1.01)	
Hypertension,									0.23
No	9,314	1.00	0.94 (0.88, 1.00)	0.94 (0.88, 1.00)	0.95 (0.89, 1.02)	0.93 (0.87, 1.00)	0.12	0.97 (0.93, 1.02)	
Yes	6,907	1.00	1.03 (0.96, 1.11)	0.97 (0.90, 1.04)	0.95 (0.88, 1.02)	0.95 (0.88, 1.03)	0.06	0.94 (0.90, 0.99)	
High Cholesterol									0.10
No	9,651	1.00	0.99 (0.93, 1.05)	0.99 (0.93, 1.06)	0.99 (0.93, 1.06)	0.97 (0.91, 1.04)	0.44	0.98 (0.94, 1.02)	
Yes	6,750	1.00	0.96 (0.89, 1.03)	0.90 (0.83, 0.97)	0.89 (0.83, 0.97)	0.89 (0.83, 0.97)	0.002	0.92 (0.88, 0.97)	

Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), menopausal status and menopausal hormone use, oral contraceptive use, baseline hypertension, baseline hypercholesterolemia, family history of diabetes, AHEI, and dietary intakes of protein, vegetable fat, and animal fat without dairy fat

1. *P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable
2. Interaction p-values were calculated using a categorical dairy fat variable (quintiles) by comparing the models with and without interaction terms using log-likelihood ratio test

Table 1.4 RRs (95% CI) of Type-2 diabetes associated with isocaloric substitutions of selected nutrients for dairy fat in HPFS, NHS, and NHS II

	Substitution for 5% calories of dairy fat					
	Other Animal fat[1]	Vegetable fat[1]	Polyunsaturated fat[1]	Total carbohydrates[2]	Carbohydrates from whole grains[2]	Carbohydrates from refined grains[2]
HPFS						
Multivariate model	**1.12 (1.04, 1.20)**	0.94 (0.88, 1.02)	0.97 (0.84, 1.11)	**0.91 (0.85, 0.99)**	**0.86 (0.78, 0.95)**	0.97 (0.90, 1.05)
NHS						
Multivariate model	**1.12 (1.06, 1.19)**	0.97 (0.91, 1.02)	1.00 (0.89, 1.12)	1.00 (0.95, 1.05)	0.94 (0.87, 1.02)	**1.06 (1.00, 1.13)**
NHS II						
Multivariate model	**1.19 (1.12, 1.26)**	1.05 (0.99, 1.12)	1.07 (0.95, 1.21)	1.02 (0.95, 1.09)	0.95 (0.88, 1.04)	**1.06 (1.00, 1.12)**
Pooled analysis						
Multivariate model	**1.14 (1.10, 1.19)**	0.99 (0.95, 1.03)	1.01 (0.94, 1.09)	0.99 (0.95, 1.02)	**0.93 (0.88, 0.97)**	**1.04 (1.00, 1.07)**
P for heterogeneity	*0.29*	*0.06*	*0.53*	*0.08*	*0.27*	*0.09*

Table 1.4 - Continued

	Substitution for 1% of calories of dairy fat			Substitution for 0.3% of calories of dairy fat	
	Omega-6 fatty acids[1]	Linoleic Acid[1]	MUFA from plant sources[1]	Omega-3 fatty acids[1]	Alpha-Linolenic acid[1]
HPFS					
Multivariate model	0.97 (0.94, 1.01)	0.97 (0.94, 1.01)	0.98 (0.96, 1.00)	1.06 (1.00, 1.12)	1.08 (0.97, 1.21)
NHS					
Multivariate model	0.99 (0.96, 1.02)	1.00 (0.97, 1.03)	**1.03 (1.01, 1.05)**	1.03 (0.98, 1.09)	0.99 (0.91, 1.08)
NHS II					
Multivariate model	1.02 (0.99, 1.06)	1.02 (0.99, 1.06)	1.01 (0.99, 1.03)	0.98 (0.91, 1.05)	**0.90 (0.82, 0.99)**
Pooled analysis					
Multivariate model	0.99 (0.98, 1.01)	1.00 (0.98, 1.02)	1.01 (1.00, 1.02)	1.03 (0.99, 1.06)	0.98 (0.93, 1.04)
P for heterogeneity	*0.12*	*0.15*	*0.002*	*0.19*	*0.04*

1. Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS and NHSII participants only), oral contraceptive use (NHSII participants only), baseline hypertension, baseline hypercholesterolemia, family history of diabetes, and dietary intake of *trans* fat.
2. Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS I and II participants only), oral contraceptive use (NHS II participants only), baseline hypertension, baseline hypercholesterolemia, family history of diabetes, and adjusted for dietary intakes of vegetable fat, *trans* fat, and animal fat without dairy fat

from plant sources, total omega-3 fatty acids, or alpha-linolenic acid was also not associated with T2D risk ($P > 0.05$).

In sensitivity analyses, we examined the associations between dairy fat and T2D risk using several models (**Table S1.1**) or in substitution analyses (**Tables S1.2A and S1.2B**). Overall, the associations between dairy fat intake and T2D risk did not change substantially in these sensitivity analyses, except that the association for replacement of dairy fat with refined grains was attenuated when using the most recent [RR: 1.00; 95% CI: (0.98, 1.03)], average of two proceeding diet cycles [RR: 1.00; 95% CI: (0.97, 1.03)], or after censoring participants when they developed an intermediate outcome [RR: 1.03; 95% CI: (0.99, 1.07)]. Associations did not change materially after introducing 0-4, 4-8, 8-12 or 12-16-year lags (**Tables S1.3 and S1.4)**.

DISCUSSION

In the current analysis of three prospective cohorts of U.S. men and women intake of dairy fat was not significantly associated with T2D risk compared with energy from carbohydrates. However, replacing calories from dairy fat with equivalent calories from animal fat from other sources or carbohydrates from refined grains was significantly associated with a higher T2D risk, whereas replacing dairy fat with carbohydrates from whole grains was significantly associated with a lower T2D risk. These observations largely persisted among individuals with various T2D risk profiles or when different analytic approaches were used.

To our knowledge, this is the first study that prospectively examined the association between dairy fat intake and risk of T2D and modeled the substitution effects between dairy fat and other macronutrient sources. A recent meta-analysis of 22 cohorts reported a modest inverse association for total dairy [RR: 0.97; 95% CI: 0.95, 1.00 (P = 0.04) per 200-g/d increment], a non-significant inverse association for low-fat dairy products [RR: 0.96; 95% CI: 0.92, 1.00 (P = 0.07)], and no association for high-fat dairy products with T2D risk.[8] A meta-analysis of four cohort studies reported an inverse association between butter intake and T2D risk [RR of 0.96; 95%CI: 0.93-0.99 per 14g/day increment].[31] It is difficult to extrapolate these findings to estimate the health effects of dairy fat intake. Other nutrients in dairy products, such as calcium and vitamin D, may also determine the associations with T2D, because they may enhance insulin secretion, reduce systemic inflammation, and aid in the regulation of body weight.[32,33] In addition, dairy may be used as ingredients in many food products, and thus associations with specific high-fat dairy products may not reflect the effects of total dairy fat intake.

In the current analysis, overall dairy fat was not significantly associated with T2D risk when replacing calories from total carbohydrates. However, replacing dairy fat with different carbohydrate sources showed divergent associations. The findings that replacing dairy fat with refined carbohydrates was associated with a higher risk of T2D deserve discussion. Refined carbohydrate intake may lead to elevated risk of developing T2D through several pathways such as increased insulin resistance and the loss of β-cell function stemming from exhaustion or cell toxicity.[34,35] Furthermore, intake of refined carbohydrates may result in elevated free fatty acid levels which may impair β-cell function. Randomized controlled trial evidence shows that

substituting SFA with refined carbohydrates resulted in similar plasma insulin and glucose levels.[37] Our findings may be explained by the fact that the main contributors of dairy fat (cheese, pizza, dairy desserts, milk, and butter) only account for 30% of total saturated fat intake in the US according to NHANES data. Although dairy fat is high in saturated fat, it also contains other fatty acids with various health effects.[38] For example, in addition to SFAs, dairy fat contains 25-30% of MUFAs, 2-3% PUFAs, 2% TFAs, and about 0.5% conjugated linoleic acid (CLA) from ruminant fermentation.[39–42] Thus, replacing dairy fat with refined carbohydrates may not be equivalent replacing SFA's with other sources of energy. Of note, intakes of refined carbohydrate and SFA can promote insulin resistance through impairing insulin signaling pathways and promoting inflammation.[43,44] Thus, these two sources of energy may have similar effects on T2D risk. In contrast, replacing dairy fat with carbohydrates from whole grains was associated with a lower T2D risk. In controlled feeding trial, intake of whole grains improves glucose tolerance and peripheral insulin sensitivity compared with intake of refined grains.[45–47] In cross-sectional analyses, greater intake of whole grains was associated with lower fasting glucose and insulin.[48] These health effects may be explained by the physical properties of the grains (composition, particle size and type of fiber) that enhance satiety and slow absorption of simple carbohydrates.[49] In addition, beneficial phytochemicals in whole grains, such as lignans and alkylresorcinols, [50] as well as the production of short-chain fatty acids in the colon,[51] may also underlie the health benefits of consuming whole grains.

Replacing dairy fat with other fats may help to meet the dietary guidelines that recommend reducing SFA intake within 10% of total energy. In the current analysis, substituting

5% of calories from dairy fat with the equivalent calories from other animal fats was positively associated with T2D risk, which was expected given the positive association between animal fat intake and T2D risk.[52–54] The SFA contents of other animal fats (e.g., lard or tallow) are in the range of 40-50%,[55] which is lower than the SFA content in dairy fat, although the SFA composition differs between dairy fat and other animal fat. Other animal fat sources contain almost exclusively C14:0 – 18:0 which makes their contents in both sources comparable. However about 10% of SFA in dairy fat are short and medium chain SFA (C4:0 – 10:0) which are absent in other animal fat.[42] These SFAs may have different health effects. For example, positive associations for C12:0-18:0 and null associations for C4:0 – 10:0 have been demonstrated with CVD outcomes.[6] In a feeding trial, intake of C6:0-10:0 together with dairy proteins for 12 weeks did not increase levels of inflammatory markers.[56] However, it is unclear if the effects are due to fatty acid or dairy protein intake. Thus, more evidence is needed to further substantiate the associations between individual SFAs with different chain length and metabolic health outcomes.

The isocaloric replacement of dairy fat with MUFAs from different food sources was not associated with T2D risk. In prospective cohort studies, total MUFA intake was not associated with T2D risk.[57] The primary MUFA in the overall diet is oleic acid (C18:1), which on average accounts for 35% of total fat in a typical American diet in data from NHANES.[58] Oleic acid intake has been associated with increased fat oxidation, energy expenditure, glycemic response regulation and insulin sensitivity.[59] Results from 30 feeding trials showed that substituting MUFAs for SFAs resulted in decreases in fasting glucose, HOMA-IR, and Hemoglobin A1c.[60] Of note, oleic acid is also the primary MUFA in milk fat, accounting for 25% of total dairy fat. It is likely that

replacing the same MUFA between dairy fat and other sources may not lead to significant change in diabetes risk. Surprisingly, replacing calories from dairy fat for calories from PUFAs was not significantly associated with T2D risk in the current analysis. This finding was unexpected given the reported inverse associations between self-reported PUFA intake, [52,54] as well as biomarkers of omega-6 fatty acids, and T2D risk.[61] In addition, dairy fat only contains 2-3% PUFAs and therefore is not a significant source of total PUFA intake. In feeding trials, replacing 2% of SFAs with PUFAs decreased plasma glucose, hemoglobin A1c, and HOMA-IR.[60] Thus, this finding in our analysis warrants replications in future studies.

The validity of dietary intake assessment is critical in nutritional epidemiology, and biomarkers can complement the assessment of intake by questionnaires. Our findings stand in contrast with the inverse associations reported for blood levels of fatty acids found in dairy fat, such as pentadecanoic (C15:0), heptadecanoic (C17:0) ,and *trans*-palmitoleic acids (*trans* 16:1 n-7), and T2D risk.[9–15] These fatty acids have been considered biomarkers of dairy intake because their concentrations in plasma and erythrocyte are positively associated with dairy intake in a dose-response fashion in observational studies[14,22,62] and in feeding trials.[63,64] However, the correlations between these fatty acids and dairy fat intake were in general weak-to-modest. For example, in the NHS and HPFS, we observed correlation coefficients of 0.29 for C15:0, 0.21 for C17:0, and 0.22 for *trans* 16:1 n-7 when dairy fat was assessed using FFQs.[15] In a more recent analysis in a subset of NHS participants, an even lower correlation (0.05) was reported between dietary record assessments of 17:0 and plasma concentrations).[65] In addition, dairy fat is not the only source of these fatty acids. For example, recent evidence has suggested that fish also

contains odd-chain fatty acids.[16,66] Furthermore, plasma concentrations of C15:0 and C17:0 have been detected in a study among vegan women, suggesting that these fatty acids can be derived from other potential sources other than dairy or animal products.[67] Gut microbiota processing of soluble fiber[68] and endogenous elongation[69] may explain the production of 17:0 from a vegan diet. Thus, more sensitive and specific markers of dairy fat are needed to facilitate the examination of associations between dairy fat intake and cardiometabolic diseases.

The main strengths of our study include large sample size, the high rate of follow-up in our cohorts, and the repeated assessments of dietary and lifestyle variables. However, the study also has some limitations to acknowledge. Our participants are exclusively health professionals and 95% of them are of European ancestry. Although the participants' educational attainment, interests in health, and relatively homogeneous socioeconomic status may aid in the collection of high-quality data through self-administered questionnaires and help alleviate confounding by socioeconomic status, such homogeneity may also limit the generalizability of study findings to other populations with different characteristics. There is inevitable measurement error in the intake assessment of dairy fat and other nutrients. The FFQ has been extensively validated against dietary records, and the assessments of dairy products have been demonstrated to be quite accurate. In addition, we used repeated measurements to reduce random measurement errors and represent long-term diets. Given the prospective nature of the current analysis, such measurement errors were independent of disease ascertainment and therefore are more likely to attenuate the true association toward the null. Finally, due to the observational nature of the current analysis, we cannot exclude the possibility that residual or unmeasured confounding

accounts for some of the associations, although we controlled an array of established and potential risk factors of T2D.

CONCLUSION

In this prospective investigation among US men and women, intake of dairy fat was not associated with T2D risk, compared with calories from carbohydrates. In substitution analyses replacing dairy fat with other animal fats or carbohydrates from refined grains was associated with higher T2D risk, whereas replacement by calories from whole grains was inversely associated with T2D risk. These results support add further evidence that the source and type of macronutrients is important for metabolic health and prevention of type 2 diabetes.

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Table S1.1 – Sensitivity analysis for type-2 diabetes risk according to quintiles of dairy fat intake in HPFS, NHS, and NHS II using different methods of updating diets

	Quintile of dairy fat consumption						HR (95% CI) for 5% of energy
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	
HPFS							
Cumulative average + stop update[1]	1.00	1.08 (0.97, 1.21)	1.04 (0.93, 1.16)	1.05 (0.93, 1.17)	1.09 (0.98, 1.23)	0.25	1.04 (0.97, 1.11)
Cumulative average + continuous update[2]	1.00	1.08 (0.97, 1.21)	1.04 (0.93, 1.16)	1.05 (0.94, 1.17)	1.10 (0.98, 1.23)	0.24	1.04 (0.97, 1.11)
Simple update[3]	1.00	1.08 (0.96, 1.21)	1.04 (0.93, 1.17)	1.05 (0.94, 1.18)	1.09 (0.98, 1.23)	0.25	1.04 (0.97, 1.11)
Average of two most recent cycles[4]	1.00	1.04 (0.93, 1.16)	1.04 (0.93, 1.16)	1.00 (0.89, 1.12)	1.06 (0.95, 1.19)	0.50	1.03 (0.97, 1.09)
Censor after intermediate outcomes[5]	1.00	1.08 (0.96, 1.21)	1.04 (0.93, 1.17)	1.05 (0.94, 1.18)	1.10 (0.98, 1.23)	0.22	1.04 (0.97, 1.11)
NHS							
Cumulative average + stop update[1]	1.00	1.00 (0.93, 1.08)	0.92 (0.86, 1.00)	0.97 (0.90, 1.04)	0.96 (0.88, 1.03)	0.21	0.98 (0.93, 1.03)
Cumulative average + continuous update[2]	1.00	0.99 (0.92, 1.07)	0.96 (0.89, 1.04)	0.95 (0.88, 1.02)	0.98 (0.91, 1.06)	0.50	0.98 (0.93, 1.04)
Simple update[3]	1.00	0.97 (0.89, 1.04)	1.01 (0.93, 1.09)	1.01 (0.93, 1.09)	1.08 (1.00, 1.17)	0.009	1.05 (1.01, 1.09)
Average of two most recent cycles[4]	1.00	0.97 (0.90, 1.05)	1.00 (0.93, 1.08)	0.99 (0.92, 1.07)	1.06 (0.98. 1.14)	0.07	1.05 (1.00, 1.09)
Censor after intermediate outcomes[5]	1.00	1.00 (0.92, 1.09)	0.92 (0.84, 1.00)	0.97 (0.89, 1.05)	0.98 (0.90, 1.07)	0.59	0.99 (0.94, 1.05)
NHS II							
Cumulative average + stop update[1]	1.00	0.93 (0.86, 1.01)	0.97 (0.89, 1.05)	0.96 (0.88, 1.04)	0.91 (0.83, 0.98)	0.06	0.93 (0.88, 0.99)
Cumulative average + continuous update[2]	1.00	0.95 (0.88, 1.03)	0.98 (0.90, 1.06)	0.96 (0.88, 1.04)	0.93 (0.86, 1.01)	0.15	0.94 (0.89, 1.00)
Simple update[3]	1.00	0.91 (0.83, 0.98)	0.96 (0.88, 1.04)	0.95 (0.88, 1.03)	0.92 (0.84, 1.00)	0.18	0.98 (0.94, 1.02)
Average of two most recent cycles[4]	1.00	0.99 (0.91, 1.08)	0.95 (0.87, 1.04)	1.01 (0.92, 1.10)	0.92 (0.84, 1.01)	0.11	0.97 (0.92, 1.02)
Censor after intermediate outcomes[5]	1.00	0.95 (0.88, 1.03)	0.96 (0.89, 1.05)	0.97 (0.89, 1.05)	0.92 (0.84, 1.01)	0.13	0.94 (0.89, 1.00)
Pooled analysis							
Cumulative average + stop update[1]	1.00	0.99 (0.94, 1.04)	0.96 (0.92, 1.01)	0.98 (0.93, 1.03)	0.96 (0.91, 1.01)†	0.17	0.98 (0.94, 1.01)
Cumulative average + continuous update[2]	1.00	0.99 (0.94, 1.04)	0.98 (0.94, 1.03)	0.97 (0.92, 1.02)	0.98 (0.93, 1.04)	0.46	0.98 (0.95, 1.01)
Simple update[3]	1.00	0.96 (0.92, 1.01)†	0.99 (0.95, 1.04)	0.99 (0.94, 1.04)	1.02 (0.97, 1.07)†	0.17	1.02 (0.99, 1.04)†
Average of two most recent cycles[4]	1.00	1.00 (0.95, 1.05)	1.00 (0.95, 1.06)	1.01 (0.96, 1.06)	1.03 (0.97. 1.08)†	0.19	1.03 (0.99, 1.05)†
Censor after intermediate outcomes[5]	1.00	1.00 (0.95, 1.05)	0.96 (0.91, 1.01)	0.98 (0.93, 1.04)	0.98 (0.93, 1.04)	0.50	0.98 (0.95, 1.02)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study. ‡ *P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

1 Main model: diet no longer updated at diagnosis of intermediate outcome (heart disease, stroke, cancer, and coronary artery surgery)

2 Continuous update: cumulative averages of dairy fat intake continually updated throughout duration of follow-up

3 Dairy fat intake represents the most recently reported intake

4 Dairy fat intake represents the average intake of the two most recent reports

5 Participants censored after diagnosis of intermediate outcome (heart disease, stroke, cancer, and coronary artery surgery)

All models were adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), menopausal status and menopausal hormone use, oral contraceptive use, baseline hypertension, baseline hypercholesterolemia, family history of diabetes, AHEI, and dietary intakes of protein, vegetable fat, and animal fat without dairy fat

Table S1.2A Sensitivity analysis RRs (95% CI) of Type-2 diabetes associated with isocaloric substitutions (5% of energy) of selected nutrients for dairy fat in HPFS, NHS, and NHS II using different methods of updating diets

	RR (95% CI) of T2D substitution 5% of calories for dairy fat					
	Other Animal fat±	Vegetable fat±	Polyunsaturated fat±	Total carbohydrates¥	Carbohydrates from whole grains¥	Carbohydrates from refined grains¥
HPFS						
Cumulative average + stop update[1]	**1.12 (1.04, 1.20)**	0.94 (0.88, 1.02)	0.97 (0.84, 1.11)	**0.91 (0.85, 0.99)**	**0.86 (0.78, 0.95)**	0.97 (0.90, 1.05)
Cumulative average + continuous update[2]	**1.14 (1.05, 1.22)**	0.96 (0.89, 1.03)	0.99 (0.86, 1.13)	**0.92 (0.85, 1.00)**	**0.87 (0.79, 0.96)**	0.98 (0.91, 1.06)
Simple update[3]	**1.11 (1.04, 1.18)**	0.98 (0.93, 1.05)	1.05 (0.94, 1.17)	0.94 (0.88, 1.00)	**0.89 (0.82, 0.96)**	1.01 (0.94, 1.07)
Average of two most recent cycles[4]	**1.11 (1.04, 1.19)**	0.96 (0.90, 1.03)	0.99 (0.88, 1.12)	**0.92 (0.86, 0.99)**	**0.89 (0.82, 0.96)**	0.97 (0.91, 1.04)
Censor after intermediate outcomes[5]	**1.09 (1.01, 1.18**)	0.93 (0.86, 1.00)	0.92 (0.80, 1.06)	**0.90 (0.83, 0.97)**	**0.86 (0.78, 0.95)**	0.95 (0.87, 1.03)
NHS						
Cumulative average + stop update[1]	**1.12 (1.06, 1.19)**	0.97 (0.91, 1.02)	1.00 (0.89, 1.12)	1.00 (0.95, 1.05)	0.94 (0.87, 1.02)	**1.06 (1.00, 1.13)**
Cumulative average + continuous update[2]	**1.20 (1.13, 1.28)**	0.98 (0.92, 1.04)	1.10 (0.97, 1.24)	1.00 (0.95, 1.05)	1.01 (0.94, 1.10)	**1.09 (1.02, 1.16)**
Simple update[3]	**1.06 (1.01, 1.10)**	0.96 (0.92, 1.00)	1.05 (0.98, 1.14)	**0.94 (0.91, 0.98)**	0.96 (0.92, 1.01)	0.98 (0.94, 1.02)
Average of two most recent cycles[4]	1.05 (1.00, 1.10)	0.96 (0.92, 1.01)	**1.10 (1.00, 1.20)**	**0.94 (0.90, 0.98)**	0.96 (0.90, 1.01)	0.97 (0.93, 1.02)
Censor after intermediate outcomes[5]	**1.15 (1.08, 1.23)**	0.95 (0.89, 1.01)	0.98 (0.86, 1.11)	1.00 (0.94, 1.05)	0.99 (0.91, 1.08)	1.05 (0.98, 1.13)
NHS II						
Cumulative average + stop update[1]	**1.19 (1.12, 1.26)**	1.05 (0.99, 1.12)	1.07 (0.95, 1.21)	1.02 (0.95, 1.09)	0.95 (0.88, 1.04)	**1.06 (1.00, 1.12)**
Cumulative average + continuous update[2]	**1.18 (1.11, 1.26)**	1.04 (0.87, 1.12)	1.07 (0.94, 1.20)	1.01 (0.94, 1.09)	0.95 (0.87, 1.04)	1.05 (0.99, 1.11)
Simple update[3]	**1.11 (1.06, 1.16)**	1.00 (0.96, 1.05)	0.99 (0.92, 1.08)	1.00 (0.95, 1.06)	0.95 (0.89, 1.01)	1.01 (0.96, 1.05)
Average of two most recent cycles[4]	**1.13 (1.07, 1.20)**	1.03 (0.97, 1.09)	1.02 (0.92, 1.14)	1.01 (0.96, 1.06)	0.96 (0.90, 1.01)	1.02 (0.98. 1.07)
Censor after intermediate outcomes[5]	**1.18 (1.11, 1.26)**	1.06 (0.99. 1.13)	1.10 (0.97, 1.24)	1.00 (0.93, 1.08)	0.96 (0.88, 1.05)	1.05 (1.00, 1.12)
Pooled analysis						
Cumulative average + stop update[1]	**1.14 (1.10, 1.19)**	0.99 (0.95, 1.03)	1.01 (0.94, 1.09)	0.99 (0.95, 1.02)	**0.93 (0.88, 0.97)**	**1.04 (1.00, 1.07)**
Cumulative average + continuous update[2]	**1.18 (1.13, 1.22)**	0.99 (0.96, 1,03)	1.06 (0.98, 1.13)	0.98 (0.95, 1.02)	0.95 (0.91, 1.00)	**1.05 (1.01, 1.09)**
Simple update[3]	**1.08 (1.06, 1.12)**	0.98 (0.95, 1.01)	1.03 (0.98, 1.08)	**0.96 (0.93, 0.99)**	**0.94 (0.91, 0.98)**	1.00 (0.98, 1.03)
Average of two most recent cycles[4]	**1.08 (1.05, 1.12)**	0.98 (0.95, 1.02)	1.05 (0.99, 1.12)	**0.95 (0.92, 0.98)**	**0.94 (0.90, 0.98)**	1.00 (0.97, 1.03)
Censor after intermediate outcomes[5]	**1.15 (1.10, 1.19)**	0.98 (0.94, 1.02)†	1.00 (0.93, 1.08)	0.97 (0.93, 1.01)	**0.94 (0.89, 0.99)**	1.03 (0.99, 1.07)

± Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS and NHSII participants only), oral contraceptive use (NHSII participants only), baseline hypertension, baseline hypercholesterolemia, family history of diabetes, and dietary intake of *trans* fat

¥ Model additionally adjusted for dietary intakes of vegetable fat, *trans* fat, and animal fat without dairy fat

1 Diet no longer updated at diagnosis of intermediate outcome (heart disease, stroke, cancer, and coronary artery surgery)

2 Continuous update: cumulative averages of dairy fat intake continually updated throughout duration of follow-up

3 Dairy fat intake represents the most recently reported intake

4 Dairy fat intake represents the average intake of the two most recent reports

sored after diagnosis of intermediate outcome (heart disease, stroke, cancer, and coronary artery surgery)

Table S1.2B Sensitivity analysis RRs (95% CI) of Type-2 diabetes associated with isocaloric substitutions of selected nutrients for dairy fat in HPFS, NHS, and NHS II using different methods of updating diets

	Substitution of 1% of calories for dairy fat			Substitution of 0.3% of calories for dairy fat	
	Omega-6 fatty acids±	Linoleic Acid±	MUFA from plant sources±	Omega-3 fatty acids±	Alpha-Linolenic Acid±
HPFS					
Cumulative average + stop update[1]	0.97 (0.94, 1.01)	0.97 (0.94, 1.01)	0.98 (0.96, 1.00)	1.06 (1.00, 1.12)	1.08 (0.97, 1.21)
Cumulative average + continuous update[2]	0.98 (0.95, 1.01)	0.98 (0.95, 1.02)	0.99 (0.96, 1.01)	**1.07 (1.01, 1.13)**	1.09 (0.97, 1.21)
Simple update[3]	1.00 (0.98, 1.03)	1.00 (0.97, 1.03)	1.00 (0.98, 1.01)	1.03 (0.99, 1.07)	**1.09 (1.02. 1.16)**
Average of two most recent cycles[4]	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)	0.99 (0.97, 1.01)	1.03 (0.98, 1.08)	1.06 (0.97, 1.16)
Censor after intermediate outcomes[5]	0.97 (0.92, 1.00)	0.97 (0.94, 1.00)	0.98 (0.96, 1.01)	1.05 (0.99, 1.12)	1.05 (0.93, 1.18)
NHS					
Cumulative average + stop update[1]	1.00 (0.97, 1.02)	1.00 (0.98, 1.03)	1.03 (1.01, 1.05)	1.03 (0.97, 1.09)	0.99 (0.91, 1.07)
Cumulative average + continuous update[2]	**1.03 (1.00, 1.07)**	**1.03 (1.00, 1.07)**	1.01 (0.99, 1.03)	0.99 (0.93, 1.05)	0.93 (0.85, 1.02)
Simple update[3]	1.00 (0.98, 1.02)	1.01 (0.99, 1.03)	1.00 (0.99, 1.02)	1.03 (1.00, 1.07)	1.01 (0.96, 1.07)
Average of two most recent cycles[4]	1.02 (0.99, 1.04)	1.02 (1.00, 1.05)	1.01 (0.99, 1.02)	1.03 (0.98, 1.07)	1.00 (0.93, 1.07)
Censor after intermediate outcomes[5]	0.99 (0.96, 1.03)	1.00 (0.97, 1.03)	1.02 (1.00, 1.04)	1.03 (0.97, 1.09)	0.95 (0.86, 1.05)
NHS II					
Cumulative average + stop update[1]	1.02 (0.99, 1.06)	1.02 (0.99, 1.06)	1.01 (0.99, 1.03)	0.98 (0.91, 1.05)	**0.90 (0.82, 0.99)**
Cumulative average + continuous update[2]	1.02 (0.99, 1.06)	1.02 (0.98, 1.06)	1.01 (0.99, 1.03)	0.98 (0.91, 1.04)	**0.90 (0.80, 0.97)**
Simple update[3]	1.01 (0.98, 1.03)	1.01 (0.98, 1.03)	1.00 (0.99, 1.01)	0.99 (0.95, 1.02)	0.95 (0.91, 1.00)
Average of two most recent cycles[4]	1.02 (0.99, 1.05)	1.02 (0.99, 1.05)	1.01 (0.99, 1.02)	0.96 (0.91, 1.02)	**0.91 (0.84, 0.98)**
Censor after intermediate outcomes[5]	1.03 (0.99, 1.07)	1.03 (0.99, 1.07)	1.01 (0.99, 1.03)	0.97 (0.91, 1.04)	**0.90 (0.81, 0.99)**
Pooled analysis					
Cumulative average + stop update[1]	0.99 (0.98, 1.01)	1.00 (0.98, 1.02)	1.01 (1.00, 1.02)	1.03 (0.99, 1.06)	0.98 (0.93, 1.04)
Cumulative average + continuous update[2]	1.01 (0.99, 1.03)†	1.01 (0.99, 1.03)	1.00 (0.99, 1.02)	1.01 (0.98, 1.05)	0.95 (0.90, 1.01)†
Simple update[3]	1.00 (0.99, 1.01)	1.00 (0.99, 1.02)	1.00 (0.99, 1.01)	1.01 (0.99, 1.04)	1.01 (0.97, 1.04)†
Average of two most recent cycles[4]	1.01 (1.00, 1.03)	1.01 (1.00, 1.03)	1.00 (0.99, 1.01)	1.01 (0.98, 1.04)	0.98 (0.94, 1.02)†
Censor after intermediate outcomes[5]	0.99 (0.98, 1.01)†	0.99 (0.97, 1.01)†	1.01 (1.00, 1.02)†	1.02 (0.99, 1.06)	0.96 (0.90, 1.02)

± Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS and NHSII participants only), oral contraceptive use (NHSII participants only), baseline hypertension, baseline hypercholesterolemia, family history of diabetes, and dietary intake of *trans* fat

1 Diet no longer updated at diagnosis of intermediate outcome (heart disease, stroke, cancer, and coronary artery surgery)

2 Continuous update: cumulative averages of dairy fat intake continually updated throughout duration of follow-up

3 Dairy fat intake represents the most recently reported intake

4 Dairy fat intake represents the average intake of the two most recent reports

5 Participants censored after diagnosis of intermediate outcome (heart disease, stroke, cancer, and coronary artery surgery)

Table S1.3 HRs (95% CI) of type-2 diabetes risk according to quintile of dairy fat in HPFS, NHS, and NHS II* assessed by 0-4-year, 4-8-year, 8-12-year, and 12-16--year lags

		Quintile of dairy fat consumption						
	Cases	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for 5% of energy
HPFS								
Main model	3,400	1.00	1.08 (0.97, 1.21)	1.04 (0.93, 1.16)	1.05 (0.93, 1.17)	1.09 (0.98, 1.23)	0.25	1.04 (0.97, 1.11)
0-4 yr. lag[1]	3,400	1.00	1.08 (0.96, 1.21)	1.04 (0.93, 1.17)	1.05 (0.94, 1.18)	1.09 (0.98, 1.23)	0.25	1.04 (0.97, 1.11)
4-8 yr. lag[1]	3,023	1.00	1.08 (0.96, 1.21)	1.02 (0.91, 1.15)	1.03 (0.91, 1.16)	1.06 (0.94, 1.20)	0.56	1.04 (0.98, 1.10)
8-12 yr. lag[1]	2,389	1.00	1.08 (0.95, 1.23)	1.03 (0.90, 1.17)	0.96 (0.84, 1.10)	0.97 (0.85, 1.11)	0.22	1.01 (0.95, 1.08)
12-16 yr. lag[1]	1,681	1.00	1.07 (0.92, 1.25)	1.09 (0.94, 1.28)	0.98 (0.83, 1.15)	1.05 (0.89, 1.23)	0.99	1.03 (0.95, 1.12)
NHS								
Main model	7,002	1.00	1.00 (0.93, 1.08)	0.92 (0.86, 1.00)	0.97 (0.90, 1.04)	0.96 (0.88, 1.03)	0.21	0.98 (0.93, 1.03)
0-4 yr. lag[1]	7,002	1.00	0.97 (0.89, 1.04)	1.01 (0.93, 1.09)	1.01 (0.93, 1.09)	1.08 (1.00, 1.17)	0.009	1.05 (1.01, 1.09)
4-8 yr. lag[1]	6,091	1.00	0.98 (0.90, 1.06)	1.00 (0.92, 1.09)	0.99 (0.92, 1.08)	1.07 (0.98, 1.16)	0.06	1.05 (1.00, 1.09)
8-12 yr. lag[1]	4,762	1.00	0.99 (0.90, 1.09)	1.03 (0.94, 1.13)	0.98 (0.90, 1.08)	1.03 (0.94, 1.13)	0.59	1.02 (0.98, 1.07)
12-16 yr. lag[1]	3,929	1.00	1.01 (0.91, 1.12)	1.02 (0.92, 1.13)	0.98 (0.88, 1.08)	1.02 (0.92, 1.13)	0.93	1.00 (0.95, 1.05)
NHS II								
Main model	5,944	1.00	0.93 (0.86, 1.01)	0.97 (0.89, 1.05)	0.96 (0.88, 1.04)	0.91 (0.83, 0.98)	0.06	0.93 (0.88, 0.99)
0-4 yr. lag[1]	5,944	1.00	0.91 (0.83, 0.98)	0.96 (0.88, 1.04)	0.95 (0.88, 1.03)	0.92 (0.84, 1.00)	0.18	0.98 (0.94, 1.02)
4-8 yr. lag[1]	5,487	1.00	1.02 (0.93, 1.11)	1.08 (0.99, 1.17)	1.04 (0.96, 1.14)	1.06 (0.97, 1.16)	0.19	1.02 (0.98, 1.06)
8-12 yr. lag[1]	4,805	1.00	1.00 (0.91, 1.09)	0.99 (0.91, 1.09)	0.96 (0.88, 1.06)	0.99 (0.90, 1.09)	0.69	0.98 (0.94, 1.02)
12-16 yr. lag[1]	3,863	1.00	1.01 (0.91, 1.12)	0.97 (0.87, 1.07)	1.00 (0.90, 1.11)	1.00 (0.90, 1.11)	0.92	1.00 (0.95, 1.06)
Pooled analysis								
Main model	16,346	1.00	0.99 (0.94, 1.04)	0.96 (0.92, 1.01)	0.98 (0.93, 1.03)	0.96 (0.91, 1.01)†	0.17	0.98 (0.94, 1.01)
0-4 yr. lag[1]	16,346	1.00	0.96 (0.92, 1.01)†	0.99 (0.95, 1.04)	0.99 (0.94, 1.04)	1.02 (0.97, 1.07)†	0.17	1.02 (0.99, 1.04)†
4-8 yr. lag[1]	14,601	1.00	1.02 (0.97, 1.08)	0.97 (0.92, 1.02)	1.02 (0.96, 1.07)	0.99 (0.94, 1.05)	0.73	1.00 (0.97, 1.04)
8-12 yr. lag[1]	11,956	1.00	1.01 (0.95, 1.07)	0.96 (0.91, 1.02)†	0.99 (0.93, 1.05)	0.96 (0.90, 1.02)	0.15	0.99 (0.96, 1.03)
12-16 yr. lag[1]	9,473	1.00	1.01 (0.94, 1.08)	0.95 (0.89, 1.01)	0.95 (0.88, 1.02)	0.95 (0.88, 1.02)†	0.15	0.98 (0.94, 1.02)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

‡ *P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

§ Median values

1 Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS I and II participants only), oral contraceptive use (NHS II participants only), baseline hypertension, baseline hypercholesterolemia, and family history of diabetes, AHEI and dietary intakes of protein, vegetable fat, and animal fat without dairy fat

†*P* for heterogeneity <0.05

Table S1.4 Sensitivity analysis RRs (95% CI) of Type-2 diabetes associated with isocaloric substitutions (5% of energy) of selected nutrients for dairy fat in HPFS, NHS, and NHS II assessed by 0-4-year, 4-8-year, 8-12-year, and 12-16--year lags

	RR (95% CI) of T2D substitution 5% of calories for dairy fat					
	Other Animal fat±	Vegetable fat±	Polyunsaturated fat±	Total carbohydrates¥	Carbohydrates from whole grains¥	Carbohydrates from refined grains¥
HPFS						
Main model	**1.12 (1.04, 1.20)**	0.94 (0.88, 1.02)	0.97 (0.84, 1.11)	**0.91 (0.85, 0.99)**	**0.86 (0.78, 0.95)**	0.97 (0.90, 1.05)
0-4 yr. lag[1]	**1.11 (1.04, 1.18)**	0.98 (0.93, 1.05)	1.05 (0.94, 1.17)	0.94 (0.88, 1.00)	**0.89 (0.82, 0.96)**	1.01 (0.94, 1.07)
4-8 yr. lag[1]	**1.08 (1.01, 1.16)**	0.97 (0.91, 1.04)	1.06 (0.95, 1.19)	**0.91 (0.85, 0.98)**	**0.90 (0.83, 0.98)**	0.98 (0.92, 1.05)
8-12 yr. lag[1]	**1.09 (1.01, 1.18)**	0.96 (0.89, 1.03)	0.96 (0.83, 1.10)	0.93 (0.87, 1.01)	**0.90 (0.83, 0.99)**	1.01 (0.94, 1.10)
12-16 yr. lag[1]	**1.06 (0.96, 1.16)**	0.96 (0.87, 1.06)	0.95 (0.80, 1.13)	**0.90 (0.81, 1.00)**	**0.95 (0.85, 1.06)**	0.96 (0.88, 1.06)
NHS						
Main model	**1.12 (1.06, 1.19)**	0.97 (0.91, 1.02)	1.00 (0.89, 1.12)	1.00 (0.95, 1.05)	0.94 (0.87, 1.02)	**1.06 (1.00, 1.13)**
0-4 yr. lag[1]	**1.06 (1.01, 1.10)**	0.96 (0.92, 1.00)	1.05 (0.98, 1.14)	**0.94 (0.91, 0.98)**	0.96 (0.92, 1.01)	0.98 (0.94, 1.02)
4-8 yr. lag[1]	**1.08 (1.03, 1.13)**	0.97 (0.93, 1.02)	**1.10 (1.02, 1.19)**	**0.95 (0.91, 0.99)**	0.98 (0.93, 1.03)	0.99 (0.95, 1.03)
8-12 yr. lag[1]	**1.07 (1.02, 1.13)**	0.99 (0.94, 1.04)	1.09 (0.99, 1.19)	0.97 (0.93, 1.02)	0.99 (0.93, 1.05)	1.00 (0.95, 1.06)
12-16 yr. lag[1]	**1.08 (1.02, 1.15)**	0.99 (0.93, 1.05)	1.06 (0.96, 1.18)	0.99 (0.94, 1.04)	0.97 (0.91, 1.04)	1.00 (0.95, 1.06)
NHS II						
Main model	**1.19 (1.12, 1.26)**	1.05 (0.99, 1.12)	1.07 (0.95, 1.21)	1.02 (0.95, 1.09)	0.95 (0.88, 1.04)	**1.06 (1.00, 1.12)**
0-4 yr. lag[1]	**1.11 (1.06, 1.16)**	1.00 (0.96, 1.05)	0.99 (0.92, 1.08)	1.00 (0.95, 1.06)	0.95 (0.89, 1.01)	1.01 (0.96, 1.05)
4-8 yr. lag[1]	1.00 (0.95, 1.04)	0.99 (0.95, 1.04)	1.00 (0.93, 1.09)	0.97 (0.92, 1.02)	0.99 (0.93, 1.05)	0.97 (0.93, 1.01)
8-12 yr. lag[1]	1.03 (0.98, 1.08)	1.03 (0.99, 1.09)	1.08 (0.99, 1.18)	1.00 (0.94, 1.07)	1.05 (0.99, 1.11)	1.02 (0.98. 1.06)
12-16 yr. lag[1]	0.99 (0.93, 1.04)	1.02 (0.96. 1.08)	1.06 (0.96, 1.17)	1.03 (0.96, 1.11)	0.99 (0.92, 1.06)	1.00 (0.95, 1.05)
Pooled analysis						
Main model	**1.14 (1.10, 1.19)**	0.99 (0.95, 1.03)	1.01 (0.94, 1.09)	0.99 (0.95, 1.02)	**0.93 (0.88, 0.97)**	**1.04 (1.00, 1.07)**
0-4 yr. lag[1]	**1.08 (1.06, 1.12)**	0.98 (0.95, 1.01)	1.03 (0.98, 1.08)	**0.96 (0.93, 0.99)**	**0.94 (0.91, 0.98)**	1.00 (0.98, 1.03)
4-8 yr. lag[1]	**1.04 (1.01, 1.08)**†	0.98 (0.95, 1,01)	**1.05 (1.00, 1.11)**	**0.95 (0.92, 0.98)**	**0.97 (0.93, 1.00)**	0.98 (0.95, 1.01)
8-12 yr. lag[1]	**1.06 (1.02, 1.09)**	1.00 (0.97, 1.04)	1.06 (1.00, 1.12)	0.99 (0.96, 1.02)†	0.99 (0.95, 1.03)	1.01 (0.98, 1.04)
12-16 yr. lag[1]	1.04 (1.01, 1.08)	1.00 (0.96, 1.03)	1.04 (0.98, 1.11)	0.99 (0.95, 1.03)	0.98 (0.93, 1.02)	0.99 (0.96, 1.03)

HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

± Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS and NHSII participants only), oral contraceptive use (NHSII participants only), baseline hypertension, baseline hypercholesterolemia, family history of diabetes, and dietary intake of *trans* fat

¥ Model adjusted for age (continuous), BMI (8 categories), total energy intake (quintiles), race, smoking, physical activity (quintiles), alcohol consumption (quartiles), menopausal status and menopausal hormone use (NHS I and II participants only), oral contraceptive use (NHS II participants only), baseline hypertension, baseline hypercholesterolemia, family history of diabetes, and adjusted for dietary intakes of vegetable fat, *trans* fat, and animal fat without dairy fat

†*P* for heterogeneity <0.05

Table S1.5 Changes in nutrient intakes after diagnosis of cancer, CHD, stroke, hypertension, or hypercholesterolemia

	Dairy Fat		Vegetable Fat		Animal fat other than dair		PUFA	
	Change (percent calories)	*P*-value	Change (percent calories)	*P*-value	Change (percent calories)	*P*-value	Change (percent calories)	*P*-value
HPFS								
Cancer	0.10 (0.01, 0.18)	0.03	0.22 (0.07, 0.37)	<0.0001	-0.27 (-0.37 -0.16)	<0.0001	-0.03 (-0.08 0.02)	0.22
CHD[1]	-0.27 (-0.50, -0.05)	0.02	-0.19 -(0.58, 0.20)	0.35	-0.53 (-0.86, -0.20)	0.002	-0.05 (-0.18, 0.07)	0.44
Stroke	0.22 (-0.11, 0.55)	0.19	-0.53 (-1.11, 0.04)	0.07	-0.21 (-0.64, 0.23)	0.36	-0.05 (-0.18 0.08)	0.44
CABG[2]	-0.83 (-1.03 -0.64)	<0.0001	-0.98 (-1.33, -0.63)	<0.0001	-0.93 (-1.20 -0.64)	<0.0001	-0.26 (-0.38, -0.15)	<0.0001
HTN[3]	0.05 (-0.02, 0.12)	0.18	-0.08 -(0.20, 0.05)	0.23	-0.01 (-0.11, 0.10)	0.96	-0.03 (-0.07, 0.02)	0.22
HC[4]	-0.54 (-0.60 -0.49)	<0.0001	0.18 (0.08, 0.27)	0.0003	-0.67 (-0.74, -0.58)	<0.0001	0.01 (-0.02, 0.04)	0.60
NHS								
Cancer	0.03 (-0.07, 0.13)	0.25	-0.12 (-0.28, 0.03)	0.13	-0.18 (-0.31, -0.05)	0.01	-0.05 (-0.10, 0.01)	0.08
CHD[1]	-0.01 (-0.29, 0.28)	0.98	0.08 (-0.41, 0.58)	0.74	-0.39 (-0.82, 0.03)	0.81	0.05 (-0.12, 0.22)	0.57
Stroke	-0.39 (-0.76, -0.03)	0.04	-0.96 (-1.50, -0.41)	0.0005	-0.04 (-0.65, 0.57)	0.82	-0.38 (-0.57, -0.19)	<0.001
CABG[2]	-0.76 (-1.08, -0.44)	<0.0001	-0.66 (-1.21, -0.11)	0.02	-0.33 (-0.75, 0.09)	0.13	-0.15 (-0.35, 0.045)	0.13
HTN[3]	-0.01 (-0.07, 0.05)	0.67	-0.31 (-0.41, -0.22)	<0.0001	0.12 (0.036, 0.20)	0.005	-0.05 (-0.08, -0.02)	0.002
HC[4]	-0.58 (-0.62, -0.54)	<0.0001	0.17 (0.11, 0.24)	<0.0001	-0.06 (-0.13, 0.01)	0.08	0.08 (0.06, 0.10)	<0.0001

(1)CHD: coronary heart disease, (2) CABG: coronary artery bypass graft; (3) HTN: hypertension; (4) HC: hypercholesterolemia
Results were generated from generalized linear models on changes in dairy products intake from one FFQ circle to next FFQ circle. Covariates included cancer diagnosis, coronary heart disease diagnosis, angina diagnosis, coronary artery bypass graft, stroke diagnosis, hypertension diagnosis, hypercholesterolemia diagnosis, calorie intake, FFQ cycles, nutrient intake of last FFQ cycle

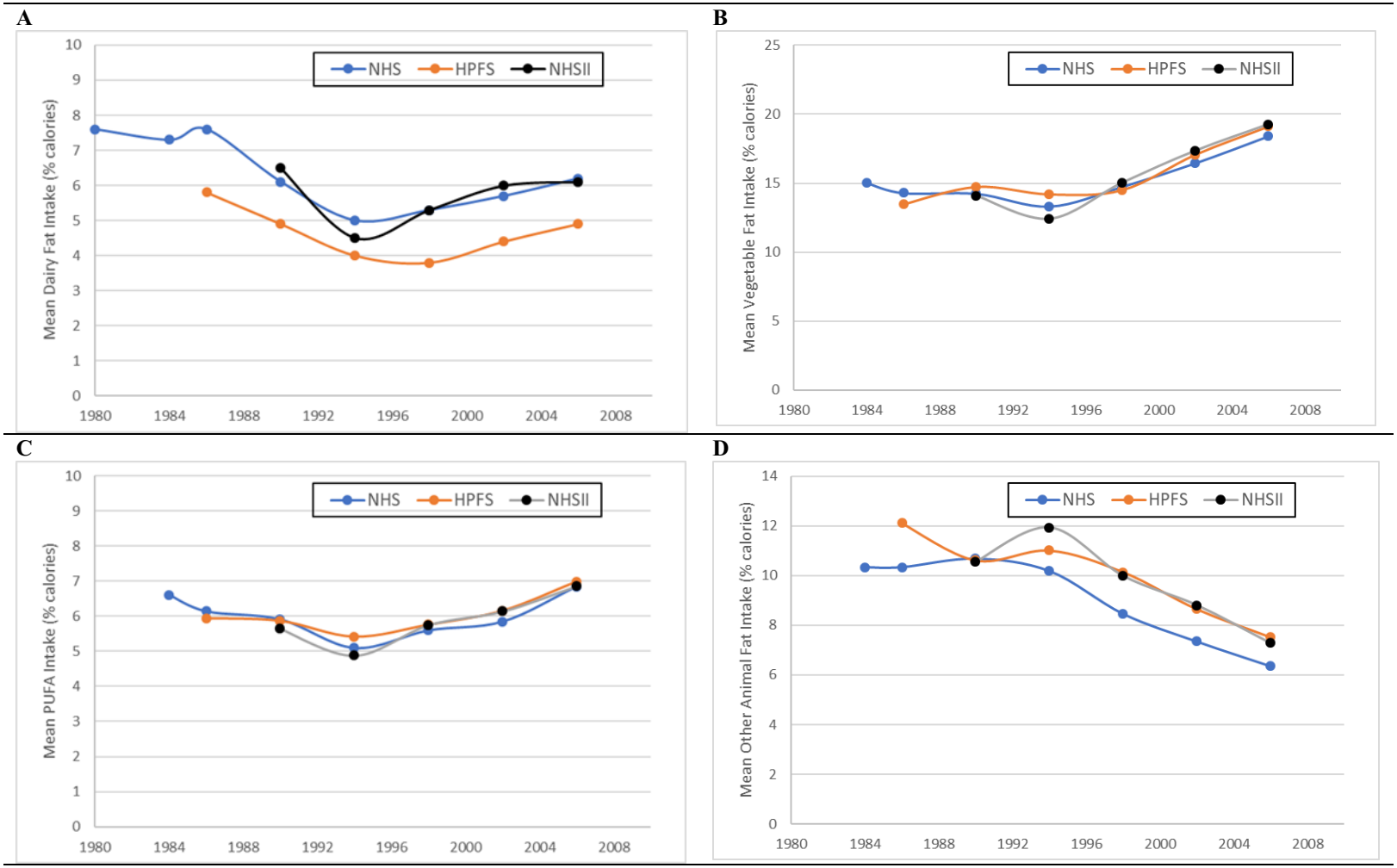


Figure S1.1. Mean intakes (percent calories) of Dairy fat (A), Vegetable fat (B), PUFA (C), Animal fat other than dairy (D)

CHAPTER 2:

Consumption of dairy products and cardiovascular disease risk among diabetes patients in two cohorts of US men and women

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ABSTRACT

Background: There is sparse epidemiological evidence supporting dietary recommendations for patients diagnosed with type 2 diabetes (T2D). Dairy products are among major food sources of saturated fat, although given the complexity of the nutritional profile of dairy products, further evidence is needed to elucidate their role in CVD incidence among diabetes patients.

Objective: The aim of this study is to examine the association between dairy consumption (total and specific dairy products) and incident CVD among participants with T2D in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS). We also examined associations for isocalorically replacing dairy fat with other macronutrients.

Methods: We prospectively followed up 15,857 participants with diabetes (4,980 from HPFS and 10,877 from NHS). Diet was assessed every 4 years with the use of validated food-frequency questionnaires. A time-dependent Cox proportional hazards regression was used to estimate the hazard ratio (HR) of CVD risk for total dairy intake, dairy subtypes, and dairy fat.

Results: During 199,168 person-years of follow-up, we documented 3,873 incident CVD cases, including 3,126 coronary heart disease (CHD) and 747 stroke cases. In multivariate models, total dairy product intake was not associated with total CVD risk [RR for extreme quintiles: 0.96; 95% CI: 0.85, 1.09]. Intake of individual dairy products was not associated with CVD outcomes, except that ice-cream intake was inversely associated with total CVD risk. In substitution analyses, replacing one serving of dairy products with red meat or processed red meat was associated with a 6% increase [RR: 1.06; 95% CI: 1.01, 1.11] and 13% increase of CVD risk [RR: 1.13; 95% CI: 1.04, 1.23], respectively. Conversely, replacing one serving of total dairy products

with one serving of nuts was associated with a 12% risk reduction of CVD [RR: 0.88; 95% CI: 0.79, 0.97]. The replacement of 5% of calories from dairy fat with the equivalent energy from other sources of animal fat was associated with an 8% increased CVD risk [RR: 1.08; 95% CI: 1.02, 1.14] and a 1% calorie substitution of omega-6 PUFAs was associated with a 3% CVD risk reduction [RR: 0.97; 95% CI: 0.94, 0.99]. Replacing dairy fat with other energy sources was not associated with CVD risk.

Conclusions:

Total dairy product intake was not associated with risk of CVD in participants with diabetes. Replacing dairy products with red and processed read meat is associated with higher CVD risk, whereas replacing dairy product intake with nuts may likely lead to lower CVD risk. These data provide initial evidence regarding the health consequences of consuming dairy product intake among patients of diabetes and need replications in future studies.

INTRODUCTION

The prevalence of type 2 diabetes (T2D) has reached the epidemic level in the U.S. and globally (8.8% of global adult population).[1] Among the numerous complications resulting from T2D, cardiovascular disease (CVD) is the primary cause of deaths in diabetes patients.[2] General lifestyle recommendations have been issued by the American Diabetes Association (ADA) and American Heart Association (AHA) for people with diabetes, including guidelines for body weight and physical activity, although the current dietary guidance is quite limited and focuses on restricting intakes of saturated fatty acids (SFAs) and *trans* fatty acids (TFAs) and emphasizing dietary fat quality by promoting the intake of mono-unsaturated fatty acids (MUFAs) and poly-

unsaturated fatty acids (PUFAs)[2,3] which is largely based on evidence from clinical trials.[4] Overall, evidence regarding dietary factors in relation to CVD risk among diabetes patients is sparse. A study from the Physicians Health Study (PHS) showed that adherence to risk factors as smoking avoidance, physical activity, consuming alcohol in moderation, and eating a healthy diet were inversely associated with total mortality among men with diabetes.[5] Regarding individual food groups, only a few studies reporting inverse associations between intakes of fruits and vegetables,[6,7] whole grains and cereal fiber and CVD incidence and mortality among diabetes patients.[8]

Intake of dairy products in this population warrants examination because dairy products play an important role in American diet and contribute nutrients such as protein, calcium, and vitamins to the diet in addition to dairy fat which is comprised primarily of saturated fatty acids (SFAs) (60-65%).[9–12] In the general population, the association between dairy product intake and CVD risk remains inconclusive. A recent meta-analysis of 29 prospective cohorts found no associations between total dairy, high-fat, and low-fat dairy product intake and coronary heart disease (CHD).[13] However, there seems to be evidence suggesting that the inclusion of dairy products in the diets of high-risk populations might be associated with reduced risk of T2D, especially for low-fat dairy products.[14] Further evidence is needed to understand the role of dairy products in populations with T2D in managing the risk of CVD outcomes. To address this gap in knowledge, we examined the association between dairy consumption (total and specific dairy products), dairy fat and incident CVD among T2D patients in the NHS and the HPFS.

We hypothesized that total dairy product intake was not associated with incident CVD among T2D patients, and we expect heterogeneity among the associations for individual products in relation to CVD risk. We further hypothesized that intake of low-fat dairy products, yogurt, and fermented dairy products was inversely associated with lower CVD risk, whereas high-fat dairy products were associated with increased CVD risk.

METHODS

Study population: We followed men and women with diagnosed T2D in two prospective cohorts: The Health Professionals Follow-Up Study (HPFS) and the Nurses' Health Study (NHS). The HPFS was established in 1986 with 51,529 male health professionals, aged 40 – 75 who returned a questionnaire with information on diet and medical history.[15] In 1976, 121,700 female nurses, aged 30-55, were enrolled in the NHS after returning a questionnaire with detailed information on their diet, lifestyle, and medical history.[16] Lifestyle and disease incidence were updated every two years and diet was updated quadrennially. The cumulative follow-up exceeds 90% of person-years in both cohorts.[17,18]

Our analyses included participants with self-reported T2D since baseline (1980 for the NHS and 1986 for the HPFS) to June 2012 in the NHS and January 2012 in the HPFS. We excluded participants with diagnosed coronary heart disease, stroke, coronary artery bypass graft surgery, angina and cancer at baseline for each cohort or before the diagnosis of T2D. We additionally excluded participants who left more than 70 blank food items in the baseline food frequency questionnaire for HPFS participants and more than 10 for NHS participants and those with

unusual self-reported calorie intake levels (<800 or >4200 kcal/day for men, and <500 or >3500 kcal/day for women). We also excluded participants without baseline dairy consumption information or follow-up information on diabetes diagnosis date, leaving a sample of 15,857 participants (4,980 from HPFS and 10,877 from NHS) in the analysis. Data from both cohorts were pooled to increase statistical power because there was no significant heterogeneity in the associations between the two cohorts. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

Ascertainment of T2D cases: Incident T2D cases were identified by self-reports on the biennial questionnaires. A supplementary questionnaire enquiring symptoms, diagnostic tests, and hypoglycemic therapy was sent to participants who reported a diabetes diagnosis and we used the criteria from the National Diabetes Data Group (NDDG) up until 1998[19]: (1) manifestation of classic symptoms such as excessive thirst, polyuria, weight loss and hunger, in conjunction with elevated fasting glucose ≥140 mg/dL (7.77 mmol/L) or non-fasting glucose levels ≥200 mg/dL (11.1 mmol/L) (2) asymptomatic but elevated plasma glucose in two separate occasions or abnormal glucose tolerance test results and (3) receiving any hypoglycemic treatment for diabetes. After 1998, a fasting glucose concentration ≥126 mg/dL (6.99 mmol/L) was adopted per by the American Diabetes Association (ADA). Previous studies in a subset of NHS and HPFS participants have demonstrated that the supplementary questionnaire is a highly-reliable confirmation tool (98% of questionnaire-confirmed cases were re-confirmed by medical record review in the NHS and 97% in the HPFS).[20,21] In this analysis we used both confirmed and

self-reported cases identified at baseline and during follow-up interval. At the baseline questionnaire, NHS and HPFS participants were asked to report time of physician-diagnosed diabetes in five-year intervals between 1955 and 1980. For prevalent patients at baseline, we assigned a T2D diagnosis to June of the mid-point year of these 5-year intervals.

Assessment of diet: NHS participants were sent a FFQ with 61 items in 1980 to gather information about their usual diet in the preceding year. The questionnaire was expanded to 131 items and mailed to participants in 1984, 1986, and every four years thereafter through 2010. The expanded FFQ was administered quadrennially since 1986 through 2010 in the HPFS. Dairy products included skim/ low fat milk, whole milk, ice cream, yogurt, cottage/ricotta cheese, cream cheese, other cheese, and cream. For each dairy product, there are 9 possible responses, ranging from never or <1 time per month to ≥6 times per day. The standard serving size was 8 oz. glass (240 ml) for skim, low fat milk, and whole milk, 1 cup (8 oz.) for yogurt through the 2006 questionnaire or 4-6 oz. starting in the 2010 questionnaire, 1 tablespoon (20g) for cream and sour cream, ½ cup (122.5g) for sherbet or frozen yogurt, ice cream, cottage and ricotta cheese, 1 oz. (30g) for cream cheese and other cheese. Dietary fat intake was calculated by multiplying the consumption frequency of each food in in its standard portion size by its nutrient composition, which was based on the Harvard University Food Composition Database. The exposures included in this analysis are: total dairy product intake, total high-fat (whole milk, cream, cream cheese, high-fat cheese, ice-cream) total low-fat (skim/low-fat milk, yogurt, cottage cheese, low-fat cheese, sherbet) dairy product intake, total dairy excluding yogurt, and intakes of individual dairy products, including whole milk, skim/low-fat milk, cheese, yogurt intake, fermented dairy (cheese

and yogurt), ice-cream, and sherbet. In a secondary analysis we also assessed butter intake and dairy fat intake (derived from food products that contain butter or dairy fat). Previous studies have described the reproducibility and validity of the FFQs: correlation coefficients were 0.62 between FFQs and multiple diet records for both high-fat and low-fat dairy products[22], 0.49 for cheese, and 0.74 for yogurt.[23,24]

Assessment of study outcome: Incident CVD outcomes, including nonfatal myocardial infarction (MI), fatal CHD, and stroke (fatal and non-fatal) were our primary outcomes of interest. Nonfatal MI or stroke cases were identified through self-reports. Upon self-reports of cardiovascular events, participants were asked for permission to access their medical records for confirmation. Medical records were reviewed by study physicians blinded to the participants' exposure status. Nonfatal MI cases were considered confirmed if World Health Organization criteria, including symptoms, and electrocardiographic changes or elevated cardiac enzyme concentrations, were met.[25] Stroke cases were confirmed according to the criteria of the National Survey of Stroke which include neurologic deficit with a sudden or rapid onset that persisted for 24 hours.[26] CHD and stroke events for which confirmation was self-reported without medical record review were designated as probable. The majority of deaths (>98%) were identified from reports from next of kin or postal authorities or by searching the National Death Index as demonstrated by a validation study.[27,28] Fatal CHD was identified if it was listed as the cause of death in autopsy reports, medical records or death certificates. Fatal CHD was confirmed if no other more plausible cause of death was reported. Otherwise, CHD cases were considered probable if medical records were not obtained. Fatal stroke was assessed by reviewing medical

records, autopsy reports, and death certificates. We included both confirmed and probable CHD and strokes in the analysis to maximize the number of cases, and results were similar when probable cases were excluded.[29]

Covariate Assessment: information about lifestyle factors and medical history was updated every two years, including body weight, cigarette smoking, physical activity, medication use, as well as history of chronic diseases. Among the NHS participants, we ascertained menopausal status and postmenopausal hormone use in the questionnaires. We derived and used a modified 2010 Alternative Health Index (AHEI) score to quantify overall diet quality. AHEI was developed as an alternative to the U.S. Department of Agriculture's Healthy Eating Index (HEI) originally designed to assess adherence to U.S. dietary guidelines.[30] There were 10 components included in the modified AHEI score, including high intake of fruits, vegetables, whole grains, nuts, long-chain omega-3 fatty acids, and PUFAs, and low intakes of sugar sweetened beverages, red and processed meats, *trans* fatty acids (TFAs), and sodium. Each component was scored from 0 to 10 based on intake where 10 indicated best adherence to recommended servings per day. AHEI was categorized in quintiles and used as a covariate. We adjusted for alcohol intake as a separate covariate.

Statistical analysis: we calculated person-time from the date of return of the first FFQ after diagnosis of T2D to the date of diagnosis of CVD, death, or the end of the follow-up (31 January 2014 for the HPFS; and 30 June 2014 for the NHS), whichever came first. Dairy intake for each participant was calculated as the cumulative average of intakes from the FFQ after T2D

diagnosis for incident cases, 1980 for prevalent T2D cases in NHS, or 1986 for prevalent cases in HPFS, to account for long-term diet and reduce within-person variation.[31,32] We used a Cox proportional hazards model to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of dairy intake with the risk of total CVD, CHD, and stroke incidence. The basic model included age, sex, and calendar time with updated information at each 2-y questionnaire cycle. Model 2 additionally adjusted for BMI, total energy intake, and lifestyle factors, including race, alcohol intake, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes, family history of myocardial infarction, alcohol intake, smoking status, physical activity, current aspirin use, current multivitamin use, diabetes duration, interval between T2D diagnosis and date of first FFQ returned after the diagnosis, baseline hypertension, and baseline hypercholesterolemia. Model 3 further adjusted for quintiles of AHEI. In the analysis of individual dairy foods, we additionally adjusted for other dairy products in model 3. To test for linear trend, the median value was assigned to each quintile, and this value was modeled as a continuous variable.

In our primary analysis, we stopped updating diet after diagnosis of cancer, coronary artery bypass surgery (CABG), or angina because these conditions may lead changes in diet and subsequently distort the associations of interest.[32] We conducted sensitivity analyses where we further stopped updating dietary intake after self-reported diagnosis of cancer only or where diet was further updated after diagnosis of hypertension and hypercholesterolemia during follow-up.[33] We assessed the association in changes in total dairy, high-fat dairy and low-fat dairy before/after T2D diagnosis in both categorical (tertiles) and continuous analyses. A linear trend

was evaluated by assigning a median value to each category and modeling this value as a continuous variable.

We also evaluated the associations for substitutions of one serving of dairy products for other protein sources by including both as continuous variables in the same isocaloric model. The relative risk was estimated from the difference in coefficients.[34] The protein sources we evaluated included high-fat dairy for low-fat dairy, whole milk for skim milk, and total dairy for other sources of protein including fish (canned tuna, dark and light fleshed fish, and breaded fish), poultry (chicken with and without skin, chicken sandwich), red meat (hamburger, beef hot dog, processed meat and processed meat sandwich, bacon, beef/pork/ lamb as a mixed and main dish), and processed red meat (bacon, hot dogs, sausage, salami, bologna, and other processed red meats), beans and lentils, and nuts (peanuts, other nuts).

We conducted a secondary analysis where we evaluated the associations of dairy fat intake with the risk of total CVD, CHD, and stroke incidence. The associations with risk of CVD for isocaloric substitutions of other macronutrients for dairy fat were evaluated using a multivariable Cox proportional hazards model where dairy fat intake and other macronutrients were modeled as continuous variables in the same model. We calculated the difference in coefficients from this model to estimate the relative risk associated with replacing calories from dairy fat with the equivalent calories from other macronutrients, including vegetable fat, PUFAs, omega-6 fatty acids, omega-3 fatty acids, other animal fat, total carbohydrate, carbohydrate from whole grains, and carbohydrate from refined grains.[35,36]

We evaluated the associations after adding a time lag of either one FFQ cycle (4-yr) or two cycles (8-yr) after T2D diagnosis to investigate the impact of potential reverse causation bias. For incident T2D cases, we also evaluated the association with changes of dairy intake from the questionnaires before and after T2D diagnosis. We also assessed the associations between dairy intake and CVD in a subset of participants who had self-reported Hb1Ac levels. We used the covariates in model 3 and further adjusted for Hb1Ac levels categorized in five groups and evaluated the association between total dairy products, high-fat dairy, and low-fat dairy with CVD risk. We conducted a similar analysis in a subset of participants with self-reported diabetes medication and insulin use by adjusting for medication in addition to the covariates in model 3.

RESULTS

During 199,168 person-years of follow-up, we documented 3,873 incident CVD cases, including 3,126 CHD and 747 stroke cases during a mean follow-up of 11.2 in the HPFS or 12.7 years in the NHS. At baseline, total dairy intake was positively associated with family history of myocardial infarction in men, and positively associated with self-reported white ethnicity and total energy intake in both cohorts. Total dairy was inversely associated with family history of diabetes in men and inversely associated with baseline hypertension and hypercholesterolemia, regular aspirin use, multivitamin use, and alcohol intake in both cohorts (**Table 2.1**). Total dairy consumption was not associated with risk of CVD in the age and sex-adjusted models nor in the models 2 and 3 (all P for trend > 0.05). In the multivariable model adjusted for lifestyle variables and AHEI, for one serving increase per day the relative risks were 1.00 (95% CI: 0.97, 1.02) for CVD; 0.99 (95% CI: 0.96, 1.01) for CHD; and 1.04 (95% CI: 0.99, 1.10) for stroke (**Table 2.2**).

Table 2.1 Characteristics of participants with T2D in the NHS and the HPFS cohorts at start of follow-up according to quintiles of dairy product intake

Characteristics	**HPFS** (n = 4,980)					**NHS** (n = 10,877)				
	Q1 (n=1,066)	Q2 (n=1,003)	Q3 (n=948)	Q4 (n=935)	Q5 (n=1,028)	Q1 (n=2,261)	Q2 (n=2,066)	Q3 (n=2,081)	Q4 (n=1,983)	Q5 (n=2,486)
Dairy intake (servings/day)	0.6(0.3)	1.3(0.2)	1.9(0.9)	2.5(0.4)	4.6(1.6)	0.7(0.5)	1.4(0.5)	1.9(0.8)	2.5(0.7)	4.1(1.6)
Age (y)	64.3(9.2)	64.5(9.6)	63.9(9.9)	64.9(9.9)	66.1(10.3)	63.9(10.6)	63.2(10.1)	61.8(10.7)	63.1(10.0)	62.8(10.2)
Physical activity (hours/wk.)	2.7(7.0)	2.6(4.3)	2.9(5.4)	2.9(6.3)	2.8(6.7)	1.5(2.4)	1.4(2.3)	1.5(2.7)	1.6(2.6)	1.5(2.4)
BMI (kg/m^2)	27.3(4.6)	27.8(4.8)	28.2(4.4)	27.7(4.4)	28.0(5.0)	27.6(5.5)	27.8(5.5)	27.9(5.5)	27.9(5.5)	28.0(5.7)
Race, white (%)	81.6	90.7	88.1	90.4	91.6	92.7	95.2	97.1	97.2	97.9
Current smoker (%)	7.8	6.2	8.5	6.9	9.1	15.0	13.1	12.0	11.7	12.0
Hypertension (%)	65.8	63.6	62.6	61.4	61.5	74.9	74.9	75.1	73.8	72.3
High cholesterol (%)	55.8	55.3	48.3	46.6	47.0	64.5	61.9	60.8	61.3	59.2
Family hist. of diabetes (%)	39.0	38.6	39.9	38.8	36.7	48.1	48.6	49.4	48.5	48.0
Family history of MI (%)	33.3	34.1	33.7	33.3	36.0	28.5	27.4	27.7	28.4	27.6
Aspirin use (%)	60.3	58.6	54.4	55.5	55.4	52.3	49.2	46.9	46.9	48.4
Multivitamin use (%)	60.4	56.7	58.2	54.4	54.5	61.9	56.5	53.9	49.7	50.7
Total energy (Kcal/d)	1,640(522)	1,778(550)	1,969(569)	2,059(623)	2,351(635)	1,410(478)	1,546(467)	1,669(495)	1,764(492)	1,988(540)
Alcohol (g/d)	9.8(15.5)	9.4(13.9)	10.4(15.1)	9.1(14.9)	8.7(14.4)	3.6(8.5)	3.3(7.9)	3.4(7.6)	3.0(7.3)	2.8(6.9)
AHEI	52.4(12.1)	51.5(11.6)	50.7(11.9)	50.9(11.4)	49.3(11.2)	46.6(13.9)	47.1(13.4)	47.0(13.5)	47.1(13.0)	45.8(13.0)

Values are means (SD) or percentages; NHS: Nurses' Health Study; HPFS: Health Professionals Follow-Up Study; MI: myocardial infarction

Table 2.2 HRs (95% CI) of cardiovascular disease (CVD: 3,823 cases), coronary heart disease (CHD: 3,126 cases), and stroke risk (747 cases) according to quintiles of total dairy intake in participants from both NHS and HPFS cohorts*

	Quintile of dairy intake						
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for one serving / day
			CVD				
Daily intake (servings)§	0.7	1.3	1.9	2.6	3.9		
Cases/person-years	747/38,699	776/38,857	786/39,241	779/38,934	735/38,850		
Model 1[1]	1.00	1.03 (0.93, 1.13)	1.01 (0.91, 1.11)	0.97 (0.88, 1.08)	0.95 (0.86, 1.06)	0.18	0.99 (0.96, 1.01)
Model 2[2]	1.00	1.03 (0.93, 1.14)	1.02 (0.92, 1.13)	0.99 (0.89, 1.10)	0.98 (0.88, 1.10)	0.48	1.00 (0.97, 1.02)
Model 3[3]	1.00	1.03 (0.93, 1.14)	1.02 (0.92, 1.13)	0.99 (0.89, 1.10)	0.98 (0.88, 1.10)	0.49	1.00 (0.97, 1.02)
			CHD				
Cases	603	651	643	634	595		
Model 1[1]	1.00	1.06 (0.95, 1.18)	1.02 (0.91, 1.14)	0.97 (0.87, 1.09)	0.95 (0.85, 1.06)	0.12	0.98 (0.96, 1.01)
Model 2[2]	1.00	1.05 (0.94, 1.18)	1.02 (0.91, 1.15)	0.97 (0.87, 1.10)	0.96 (0.85, 1.09)	0.24	0.99 (0.96, 1.02)
Model 3[3]	1.00	1.05 (0.94, 1.18)	1.03 (0.91, 1.15)	0.98 (0.87, 1.10)	0.96 (0.85, 1.09)	0.25	0.99 (0.96, 1.01)
			STROKE				
Cases	158	132	148	157	152		
Model 1[1]	1.00	0.86 (0.68, 1.08)	0.92 (0.73, 1.15)	0.95 (0.76, 1.19)	0.97 (0.77, 1.21)	0.82	1.02 (0.97, 1.07)
Model 2[2]	1.00	0.88 (0.69, 1.11)	0.96 (0.76, 1.21)	1.02 (0.80, 1.29)	1.05 (0.82, 1.34)	0.36	1.04 (0.99, 1.10)
Model 3[3]	1.00	0.88 (0.70, 1.11)	0.96 (0.76, 1.22)	1.02 (0.80, 1.29)	1.05 (0.82, 1.34)	0.36	1.04 (0.99, 1.10)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

‡*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

§ Median value of each quintile (all such values)

[1] Model 1 was adjusted for age (continuous) and sex

[2] Model 2 included Model 1 covariates and was additionally adjusted for BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, and lag-time between T2D diagnosis and return of first FFQ

[3]Model 3 included Model 2 covariates and was additionally adjusted for AHEI (quintiles)

We examined the association between dairy product intake categorized by fat content and CVD risk (**Table 2.3**). Neither low-fat nor high-fat dairy products were significantly associated with CVD, CHD, or stroke risk across all models (all P for trend > 0.05). We also assessed the association between individual dairy products types and CVD risk (**Table 2.4**). No significant associations observed for individual dairy products and CVD risk except for an inverse association between ice-cream intake and CVD risk in the multivariate model adjusted for AHEI and mutually adjusted for other dairy product types (P for trend < 0.05). The relative risk of CVD for one serving of ice-cream per day was 0.82 (95% CI: 0.67, 0.99). Ice-cream intake was marginally associated with CHD risk [RR per one serving per day: 0.82; 95% CI: 0.66, 1.01] and non-significantly associated with lower stroke risk [RR per one serving per day: 0.82; 95% CI: 0.53, 1.26]. All other individual dairy products were not significantly associated with risk of CVD, CHD or stroke.

We also examined the changes in dairy product intake from before to after diabetes diagnosis among incident T2D cases only. There were no significant associations for changes in total dairy, low-fat dairy or high-fat dairy intake with CVD, CHD or stroke risk in the categorical analyses. However, the continuous analysis showed that every one-serving increase in high-fat dairy intake was associated with a 5% CVD risk reduction [RR: 0.95; 95% CI: 0.90, 0.99],] but no association with CHD or stroke risk as shown in (**Table 2.5**). We further assessed whether intake changes of individual dairy products before and after diabetes were associated with CVD in (**Table 2.6**). Overall, changes in individual dairy products were not associated with CVD risk except for cheese and fermented dairy (comprised of cheese and yogurt). Changes in cheese intake were

Table 2.3 HRs (95% CI) of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke risk according to quintiles of high-fat and low-fat dairy intake in participants from both NHS and HPFS cohorts*

	Quintile of dairy intake						
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for one serving / day
				CVD			
High-fat dairy products							
Daily intake (servings)§	0.07	0.32	0.57	0.93	1.73		
Cases/person-years	764/39,294	844/38,612	743/39,047	744/38,823	728/38,803		
Model 1[1]	1.00	1.10 (1.00, 1.22)	0.97 (0.87, 1.07)	1.00 (0.90, 1.11)	0.99 (0.89, 1.09)	0.31	0.97 (0.94, 1.01)
Model 2[2]	1.00	1.03 (0.93, 1.14)	0.91 (0.82, 1.01)	0.94 (0.84, 1.04)	0.93 (0.84, 1.04)	0.10	0.96 (0.92, 1.00)
Model 3[3]	1.00	1.02 (0.92, 1.13)	0.91 (0.82, 1.01)	0.93 (0.83, 1.03)	0.94 (0.84, 1.04)	0.14	0.96 (0.92, 1.00)
Low-fat dairy products							
Daily intake (servings)§	0.14	0.68	1.06	1.57	2.76		
Cases/person-years	817/38,011	664/34,577	862/44,238	683/34,973	797/37,890		
Model 1[1]	1.00	1.00 (0.90, 1.11)	0.92 (0.83, 1.01)	1.01 (0.91, 1.12)	1.00 (0.90, 1.10)	0.85	1.01 (0.98, 1.04)
Model 2[2]	1.00	1.01 (0.91, 1.12)	1.04 (0.94, 1.16)	1.04 (0.93, 1.15)	1.03 (0.93, 1.15)	0.54	1.02 (0.99, 1.05)
Model 3[3]	1.00	1.01 (0.91, 1.12)	1.05 (0.94, 1.16)	1.03 (0.93, 1.15)	1.03 (0.93, 1.15)	0.61	1.02 (0.98, 1.05)
				CHD			
High-fat dairy products							
Cases	617	700	613	599	597		
Model 1[1]	1.00	1.13 (1.01, 1.26)	0.98 (0.87, 1.09)	0.99 (0.88, 1.11)	0.99 (0.88, 1.11)	0.25	0.96 (0.92, 1.00)
Model 2[2]	1.00	1.04 (0.93, 1.16)	0.92 (0.82, 1.03)	0.92 (0.82, 1.04)	0.92 (0.82, 1.04)	0.07	0.94 (0.90, 1.00)
Model 3[3]	1.00	1.04 (0.93, 1.16)	0.92 (0.82, 1.03)	0.91 (0.81, 1.03)	0.93 (0.82, 1.05)	0.09	0.94 (0.90, 0.99)
Low-fat dairy products							
Cases	675	532	710	559	650		
Model 1[1]	1.00	0.98 (0.87, 1.11)	0.91 (0.81, 1.01)	1.02 (0.91, 1.15)	0.98 (0.88, 1.10)	0.89	1.01 (0.98, 1.04)
Model 2[2]	1.00	0.99 (0.88, 1.11)	1.04 (0.93, 1.17)	1.04 (0.92, 1.17)	1.01 (0.90, 1.14)	0.75	1.02 (0.98, 1.06)
Model 3[3]	1.00	0.99 (0.88, 1.11)	1.04 (0.93, 1.17)	1.04 (0.92, 1.17)	1.01 (0.90, 1.13)	0.85	1.02 (0.98, 1.05)

Table 2.3 - Continued

				STROKE			
High-fat dairy products							
Cases	159	153	136	154	145		
Model 1[1]	1.00	0.99 (0.79, 1.23)	0.90 (0.72, 1.13)	1.05 (0.84, 1.30)	1.00 (0.80, 1.26)	0.75	1.06 (0.97, 1.14)
Model 2[2]	1.00	0.96 (0.76, 1.20)	0.89 (0.70, 1.13)	1.02 (0.81, 1.29)	0.99 (0.78, 1.26)	0.75	1.06 (0.97, 1.15)
Model 3[3]	1.00	0.92 (0.73, 1.16)	0.84 (0.66, 1.06)	0.99 (0.78, 1.25)	0.97 (0.76, 1.24)	0.80	1.06 (0.97, 1.16)
Low-fat dairy products							
Cases	156	141	162	126	162		
Model 1[1]	1.00	1.05 (0.83, 1.33)	0.98 (0.78. 1.22)	0.90 (0.70, 1.14)	1.06 (0.85, 1.33)	0.79	1.00 (0.93, 1.07)
Model 2[2]	1.00	1.07 (0.84, 1.36)	1.07 (0.84. 1.35)	0.95 (0.74, 1.22)	1.14 (0.90 ,1.45)	0.39	1.02 (0.95, 1.10)
Model 3[3]	1.00	1.07 (0.85, 1.37)	1.07 (0.85. 1.36)	0.96 (0.75, 1.23)	1.15 (0.91 ,1.46)	0.36	1.06 (0.85, 1.32)

[*]Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[‡]*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

[§] Median value of each quintile (all such values)

[1] Model 1 was adjusted for age (continuous) and sex

[2] Model 2 included Model 1 covariates and was additionally adjusted for BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, and lag-time between T2D diagnosis and return of first FFQ

[3]Model 3 included Model 2 covariates and was additionally adjusted for AHEI (quintiles) and mutually adjusted for other dairy product categories

Table 2.4 HRs (95% CI) of cardiovascular disease (CVD) risk according to intakes of various dairy foods in participants from both NHS and HPFS cohorts*

	Dairy intake (servings)					
	Category 1*	Category 2*	Category 3*	Category 4*	*P*-trend‡	HR (95% CI) for one serving / day
			CVD			
Cheese intake	< 1/week	1 - 4/week	5/week - 1/day	>1.0/day		
Cases/person-years	646/35,006	1,714/83,923	1,284/64,193	209/11,457		
Model 1[1]	1.00	1.06 (0.97, 1.17)	1.05 (0.95, 1.15)	0.99 (0.85, 1.16)	0.90	0.98 (0.93, 1.04)
Model 2[2]	1.00	1.02 (0.93, 1.12)	1.02 (0.92, 1.12)	0.98 (0.83, 1.15)	0.81	0.99 (0.94, 1.05)
Model 3[3]	1.00	1.06 (0.95, 1.18)	1.06 (0.95, 1.19)	1.01 (0.84, 1.20)	0.99	1.00 (0.94. 1.07)
Skim/low-fat milk	< 1/week	1 - 4/week	5/week - 1/day	>1.0/day		
Cases/person-years	897/47,977	806/41,729	1,328/64,388	822/40,496		
Model 1[1]	1.00	0.99 (0.90, 1.09)	1.04 (0.95, 1.13)	0.99 (0.90, 1.09)	0.80	1.00 (0.96, 1.03)
Model 2[2]	1.00	0.96 (0.87, 1.06)	1.02 (0.93, 1.11)	0.98 (0.89, 1.08)	0.88	1.00 (0.97, 1.04)
Model 3[3]	1.00	0.97 (0.87, 1.07)	1.05 (0.95, 1.15)	0.98 (0.88, 1.09)	0.88	1.00 (0.96, 1.04)
Whole milk	< 1/month	1 - 3/month	1/week	≥2/week		
Cases/person-years	2,885/150,377	314/14,633	228/10,298	426/19,273		
Model 1[1]	1.00	1.14 (1.01, 1.28)	1.19 (1.03, 1.36)	1.13 (0.02, 1.26)	0.02	1.08 (1.00, 1.17)
Model 2[2]	1.00	1.10 (0.97, 1.24)	1.11 (0.97, 1.28)	1.10 (0.99, 1.22)	0.07	1.06 (0.98, 1.15)
Model 3[3]	1.00	1.12 (0.99, 1.27)	1.07 (0.92, 1.24)	1.13 (1.00, 1.27)	0.06	1.04 (0.94, 1.16)
Yogurt intake	< 1/month	1 - 3/month	1/week	≥2 /week		
Cases/person-years	1,935/93,587	714/35,425	655/33,870	549/31,699		
Model 1[1]	1.00	1.04 (0.95, 1.13)	1.03 (0.94, 1.13)	0.95 (0.86, 1.05)	0.33	0.88 (0.77, 1.01)
Model 2[2]	1.00	1.04 (0.95, 1.13)	1.04 (0.95, 1.14)	1.00 (0.91, 1.11)	0.96	0.96 (0.84, 1.10)
Model 3[3]	1.00	1.06 (0.96, 1.16)	1.06 (0.96, 1.17)	1.03 (0.93, 1.15)	0.57	0.98 (0.85, 1.13)
Fermented dairy products	< 1/week	1 - 3/week	5/week - 1/day	>1.0/day		
Cases/person-years	1,965/80,886	817/48,490	813/49,386	258/15,817		
Model 1[1]	1.00	0.97 (0.83, 1.04)	0.91 (0.81, 1.02)	0.92 (0.79, 1.08)	0.34	0.98 (0.92, 1.03)
Model 2[2]	1.00	0.93 (0.83, 1.05)	0.94 (0.84, 1.06)	0.96 (0.81, 1.12)	0.73	0.98 (0.93, 1.04)
Model 3[3]	1.00	0.97 (0.86, 1.11)	0.98 (0.86, 1.11)	1.00 (0.84, 1.18)	0.93	1.00 (0.94, 1.06)

Table 2.4 - Continued

Cream	< 1/month	1 - 3/month	1/week	≥2/week		
Cases/person-years	2,724/129,242	504/29,453	223/12,770	402/23,114		
Model 1[1]	1.00	0.90 (0.81, 0.99)	0.93 (0.81, 1.06)	0.87 (0.78, 0.97)	0.02	0.98 (0.91, 1.05)
Model 2[2]	1.00	0.93 (0.84, 1.02)	0.95 (0.83, 1.09)	0.90 (0.81, 1.00)	0.05	0.98 (0.91, 1.05)
Model 3[3]	1.00	0.94 (0.85, 1.04)	0.96 (0.83, 1.10)	0.91 (0.81, 1.01)	0.11	0.98 (0.91, 1.05)
Ice cream	< 1/month	1 - 3/month	1/week	≥2/week		
Cases/person-years	1,433/69,968	1,238/62,586	717/36,432	476/25,594		
Model 1[1]	1.00	1.03 (0.95, 1.11)	0.99 (0.91, 1.09)	0.94 (0.84, 1.04)	0.15	0.86 (0.73, 1.02)
Model 2[2]	1.00	1.03 (0.95, 1.11)	0.99 (0.91, 1.09)	0.94 (0.84, 1.04)	0.11	0.86 (0.72, 1.02)
Model 3[3]	1.00	0.98 (0.90, 1.06)	0.94 (0.85, 1.03)	0.88 (0.79, 0.99)	0.03	0.82 (0.67, 0.99)
Sherbet	< 1/month	1 - 3/month	1/week	≥2/week		
Cases/person-years	1,989/99,944	986/49,105	492/25,804	386/19,728		
Model 1[1]	1.00	1.01 (0.94, 1.09)	0.95 (0.86, 1.05)	0.90 (0.80, 0.99)	0.03	0.84 (0.71, 1.00)
Model 2[2]	1.00	1.02 (0.94, 1.11)	0.95 (0.86, 1.05)	0.93 (0.83, 1.04)	0.15	0.89 (0.75, 1.06)
Model 3[3]	1.00	1.04 (0.96, 1.13)	0.99 (0.89, 1.09)	0.96 (0.85, 1.08)	0.40	0.92 (0.78, 1.11)
Butter	< 1/month	1 - 3/month	1/week	≥2/week		
Cases/person-years	1,796/86,434	633/32,683	475/24,034	949/54,29		
Model 1[1]	1.00	0.98 (0.89, 1.07)	0.99 (0.89, 1.10)	0.95 (0.88, 1.03)	0.24	1.01 (0.96, 1.06)
Model 2[2]	1.00	0.98 (0.89, 1.07)	0.97 (0.87, 1.07)	0.95 (0.88, 1.03)	0.29	1.01 (0.96, 1.07)
Model 3[¥]	1.00	0.98 (0.89, 1.08)	0.97 (0.87, 1.07)	0.94 (0.87, 1.02)	0.19	1.00 (0.95, 1.06)

[*] HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[‡] *P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

[1] Model 1 was adjusted for age (continuous) and sex

[2] Model 2 included Model 1 covariates and was additionally adjusted for BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, and lag-time between T2D diagnosis and return of first FFQ

[3] Model 3 included Model 2 covariates and was additionally adjusted for AHEI (quintiles) and mutually adjusted for other dairy product categories

[¥] Model 3 in butter analysis was additionally adjusted for AHEI (quintiles) and dairy product intake

Table 2.5: HRs (95% CI) of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke risk according to changes in total dairy product intake, high-fat and low-fat dairy intake in incident type-2 diabetes (T2D) cases only from both NHS and HPFS cohorts*

	CVD Incidence (2,222 cases)		CHD Incidence (1,775 cases)		Stroke Incidence (472 cases)	
	Cases	**HR* (95% CI)**	**Cases**	**HR* (95% CI)**	**Cases**	**HR* (95% CI)**
Tertile changes in dairy product intake servings/day (mean, interquartile range)						
T1 -1.1 (-1.7, -0.4)	701	1.15 (1.04, 1.28)	555	1.20 (1.06, 1.34)	153	1.02 (0.81, 1.27)
T2 0.0 (0.0, 0.1)	817	1.00 (ref)	665	1.00 (ref)	165	1.00 (ref)
T3 1.2 (0.8, 2.1)	704	0.99 (0.89, 1.10)	555	0.98 (0.87, 1.11)	154	1.02 (0.81, 1.28)
P trend		0.59		0.47		0.89
HR *continuous*		0.99 (0.97, 1.02)		1.00 (0.97, 1.03)		1.02 (0.95, 1.07)
$P_{continuous}$		0.78		0.75		0.88
Tertile changes in high-fat dairy intake servings/day (mean, interquartile range)¥						
T1 -0.8 (-0.9, -0.2)	772	0.97 (0.87, 1.08)	604	1.01 (0.89, 1.13)	174	0.86 (0.69, 1.09)
T2 0.0 (0.0, 0.0)	722	1.00 (ref)	584	1.00 (ref)	148	1.00 (ref)
T3 0.8 (0.4, 1.0)	728	0.96 (0.86, 1.06)	587	0.97 (0.86, 1.09)	150	0.92 (0.73, 1.15)
P trend		0.40		0.60		0.49
HR *continuous*		0.95 (0.90, 0.99)		0.95 (0.91, 1.00)		0.93 (0.85, 1.03)
$P_{continuous}$		0.01		0.06		0.17
Tertile changes in low-fat dairy intake servings/day (mean, interquartile range)¥						
T1 -0.8 (-1.1, -0.3)	740	0.92 (0.82, 1.02)	599	0.91 (0.81, 1.02)	149	0.96 (0.76, 1.21)
T2 0.0 (0.0, 0.1)	714	1.00 (ref)	577	1.00 (ref)	148	1.00 (ref)
T3 1.2 (0.5, 1.5)	768	1.01 (0.91, 1.12)	599	0.97 (0.86, 1.09)	175	1.18 (0.95, 1.48)
P trend		0.66		0.69		0.11
HR *continuous*		1.00 (0.96, 1.03)		0.99 (0.95, 1.03)		1.03 (0.95, 1.11)
$P_{continuous}$		0.84		0.56		0.55

Model was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs/week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and AHEI (quintiles)

¥ Models for changes in high-fat and low-fat dairy were and mutually adjusted for changes in the other dairy product category

Table 2.6: HRs (95% CI) of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke risk according to in intake of various dairy foods in incident type-2 diabetes (T2D) cases only from both NHS and HPFS cohorts*

	CVD Incidence		CHD Incidence		Stroke Incidence	
	HR* (95% CI)	*P-value*	**HR* (95% CI)**	*P-value*	**HR* (95% CI)**	*P-value*
Cheese	0.89 (0.82, 0.96)	0.002	0.87 (0.80, 0.95)	0.002	0.95 (0.81, 1.12)	0.55
Skim/low-fat milk	1.01 (0.95, 1.06)	0.86	0.98 (0.92, 1.04)	0.49	1.11 (0.98, 1.25)	0.11
Whole milk	0.91 (0.73, 1.14)	0.41	0.99 (0.76, 1.27)	0.92	0.73 (0.46, 1.15)	0.18
Yogurt	0.95 (0.79, 1.14)	0.56	0.94 (0.76, 1.16)	0.57	0.94 (0.64, 1.40)	0.77
Fermented dairy	0.90 (0.84, 0.96)	0.002	0.88 (0.82, 0.95)	0.002	0.95 (0.82, 1.10)	0.50
Cream	0.99 (0.87, 1.11)	0.80	1.02 (0.89, 1.16)	0.82	0.84 (0.65, 1.10)	0.20
Ice cream	1.08 (0.85, 1.35)	0.54	1.02 (0.79, 1.32)	0.87	1.30 (0.77, 2.17)	0.33
Sherbet	1.15 (0.93, 1.41)	0.20	1.13 (0.90, 1.43)	0.30	1.23 (0.79. 1.92)	0.35

Model was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, AHEI (quintiles), and mutually adjusted for changes in other dairy product categories

inversely associated with CVD risk [RR for one serving a day change: 0.89; 95% CI: 0.82, 0.96] and CHD risk [RR: 0.87; 95% CI: 0.80, 0.95] but not with stroke risk. Changes in fermented dairy were also inversely associated with CVD risk [RR for one serving a day change: 0.90; 95% CI: 0.84, 0.96] and CHD risk [RR: 0.88; 95% CI: 0.82, 0.95] but not with stroke risk [RR: 0.93; 95% CI: 0.85, 1.03].

In substitution analyses, replacing one serving of dairy products with red meat was associated with a 6% CVD risk increase [RR: 1.06; 95% CI: 1.01, 1.11], and replacing one serving of dairy products with processed red meat was associated with a 13% risk increase of CVD [RR: 1.13; 95% CI: 1.04, 1.23]. Conversely, replacing one serving of total dairy products with one serving of nuts was associated with a 12% risk reduction of CVD [RR: 0.88; 95% CI: 0.79, 0.97] (**Table 2.7**). Replacing one serving high-fat dairy products with low-fat dairy products, dairy products excluding yogurt with yogurt, whole milk with skim milk, or total dairy with fish, poultry, or beans was not significantly associated with CVD risk. In isocaloric substitutions for selected nutrients (**Table 2.8**), the replacement of 5% of calories from dairy fat with the equivalent energy from other sources of animal fat was associated with an 8% increased CVD risk [RR: 1.08; 95% CI: 1.02, 1.14], whereas the replacement with PUFAs was marginally associated with a 10% CVD decrease [RR: 0.90; 95% CI: 0.81, 1.00; P-value = 0.05]. Substituting 1% of calories from omega-6 fatty acids for dairy fat was associated with a 3% CVD risk reduction [RR: 0.97; 95% CI: 0.94, 0.99]. Replacing dairy fat with the same energy intake from vegetable fat, omega-3 fatty acids, total carbohydrate or carbohydrates from whole grains or carbohydrates from refined grains was not associated with CVD risk (all P-values > 0.05).

Table 2.7: HR (95% CI) of CVD incidence associated with isocaloric substitutions of selected dairy products in participants from both NHS and HPFS cohorts*

Replacement	HR (95% CI) for one serving / day	*P*-value
High-fat dairy with low-fat dairy	1.04 (0.99, 1.10)	0.10
Dairy w/o yogurt with yogurt	0.97 (0.84, 1.12)	0.67
Whole milk with skim milk	0.94 (0.86, 1.02)	0.15
Dairy with fish	0.98 (0.86, 1.10)	0.69
Dairy with poultry	0.95 (0.84, 1.07)	0.39
Dairy with red meat	**1.06 (1.01, 1.11)**	0.02
Dairy with processed red meat	**1.13 (1.04, 1.23)**	0.004
Dairy with beans	0.90 (0.76, 1.06)	0.21
Dairy with nuts	**0.88 (0.79, 0.97)**	0.01

Model was adjusted for age (continuous), sex, BMI (4 categories), and total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, and lag-time between T2D diagnosis and return of first FFQ

Table 2.8: HR (95% CI) of CVD incidence associated with isocaloric substitutions of selected nutrients for dairy fat in participants from both NHS and HPFS cohorts*

Replacement of dairy fat calories with	HR (95% CI)	*P*-value
Other animal fat (5% calories)	**1.08 (1.02, 1.14)**	0.007
Vegetable fat (5% calories)	0.98 (0.93, 1.04)	0.60
Polyunsaturated fat (5% calories)	0.90 (0.81, 1.00)	0.05
Total carbohydrates (5% calories)	1.02 (0.97, 1.07)	0.53
Carbohydrates from whole grains (5% calories)	0.97 (0.91, 1.04)	0.45
Carbohydrates from refined grains (5% calories)	1.04 (0.97, 1.10)	0.29
Omega-6 fatty acids (1% of calories)	**0.97 (0.94, 0.99)**	0.01
Omega-3 fatty acids (1% of calories)	1.06 (0.91, 1.25)	0.45

Model was adjusted for age (continuous), sex, BMI (4 categories), and total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1 0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and *trans* fat intake

We conducted an additional analysis to assess the association between consumption of dairy products without yogurt and CVD and there were no significant associations with risk of CVD, CHD or stroke (**Table S2.1**).

We conducted sensitivity analyses in which we stopped updating diet after cancer only or we stopped updating diet for cancer, CABG, angina, hypertension or hypercholesterolemia. The results did not change substantially in these sensitivity analyses (**Table S2.3**). The results of substitution analyses were similar when using the same strategies to process diet data. When diet was stopped for cancer only, the replacement of one serving of high-fat dairy with low-fat dairy became statistically significant [RR: 1.06; 95% CI: 1.01, 1.11]. Conversely, the replacement of one serving of dairy products with nuts was attenuated [RR: 0.95; 95% CI: 0.85, 1.05] when we stopped updating diet after self-reports of cancer, CABG, angina, hypertension or hypercholesterolemia (**Table S2.4**).

The associations for the replacement of calories of dairy fat with calories from other nutrients were also similar in these sensitivity analyses. The main differences included an attenuation of the replacement of 5% of calories from dairy fat with calories from other animal fat in the model where diet update was stopped after self-reports of cancer, CABG, angina, hypertension or hypercholesterolemia [RR: 1.00; 95% CI: 0.95, 1.05], and a strengthening of the association for replacement of 5% of dairy fat calories with vegetable fat following the same diet update protocol [RR: 0.93; 95% CI: 0.88, 0.99], as shown in **Table S2.5**. We evaluated the associations between dairy product intake and CVD risk at different time intervals to assess

potential reverse causation (**Table S2.6).** The associations were similar for total dairy intake, high-fat and low-fat dairy intake when we only used the FFQ cycle before diagnosis or the FFQ cycle after diagnosis (incident T2D cases only). We also evaluated the associations after placing a 4-year) or 8-year lag between dietary assessments and T2D incidence. The associations for total dairy and low-fat dairy product intake were similar to those in the main model. However, the association for high-fat dairy products became significant in the 4-yr lag model [RR: 0.94; 95% CI: 0.88, 0.98] and the 8-yr lag model [RR: 0.94; 95% CI: 0.88, 0.99]. The associations were similar after adjusting for either self-reported HbA1c, insulin use, or diabetes medication use in a subset of participants for total dairy, high-fat dairy, and low-fat dairy (**Tables S2.7 and S2.8**). We conducted a similar analysis for individual dairy products and the results were not statistically significant in the main model or after adjustment for HbA1c, or insulin use or diabetes medication except for the significant inverse association for ice-cream intake and CVD risk (**Tables S2.9 and S2.10**). In our lagged analysis, the estimates were not significant for individual dairy products in the 4-year lag and 8yr analysis. The only exception was significant association for ice-cream intake in the 4-yr lag [RR: 0.79; 95% CI: 0.65, 0.97] which was attenuated after placing an 8-yr lag [RR: 0.84; 0.68, 1.05]. (**Table S2.11).**

DISCUSSION

We found that consumption of dairy products, including low-fat dairy and high-fat dairy, was not significantly associated with incident CVD, CHD, or stroke in two large prospective cohorts of U.S. men and women with type 2 diabetes. In substitution analyses, we found that replacing dairy products with red meat or processed red meat was associated with an increased CVD risk while replacing dairy products with nuts was inversely associated with CVD in this

population. In secondary analysis, we found that dairy fat intake was not associated with risk of CVD, CHD, or stroke. However, the replacement of equivalent calories of dairy fat with omega-6 PUFAs was associated with a decreased CVD risk whereas the replacement with other animal fat sources was associated with a higher CVD risk.

To our knowledge, this is the first study that explicitly examined the prospective association between dairy product intake and incident CVD risk in two cohorts of participants with T2D. A previous study in the EPIC cohorts reported no associations between intakes of milk, yogurt, or cheese and total mortality.[37] In contrast to sparse data regarding dairy intake and CVD risk among diabetes patients, evidence is abundant among general populations. A recent meta-analysis of 29 cohorts summarized the association between dairy product intake and CVD risk among participants with various baseline risk profiles, and found no association for total dairy, high fat dairy, and low-fat dairy product intake or fermented dairy intake and CHD risk.[13] Previous studies in the NHS have reported no significant associations between high-fat and low-fat dairy product intake and CHD risk in multivariate models among participants free of disease at baseline.[32,34] Consumption of total dairy, cheese, yogurt, and butter were not associated with stroke risk in a meta-analysis of 18 prospective cohorts.[38] However, high-fat milk was positively associated with an increased stroke risk [RR for 200 g/day: 1.04, 95% CI: 1.02, 1.06].[38] Taken together, the pattern of associations between dairy intake and CVD risk in populations free of disease at baseline is similar to our findings in T2D patients.

The results of our substitution analyses replacing dairy products with other protein sources such as red meats, poultry, and fish are in agreement with previous findings among more general populations, including those in the NHS.[34] For example, in two recent meta-analyses, processed and unprocessed red meat were associated with a higher risk of developing incident stroke,[39] and processed red meat was associated with CHD[40]. Processed red meat contribute dietary heme iron which is positively associated with CHD[41] and substantial amounts of sodium which in elevated quantities may be associated with CVD risk.[42] The replacement of dairy products with nuts is consistent with the inverse association reported for nut consumption and CVD incidence [RR for one serving per day: 0.71; 95% CI: 0.59, 0.85] in a meta-analysis of 18 prospective cohorts.[43]

Dairy fat intake was not associated with risk of CVD, CHD, or stroke in our analyses. These results are consistent with a previous study from the HPFS, NHS , and NHSII conducted in participants free of disease at baseline where replacing 5% of calories from dairy fat with calories from carbohydrate (excluding that from fruits and vegetables) was not associated with CVD, CHD, or stroke [RR: 1.02; 95% CI: 0.98, 1.05].[36] That study also reported a positive association for the isocaloric replacement of dairy fat calories with calories from other animal fat [1.06; 95% CI: 1.02, 1.09] and an inverse association observed for replacement with omega-6 fatty acids and CVD risk [0.76; 95% CI: 0.71, 0.81].[36] Dairy fat contains about 60-65% SFAs and about 25% MUFAs[9–12] which have not been independently associated with incident CHD when compared with calories form carbohydrates.[33]

The substitution associations of omega-6 PUFAs are in agreement with previous studies from our cohorts in general populations where replacements of energy from SFAs with PUFAs were associated with a reduction in CHD risk.[33] A meta-analysis of 11 observational studies and 8 clinical trials also supports the consistent inverse associations between PUFA intake and risk of CHD, MI, or death when replacing calories from overall diet and calories from SFAs.[44] Substituting calories from PUFA for carbohydrate calories in clinical trials reduced total cholesterol, LDL-C, triglycerides (TGs), and increased HDL-C.[45] Moreover, substituting PUFAs for butter fat decreased inflammatory markers (IL-6 and TNFα) which suggests that lipid and inflammatory pathways may explain the observed associations.[46].

Besides their fat content which can range from 1% in low-fat milk to >30% in cream, dairy foods contain some important nutrients, including dairy proteins, lactose, calcium and vitamin D.[47] Dairy proteins, particularly whey protein, can stimulate insulin secretion[48] which may help manage hyperglycemia,[49] although their role in regulating post-prandial or fasting dyslipidemia is unclear.[50] Milk-derived peptides have been hypothesized to decrease blood pressure because they can inhibit angiotensin converting enzyme (ACE) regulating the conversion of angiotensin I to angiotensin II responsible for vasoconstriction *in-vitro*.[51] However, human trials have not related blood pressure reduction to ACE inhibition.[50] Calcium intake, particularly dairy calcium, was not associated with ischemic heart disease in a previous study in the HPFS[52] or to changes in body weight or fat mass.[53] On the other hand, calcium may enhance insulin sensitivity in peripheral tissues by regulating phosphorylation processes in the insulin receptor.[54] In a recent study, intake of vitamin D was inversely associated with TGs after five years but not associated

with blood lipids.[55] In the same study, vitamin D intake was not associated with stroke, myocardial infarction or all-cause mortality over 20 years of follow-up.[55] In the NHS and HPFS, total vitamin D intake was inversely associated with CVD risk in men but not in women.[56] The mechanisms behind these associations include decreases in inflammatory markers, amelioration of insulin sensitivity, regulation of blood pressure and inhibition of vascular calcification.[57]

In our study, ice-cream intake was inversely associated with CVD risk among diabetes patients. This result was unexpected given its considerable saturated fat content (6.7 g per 100g) and sugar (21.2g of per 100g) of which, the majority is added sugar (>60%) and the rest is comprised of naturally occurring lactose.[47] Nonetheless, in a previous study conducted among HPFS, the NHS, and the NHSII participants free of disease at baseline, ice-cream intake was also associated with a lower T2D risk.[18] This result was in agreement with the those from two additional cohort studies, and in a meta-analysis it was estimated that consuming 10g of ice-cream per day vs. 0 g was associated with a 19% lower T2D risk.[58] On the other hand, Ice-cream intake was not associated with risk of total stroke or stroke subtypes.[59] We performed multiple sensitivity analyses to explore whether such a surprising inverse association was due to methodological issues. To account for severity of diabetes, we also conducted a sensitivity analysis where we adjusted for self-reported information on HbA1c levels, or medication, or insulin use in a subset of participants and the results were not different from our main analysis results. We also conducted a sensitivity analyses where we stopped diet update after the diagnosis of hypertension and hypercholesterolemia and the results were consistent with the main model. The results were similar when we placed a 4-yr lag but attenuated after placing an

8-yr lag between T2D diagnosis and assessment of dietary intake which may suggest that reverse causation maybe partially responsible for the associations as health changes related to CVD may explain dietary choices, including ice-cream intake. There are few plausible biological explanations for these results. One possibility is that in dairy products such as milk, cheese, cream and ice cream, the membrane that coats fat globules, called milk-fat globule membrane (MFGM), is intact in contrast to products like butter that are stripped of their MFGM in the manufacturing process. MFGM is comprised of proteins, lipids, enzymes, and sphingolipids that form a triple-layered membrane designed to encase milk fat inside globular particles.[60–62] In feeding trials, intake of dairy products with intact MFGM such as whole milk, cream and cheese did not raise LDL-C and TG compared to intake of dairy products without MFGM (such as butter or butter oil).[60,63,64] Decreases in LDL-C and TGs were observed in trials where MFGM components without dairy fat were fed to participants in the form of buttermilk and compared to a placebo drink.[62,65] Nevertheless, this mechanism is unlikely to explain the specific inverse association observed for ice-cream because other dairy products with higher fat content than ice-cream (which is proportional to its MFGM concentration)[47] such as cheese (35%) and cream (20-36%) were not inversely associated with CVD risk in our study. Of note, the blood lipid profile resulting from intake of other fatty acids such as PUFAs and MUFAs[66] or plant-based sources such as olive oil are more favorable than those resulting from dairy fat intake with or without MFGM.[67]

Our study's strengths include its relatively large sample size of participants with diabetes, high follow-up rates in both cohorts (>90%), and repeated measurements of diet and covariates. However, our study also has some limitations. Our participants are health professionals of

predominantly European ancestry. Although the participant's educational background and socioeconomic status homogeneity can decrease confounding, it may limit the generalizability of findings to other populations that do not share these characteristics. Our assessment of dairy intake and other dietary and nutrient variables carries measurement error. However, the FFQ has been validated against diet records and the correlation coefficients between these methods were reasonable. Additionally, we used cumulative averages to decrease random measurement error and represent long-term intake. Moreover, the measurement errors were independent of disease diagnosis and are more likely to bias the association toward the null. Due to the observational nature of our study, our observed associations do not necessarily entail causation. Another limitation in our study is the lack of measurements of glycemic control and severity of diabetes for the full cohorts. However, our results were not different when compared to a subset of participants for which we had self-reported information on HbA1c levels, or medication, or insulin use which decreases the possibility that disease severity confounded the results. We used self-reported diabetes cases, which may lead to the inclusion of participants who are misclassified as being diagnosed with T2D or introduce selection bias by including only the most severe cases. However, we know from our validation study that >97% of self-reported incident cases are confirmed through the supplementary questionnaire which minimizes the risk of including participants without T2D.[20,21] By including prevalent cases but only counting their exposure and person-time contributions from the baseline questionnaire in each cohort, we may be introducing survival bias as only the T2D cases who did not develop CVD by the baseline questionnaire were included. This is also true for the incident T2D cases as the date of assessment of exposure (dairy intake) occurs after diabetes diagnosis (1-4 years for incident cases and ≥ 4-

yrs for prevalent cases). To address this, we adjusted for this time lag and compared the analysis with and without this covariate and the results were consistent. We also compared the analysis including and excluding prevalent T2D cases and the associations did not change. Finally, despite controlling for established and potential T2D risk factors, unmeasured and residual confounding can account for some of the associations.

CONCLUSION

Our study showed that consumption of dairy products was not significantly associated with CVD incidence among participants with diabetes, although replacing dairy products with nuts was associated with lower CVD risk and the replacement of dairy with red and processed red meat was associated with higher CVD risk. These results underscore the importance of quality of foods in the prevention of CVD in diabetes patients.

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Table S2.1: HRs (95% CI) of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke risk according to quintiles of dairy intake without yogurt in participants from both NHS and HPFS cohorts[*]

	Quintile of dairy intake						
	Q1[*]	Q2[*]	Q3[*]	Q4[*]	Q5[*]	*P*-trend[‡]	HR (95% CI) for one serving / day
			CVD				
Daily intake (servings)[§]	0.6	1.1	1.6	2.3	3.6		
Cases/person-years	7771/38,783	756/38,809	736/39,306	841/38,858	749/38,825		
Model 1[1]	1.00	0.96 (0.86, 1.06)	0.91 (0.82, 1.01)	1.03 (0.93, 1.13)	0.92 (0.84, 1.03)	0.45	0.99 (0.97, 1.01)
Model 2[2]	1.00	0.96 (0.86, 1.06)	0.93 (0.84, 1.03)	1.03 (0.93, 1.15)	0.95 (0.85, 1.06)	0.87	1.00 (0.97, 1.03)
Model 3[3]	1.00	0.96 (0.86, 1.07)	0.93 (0.84, 1.03)	1.04 (0.93, 1.15)	0.95 (0.85, 1.06)	0.80	1.00 (0.97, 1.02)
			CHD				
Cases/person-years	630	623	613	680	604		
Model 1[1]	1.00	0.97 (0.86, 1.08)	0.93 (0.83, 1.04)	1.01 (0.91, 1.13)	0.91 (0.82, 1.02)	0.98	0.98 (0.97, 1.01)
Model 2[2]	1.00	0.96 (0.86, 1.08)	0.94 (0.84, 1.06)	1.01 (0.90, 1.13)	0.93 (0.82, 1.05)	0.44	0.99 (0.96, 1.02)
Model 3[3]	1.00	0.97 (0.86, 1.08)	0.94 (0.84, 1.06)	1.01 (0.90, 1.13)	0.93 (0.82, 1.04)	0.38	0.99 (0.96, 1.02)
			STROKE				
Cases	152	141	131	173	157		
Model 1[1]	1.00	0.91 (0.72, 1.14)	0.83 (0.65, 1.05)	1.09 (0.87, 1.36)	1.00 (0.80, 1.25)	0.44	1.03 (0.98, 1.09)
Model 2[2]	1.00	0.93 (0.74, 1.18)	0.87 (0.68, 1.11)	1.16 (0.92, 1.46)	1.08 (0.85, 1.38)	0.16	1.06 (0.99, 1.12)
Model 3[3]	1.00	0.93 (0.74, 1.18)	0.87 (0.68, 1.11)	1.16 (0.92, 1.46)	1.08 (0.85, 1.38)	0.16	1.06 (0.99, 1.14)

[*]Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[‡]*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

[§] Median value of each quintile (all such values)

[1] Model 1 was adjusted for age (continuous) and sex

[2] Model 2 included Model 1 covariates and was additionally adjusted for BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, and lag-time between T2D return of first FFQ

[3]Model 3 included Model 2 covariates and was additionally adjusted for AHEI (quintiles)

Table S2.2 HRs (95% CI) fatal CVD (943 cases) according to quintiles of total dairy intake, high-fat and low-fat dairy intake in participants from both NHS and HPFS cohorts*

	Quintile of dairy intake						
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for one serving / day
Total dairy							
Daily intake (servings)§	0.7	1.4	1.9	2.6	3.9		
Cases	192/35,131	160/34,855	203/36,282	194/35,052	179/35,199		
Model 1[1]	1.00	0.79 (0.64, 0.98)	0.96 (0.79, 1.17)	0.88 (0.72, 1.08)	0.82 (0.67, 1.01)	0.16	0.97 (0.92, 1.02)
Model 2[2]	1.00	0.82 (0.66, 1.01)	1.03 (0.84, 1.27)	1.01 (0.81, 1.25)	0.92 (0.74, 1.16)	0.95	0.99 (0.94, 1.05)
Model 3[3]	1.00	0.83 (0.66, 1.02)	1.04 (0.85, 1.28)	1.02 (0.82, 1.26)	0.93 (0.74, 1.16)	0.94	0.99 (0.94, 1.04)
High-fat dairy products							
Daily intake (servings)§	0.07	0.32	0.57	0.93	1.72		
Cases/person-years	191/35,554	214/34,536	162/30,107	157/35,143	204/35,179		
Model 1[1]	1.00	1.14 (0.94, 1.39)	0.89 (0.72, 1.10)	0.82 (0.66, 1.02)	1.17 (0.95, 1.44)	0.41	1.03 (0.95, 1.12)
Model 2[2]	1.00	1.06 (0.86, 1.29)	0.80 (0.64, 0.99)	0.71 (0.56, 0.88)	1.01 (0.82, 1.25)	0.72	0.99 (0.91, 1.08)
Model 3[3]	1.00	1.06 (0.86, 1.29)	0.80 (0.64, 0.99)	0.70 (0.56, 0.88)	1.01 (0.82, 1.25)	0.69	0.99 (0.91, 1.07)
Low-fat dairy products	0.14	0.64	1.07	1.61	2.77		
Cases/person-years	204/34,841	143/32,254	199/41,791	198/32,227	184/35,029		
Model 1[1]	1.00	0.78 (0.63, 0.97)	0.72 (0.59, 0.88)	1.03 (0.84, 1.26)	0.77 (0.63, 0.95)	0.23	0.96 (0.89, 1.02)
Model 2[2]	1.00	0.86 (0.69, 1.07)	0.95 (0.77, 1.16)	1.23 (1.00, 1.52)	0.90 (0.73, 1.12)	0.98	0.99 (0.92, 1.05)
Model 3[3]	1.00	0.86 (0.69, 1.07)	0.94 (0.77, 1.16)	1.23 (1.00, 1.52)	0.90 (0.73, 1.12)	0.96	0.99 (0.92, 1.05)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

‡*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

§ Median value of each quintile (all such values)

[1] Model 1 was adjusted for age (continuous) and sex

[2] Model 2 included Model 1 covariates and was additionally adjusted for BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ

[3]Model 3 included Model 2 covariates and was additionally adjusted for AHEI (quintiles) and mutually adjusted for other dairy product categories

Table S2.3 HRs (95% CI) of cardiovascular disease (CVD) risk according to intakes of various dairy foods in participants with different diet update approaches in participants from both NHS and HPFS cohorts*

	HR (95% CI) for one serving / day		
	Main model[1]	Cancer only[2]	HBP/HC[3]
Total dairy	1.00 (0.97, 1.02)	1.01 (0.98, 1.04)	0.99 (0.97, 1.02)
High-fat dairy	0.96 (0.92, 1.00)	0.97 (0.93, 1.01)	0.96 (0.93, 1.01)
Low-fat dairy	1.02 (0.98, 1.05)	1.02 (0.99, 1.05)	1.02 (0.98, 1.05)
Cheese	1.00 (0.94. 1.07)	1.00 (0.94. 1.06)	1.00 (0.92, 1.08)
Skim/low-fat milk	1.00 (0.96, 1.04)	1.01 (0.97, 1.04)	1.01 (0.96, 1.07)
Whole milk	1.04 (0.94, 1.16)	1.02 (0.91, 1.13)	1.04 (0.94, 1.14)
Yogurt	0.98 (0.85, 1.13)	0.99 (0.86, 1.15)	1.03 (0.83, 1.26)
Fermented dairy products	1.00 (0.94, 1.06)	1.00 (0.94, 1.06)	1.00 (0.90. 1.09)
Cream	0.98 (0.91, 1.05)	0.98 (0.90, 1.05)	1.03 (0.94, 1.12)
Ice cream	0.82 (0.67, 0.99)	0.79 (0.64, 0.96)	0.79 (0.64, 0.96)
Sherbet	0.92 (0.78, 1.11)	0.92 (0.76, 1.09)	1.29 (0.73, 1.26)
Butter[¥]	1.00 (0.95, 1.06)	0.99 (0.94, 1.05)	1.03 (0.98, 1.07)
Dairy fat (1% calories) [§]	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)	1.00 (0.99, 1.01)

* HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[1] Model was adjusted for age (continuous), sex, BMI (4 categories), and total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, AHEI, and mutually adjusted for other dairy products. Diet update was stopped after diagnosis of cancer, CABG, or angina

[2] Diet update was stopped after diagnosis of cancer only

[3] Diet update was stopped after diagnosis of cancer, CABG, angina, hypertension or hypercholesterolemia

[¥] Model for butter analysis was additionally adjusted for total dairy product intake

[§] Model for dairy fat analysis was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and trans fat

Table S2.4: HR (95% CI) of CVD incidence associated with isocaloric substitutions of selected dairy products with different diet update approaches in participants from both NHS and HPFS cohorts*

	HR (95% CI) for one serving / day		
Replacement	Main model[1]	Cancer only[2]	HBP/HC[3]
High-fat dairy with low-fat dairy	1.04 (0.99, 1.10)	1.06 (1.01, 1.11)	1.04 (0.99, 1.10)
Dairy w/o yogurt with yogurt	0.97 (0.84, 1.12)	0.98 (0.85, 1.13)	0.97 (0.84, 1.12)
Whole milk with skim milk	0.94 (0.86, 1.02)	0.94 (0.87, 1.04)	0.93 (0.87, 1.00)
Dairy with fish	0.98 (0.86, 1.10)	1.00 (0.89, 1.13)	1.01 (0.90, 1.14)
Dairy with poultry	0.95 (0.84, 1.07)	0.92 (0.81, 1.03)	0.99 (0.88, 1.12)
Dairy with red meat	1.06 (1.01, 1.11)	1.06 (1.01, 1.11)	1.06 (1.01, 1.11)
Dairy with processed red meat	1.13 (1.04, 1.23)	1.13 (1.04, 1.23)	1.13 (1.05, 1.22)
Dairy with beans	0.90 (0.76, 1.06)	0.92 (0.78, 1.09)	0.97 (0.83, 1.14)
Dairy with nuts	0.88 (0.79, 0.97)	0.86 (0.78, 0.95)	0.95 (0.85, 1.05)

[1] Model was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, AHEI, Diet update was stopped after diagnosis of cancer, CABG, or angina

[2] Diet update was stopped after diagnosis of cancer only 3 Diet update was stopped after diagnosis of cancer, CABG, angina, hypertension or hypercholesterolemia

Table S2.5: HR (95% CI) of CVD incidence associated with isocaloric substitutions of selected nutrients for dairy fat different diet update approaches in participants from both NHS and HPFS cohorts*

	HR (95% CI)		
Replacement of dairy fat calories with	Main model[1]	Cancer only[2]	HBP/HC[3]
Other animal fat (5% calories)	1.08 (1.02, 1.14)	1.10 (1.04, 1.16)	1.00 (0.95, 1.05)
Vegetable fat (5% calories)	0.98 (0.93, 1.04)	1.03 (0.97, 1.09)	0.93 (0.88, 0.99)
Polyunsaturated fat (5% calories)	0.90 (0.81, 1.00)	0.94 (0.85, 1.05)	0.92 (0.83, 1.02)
Total carbohydrates (5% calories)	1.02 (0.97, 1.07)	1.05 (1.00, 1.11)	0.97 (0.93, 1.02)
Carbohydrates from whole grains (5% calories)	0.97 (0.91, 1.04)	1.02 (0.95, 1.10)	0.94 (0.87, 1.02)
Carbohydrates from refined grains (5% calories)	1.04 (0.97, 1.10)	1.08 (1.01, 1.15)	0.98 (0.92, 1.04)
Omega-6 fatty acids (1% of calories)	0.97 (0.94, 0.99)	0.98 (0.95, 1.00)	0.98 (0.96, 1.00)
Omega-3 fatty acids (1% of calories)	1.06 (0.91, 1.25)	1.09 (0.93, 1.28)	0.97 (0.81, 1.18)

[1] Model was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and trans fat intake

Table S2.6: HRs (95% CI) of cardiovascular disease (CVD), according to quintiles of total dairy intake in participants from both NHS and HPFS cohorts after with various exposure timing protocols*

	Quintile of dairy intake						
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for one serving / day
Total Dairy							
Main model[1]	1.00	1.03 (0.93, 1.14)	1.02 (0.92, 1.13)	0.99 (0.89, 1.10)	0.98 (0.88, 1.10)	0.49	1.00 (0.97, 1.02)
§Intake before T2D diagnosis[2]	1.00	0.95 (0.83. 1.09)	1.06 (0.93, 1.21)	0.93 (0.81. 1.06)	0.97 (0.84, 1.11)	0.53	1.00 (0.97, 1.03)
§Intake after T2D diagnosis[3]	1.00	0.90 (0.78, 1.01)	1.05 (0.93, 1.20)	0.97 (0.85, 1.10)	0.90 (0.78, 1.02)	0.22	0.99 (0.96, 1.02)
4-yr lag[4]	1.00	0.98 (0.88, 1.10)	1.00 (0.89, 1.11)	0.91 (0.82, 1.02)	0.96 (0.86, 1.08)	0.98	0.99 (0.96, 1.02)
8-yr lag[5]	1.00	0.98 (0.87, 1.11)	0.98 (0.87, 1.11)	0.96 (0.84, 1.09)	0.93 (0.82, 1.07)	0.28	1.00 (0.97, 1.03)
High-fat Dairy							
Main model[1]	1.00	1.02 (0.92, 1.13)	0.91 (0.82, 1.01)	0.93 (0.83, 1.03)	0.94 (0.84, 1.04)	0.14	0.96 (0.92, 1.00)
§Intake before T2D diagnosis[2]	1.00	1.07 (0.93, 1.24)	1.12 (0.97, 1.28)	1.10 (0.95, 1.26)	1.07 (0.93, 1.24)	0.54	1.02 (0.97, 1.06)
§Intake after T2D diagnosis[3]	1.00	0.92 (0.81, 1.04)	0.93 (0.81, 1.06)	0.88 (0.77, 1.00)	0.92 (0.81, 1.06)	0.45	0.98 (0.94, 1.03)
4-yr lag[4]	1.00	0.98 (0.88, 1.09)	0.91 (0.81, 1.01)	0.90 (0.81, 1.00)	0.89 (0.79, 1.00)	0.03	0.94 (0.89, 0.98)
8-yr lag[5]	1.00	0.98 (0.87, 1.11)	0.91 (0.80, 1.04)	0.95 (0.83, 1.07)	0.91 (0.80, 1.04)	0.16	0.94 (0.89, 0.99)
Low-fat dairy							
Main model[1]	1.00	1.01 (0.91, 1.12)	1.05 (0.94, 1.16)	1.03 (0.93, 1.15)	1.03 (0.93, 1.15)	0.61	1.02 (0.98, 1.05)
§Intake before T2D diagnosis[2]	1.00	0.97 (0.84, 1.11)	0.97 (0.85, 1.12)	0.95 (0.82, 1.10)	0.87 (0.75, 1.00)	0.05	0.97 (0.93, 1.01)
§Intake after T2D diagnosis[3]	1.00	1.06 (0.93, 1.20)	0.97 (0.85, 1.11)	1.06 (0.93, 1.21)	0.94 (0.82, 1.07)	0.24	0.99 (0.95, 1.02)
4-yr lag[4]	1.00	1.02 (0.94, 1.18)	1.08 (0.97, 1.20)	1.02 (0.90, 1.14)	1.04 (0.93, 1.17)	0.81	1.02 (0.98, 1.05)
8-yr lag[5]	1.00	1.02 (0.89, 1.17)	1.09 (0.96, 1.24)	1.06 (0.93, 1.22)	1.07 (0.94, 1.22)	0.35	1.03 (0.99, 1.07)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

‡*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable

§ Incident cases after 1980 in the NHS and 1986 in the HPFS

[1] Main model 1 was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and AHEI (quintiles). Models for high-fat dairy and low-fat dairy product categories were mutually adjusted

[2] Model used dairy intake in the questionnaire immediately before T2D diagnosis as the exposure

[3] Model used dairy intake in the questionnaire at or immediate after T2D diagnosis as the exposure

[4] An average 4-yr lag was used between T2D diagnosis and beginning of follow-up

[5] An average 8-yr lag was used between T2D diagnosis and beginning of follow-up

Table S2.7: HRs (95% CI) of cardiovascular disease (CVD: 429 cases) risk according to quintiles of total dairy, high-fat, and low-fat dairy intake in participants from the HPFS who had self-reported HbA1c levels*

	Quintile of intake						
	Q1*	Q2*	Q3*	Q4*	Q5*	*P*-trend‡	HR (95% CI) for one serving / day
Total Dairy							
Main model[1]	1.00	1.12 (0.81, 1.55)	1.05 (0.75, 1.47)	0.77 (0.54, 1.09)	0.85 (0.59, 1.22)	0.08	0.95 (0.88, 1.03)
Adjusted for HbA1c[2]	1.00	1.12 (0.81, 1.54)	1.05 (0.75, 1.46)	0.76 (0.53, 1.08)	0.85 (0.59, 1.22)	0.08	0.95 (0.88, 1.03)
High-Fat Dairy							
Main model[1]	1.00	1.08 (0.79, 1.47)	0.86 (0.61, 1.19)	1.05 (0.75, 1.45)	0.94 (0.66, 1.35)	0.72	1.01 (0.89, 1.14)
Adjusted for HbA1c[2]	1.00	1.08 (0.79, 1.48)	0.86 (0.61, 1.19)	1.05 (0.75, 1.45)	0.94 (0.66, 1.35)	0.71	1.01 (0.89, 1.14)
Low-Fat Dairy							
Main model[1]	1.00	1.24 (0.90, 1.72)	0.72 (0.47, 1.09)	0.86 (0.61, 1.22)	0.82 (0.60, 1.12)	0.07	0.91 (0.81, 1.01)
Adjusted for HbA1c[2]	1.00	1.25 (0.90, 1.73)	0.71 (0.47, 1.08)	0.86 (0.60, 1.21)	0.81 (0.60, 1.11)	0.07	0.91 (0.81, 1.01)

*Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

‡*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

[1] Main model was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and AHEI (quintiles)

[2] Model further adjusted for HbA1c (<7, 7-7.9, 8-9.9, 10-11.9, ≥12 %)

Table S2.8: HRs (95% CI) of cardiovascular disease (CVD: 1,485 cases) risk according to quintiles of total dairy, high-fat, and low-fat dairy intake in participants from the NHS and the HPFS who use diabetes medication and/or insulin[*]

	Quintile of intake						
	Q1[*]	Q2[*]	Q3[*]	Q4[*]	Q5[*]	*P*-trend[‡]	HR (95% CI) for one serving / day
Total Dairy							
Main model[1]	1.00	1.05 (0.89, 1.23)	1.05 (0.88, 1.24)	0.99 (0.83, 1.18)	0.99 (0.83, 1.19)	0.67	1.00 (0.96, 1.04)
Adjusted for medication and insulin use[2]	1.00	1.05 (0.88, 1.23)	1.05 (0.88, 1.24)	0.99 (0.83, 1.18)	0.99 (0.82, 1.18)	0.66	1.00 (0.96, 1.04)
High-Fat Dairy							
Main model[1]	1.00	0.98 (0.84, 1.15)	0.85 (0.72, 1.00)	0.82 (0.69, 0.97)	0.85 (0.71, 1.00)	0.05	0.94 (0.88, 1.01)
Adjusted for medication and insulin use[2]	1.00	0.98 (0.83, 1.14)	0.85 (0.72, 1.00)	0.82 (0.69, 0.97)	0.85 (0.71, 1.00)	0.05	0.94 (0.88, 1.01)
Low-Fat Dairy							
Main model[1]	1.00	0.98 (0.82, 1.17)	1.14 (0.97, 1.35)	1.02 (0.86, 1.21)	1.08 (0.91, 1.28)	0.39	1.03 (0.98, 1.08)
Adjusted for medication and insulin use[2]	1.00	0.99 (0.83, 1.17)	1.15 (0.98, 1.36)	1.02 (0.86, 1.22)	1.08 (0.91, 1.29)	0.39	1.03 (0.98, 1.08)

[*]Q is quintile, HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[‡]*P*-trend was calculated by assigning median values to each quintile and was treated as continuous variable.

[1] Main model was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, and AHEI (quintiles)

[2] Model further adjusted for insulin use and diabetes medication use (Sulfonylurea, Metformin, Troglitazone, Rosiglitazone, Pioglitazone, Acarbose, or other diabetes medication)

Table S2.9 HRs (95% CI) of cardiovascular disease (CVD: 429 cases) risk according to intake of various dairy foods in participants from both NHS and HPFS cohorts who had self-reported HbA1c levels *

	HR (95% CI) for one serving / day	
	Model 1[1]	Model 2[2]
Cheese	1.03 (0.88, 1.20)	1.03 (0.88, 1.21)
Skim/low-fat milk	0.92 (0.83, 1.03)	0.92 (0.83, 1.03)
Whole milk	0.95 (0.68, 1.32)	0.94 (0.68, 1.32)
Yogurt	0.75 (0.44, 1.27)	0.75 (0.44, 1.27)
Fermented dairy products	0.98 (0.79, 1.16)	0.98 (0.80, 1.17)
Cream	1.02 (0.83, 1.26)	1.02 (0.83, 1.26)
Ice cream	0.61 (0.30, 1.26)	0.61 (0.30, 1.26)
Sherbet	0.93 (0.60, 1.43)	0.92 (0.59, 1.42)

* HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[1] Model 1 was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, AHEI, and mutually adjusted for other dairy products.

[2] Model 2 was further adjusted for HbA1c (<7, 7-7.9, 8-9.9, 10-11.9, ≥12 %)

Table S2.10 HRs (95% CI) of cardiovascular disease (CVD: 1,485 cases) risk according to intake of various dairy foods in participants from both NHS and HPFS cohorts who reported insulin use, or diabetes medication use*

	HR (95% CI) for one serving / day		
	Model 1[1]	Model 2[2]	Model 3[3]
Cheese	0.97 (0.87, 1.07)	0.96 (0.87, 1.07)	0.97 (0.87, 1.07)
Skim/low-fat milk	0.98 (0.91, 1.06)	0.98 (0.90, 1.06)	0.98 (0.91, 1.06)
Whole milk	0.96 (0.74, 1.24)	0.96 (0.74, 1.25)	0.95 (0.73, 1.24)
Yogurt	1.10 (0.87, 1.40)	1.09 (0.86, 1.39)	1.10 (0.86, 1.39)
Fermented dairy products	0.96 (0.88, 1.05)	0.96 (0.88, 1.04)	0.96 (0.88, 1.05)
Cream	0.92 (0.78, 1.09)	0.93 (0.78, 1.09)	0.92 (0.78, 1.09)
Ice cream	0.67 (0.48, 0.93)	0.67 (0.48, 0.94)	0.67 (0.49, 0.95)
Sherbet	1.16 (0.86, 1.58)	1.17 (0.87, 1.59)	1.17 (0.86, 1.58)

* HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[1] Model 1 was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, AHEI, and mutually adjusted for other dairy products.

[2] Model 2 was further adjusted for insulin use

[3] Model 3 included covariates in model 1 and diabetes medication use (Sulfonylurea, Metformin, Troglitazone, Rosiglitazone, Pioglitazone, Acarbose, or other diabetes medication)

Table S2.11 HRs (95% CI) of cardiovascular disease (CVD) risk according to intake of various dairy foods in participants from both NHS and HPFS cohorts after with various exposure timing protocols*

	HR (95% CI) for one serving / day				
	Model 1[1]	Model 2[2]	Model 3[3]	Model 4[4]	Model 5[5]
Cheese	1.00 (0.94, 1.07)	0.94 (0.84, 1.05)	0.92 (0.82, 1.03)	1.00 (0.93, 1.07)	1.01 (0.93, 1.08)
Skim/low-fat milk	1.00 (0.96, 1.04)	0.97 (0.90, 1.04)	0.99 (0.92, 1.06)	1.02 (0.94, 1.10)	0.98 (0.87, 1.08)
Whole milk	1.04 (0.94, 1.16)	1.08 (0.97, 1.19)	1.06 (0.95, 1.17)	1.14 (0.98, 1.33)	1.17 (0.99, 1.37)
Yogurt	0.98 (0.85, 1.13)	0.96 (0.86, 1.20)	1.00 (0.90, 1.10)	1.06 (0.92, 1.22)	1.08 (0.88, 1.23)
Fermented dairy products	1.00 (0.94, 1.06)	0.93 (0.88, 1.03)	0.91 (0.86, 1.01)	1.01 (0.95, 1.08)	1.01 (0.94, 1.09)
Cream	0.98 (0.91, 1.05)	0.97 (0.83, 1.15)	0.96 (0.81, 1.13)	0.89 (0.77, 1.00)	0.88 (0.90, 1.00)
Ice cream	0.82 (0.67, 0.99)	0.80 (0.65, 0.99)	0.78 (0.63, 0.98)	0.79 (0.65, 0.97)	0.84 (0.68, 1.05)
Sherbet	0.92 (0.78, 1.11)	1.00 (0.75, 1.34)	0.98 (0.73, 1.31)	0.90 (0.70, 1.16)	0.87 (0.65, 1.16)

* HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[1] Model 1 was adjusted for age (continuous), sex, BMI (4 categories), total energy intake (quintiles), race, menopausal status [pre or postmenopausal (never, past or current menopausal hormone use)], family history of diabetes (yes/no), family history of myocardial infarction (yes/no), alcohol intake (0, 1-4.9, 5-14.9, >15 g/day), smoking status (never, past, current 1-15 cigarettes/day, >15 cigarettes/day), physical activity (0, 0.1-0.9, 1-3.5, >3.5 hrs./week) current aspirin use (yes/no), current multivitamin use (yes/no), diabetes duration (<5, 5-10, >10 years), baseline hypertension, baseline hypercholesterolemia, lag-time between T2D diagnosis and return of first FFQ, AHEI, and mutually adjusted for other dairy products.

[2] Model used dairy intake in the questionnaire immediately before T2D diagnosis as the exposure (incident T2D cases sonly)

[3] Model used dairy intake in the questionnaire at or immediate after T2D diagnosis as the exposure (incident T2D cases only)

[4] An average 4-yr lag was used between T2D diagnosis and beginning of follow-up

[5] An average 8-yr lag was used between T2D diagnosis and beginning of follow-up

Table S2.12 HRs (95% CI) Spearman correlations between intakes of various dairy products after T2D diagnosis

Correlation (p- value)	Total Dairy	High-fat Dairy	Low-fat Dairy	Cheese	Cream	Fermented Dairy	Ice Cream	Sherbet	Skim Milk	Whole Milk	Yogurt
Total Dairy	1.00										
High-Fat Dairy	0.45 <0.001	1.00									
Low-fat Dairy	0.77 <0.0001	-0.08 <0.0001	1.00								
Cheese	0.49 <0.0001	0.56 <0.0001	0.24 <0.0001	1.00							
Cream	0.26 <0.0001	0.41 <0.0001	0.01 0.0004	0.12 <0.0001	1.00						
Fermented Dairy	0.57 <0.0001	0.47 <0.0001	0.37 <0.0001	0.89 <0.0001	0.12 <0.0001	1.00					
Ice Cream	0.17 <0.0001	0.46 <0.0001	-0.06 <0.0001	0.11 <0.0001	0.10 <0.0001	0.07 <0.001	1.00				
Sherbet	0.21 <0.0001	-0.07 <0.0001	0.32 <0.0001	0.05 <0.0001	0.04 <0.0001	0.12 <0.0001	-0.02 <0.0001	1.00			
Skim Milk	0.63 <0.0001	-0.07 <0.0001	0.83 <0.0001	0.03 <0.0001	0.12 <0.0001	0.11 <0.0001	-0.01 0.003	0.12 <0.0001	1.00		
Whole Milk	0.08 <0.0001	0.37 <0.0001	-0.20 <0.0001	0.03 <0.0001	0.09 <0.0001	0.001 0.92	0.17 <0.0001	-0.06 <0.0001	-0.20 <0.0001	1.00	
Yogurt	0.22 <0.0001	-0.01 0.0002	0.28 <0.0001	0.12 <0.0001	0.05 <0.0001	0.32 <0.0001	-0.04 <0.0001	0.21 <0.0001	0.12 <0.0001	-0.04 <0.0001	1.00

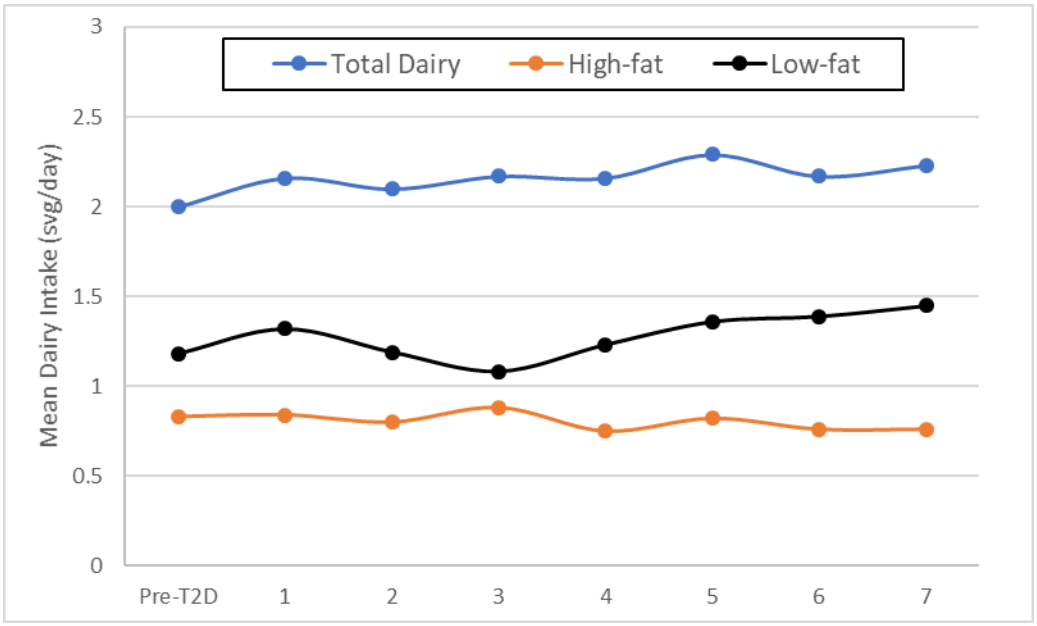


Figure S2.1. Trends of dairy consumption in T2D Patients (NHS and HPFS)

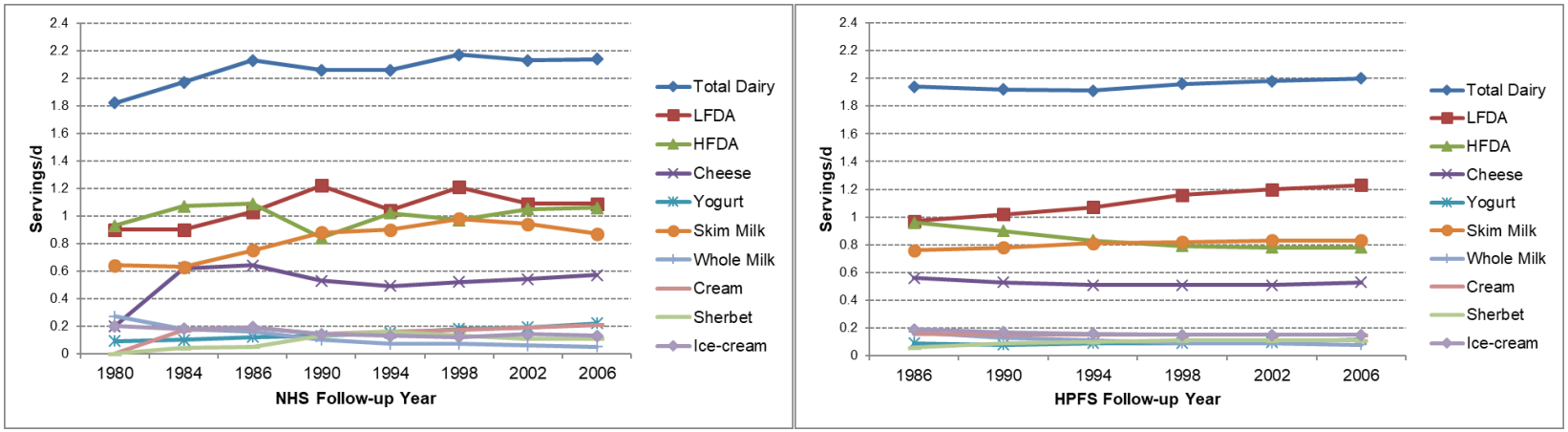


Figure S2.2: Trends of dairy consumption in NHS and HPFS (full cohorts) Chen et al. 2014

CHAPTER 3:

Circulating Very-Long Chain Saturated Fatty Acids and Incident Type 2 Diabetes in U.S. Men and Women

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ABSTRACT

Background: very-long chain saturated fatty acids (VLCSFAs), such as 20:0, 22:0, and 24:0, are robustly associated with multiple cardiometabolic conditions, although data for type 2 diabetes (T2D) are still sparse. In this study, we examined the association between plasma and erythrocyte levels of VLCSFAs and incident T2D risk.

Methods: We used existing measurements of fatty acid levels in plasma and erythrocytes among 1,392 women in the Nurses' Health Study and 1,462 men in the Health Professionals Follow-Up Study. VLCSFAs were measured using gas-liquid chromatography and levels of individual fatty acids were expressed as a percentage of total fatty acids. Incident T2D cases were identified by self-reports and confirmed by a validated supplementary questionnaire. Cox proportional hazards regression was used to evaluate the association between each VLCSFA and T2D, adjusting for demographic, lifestyle, and dietary variables.

Results: The mean (standard deviation) plasma level of 20:0, 22:0, and 24:0 was 0.20% (0.06%), 0.52% (0.17%), and 0.40% (0.17%), respectively. Multiple dietary factors, including peanuts, peanut butter, vegetable fat, dairy fat, and dietary 16:0-20:0, were significantly correlated with plasma and erythrocyte VLCSFA levels. In addition, plasma VLCSFAs were positively correlated with physical activity. After controlling for these factors and other covariates, comparing extreme quartiles of plasma levels, 20:0, 22:0, 24:0, and their sum were associated with a lower T2D risk; the pooled hazard ratios (HR; 95% CIs) were 0.50 (0.34, 0.73) for 20:0, 0.42 (0.28, 0.64) for 22:0; 0.40 (0.26, 0.61) for 24:0; and 0.40 (0.26, 0.61) for the sum of VLCSFAs. Levels of 20:0 and 22:0, but not 24:0 in erythrocyte membranes, were significantly

associated with a decreased T2D risk: pooled HRs (95% CIs) were: 0.58 (0.39, 0.87) for 20:0 and 0.51 (0.34, 0.77) for 22:0, comparing extreme quartiles.

Conclusions: Our findings suggest that in US men and women, plasma levels VLCSFAs are associated with a lower risk of T2D. More research is needed to understand the mechanistic pathways underlying these associations.

INTRODUCTION

Type 2 diabetes (T2D) is a leading public health issue and has numerous complications ranging from cardiovascular disease, renal disease, retinopathy and amputations.[1] Many dietary, genetic, lifestyle, and metabolic risk factors of T2D have been identified in large epidemiological studies.[2,3] However, fatty acids, exogenously ingested or endogenously synthesized, have been explored only recently as potential risk factors for T2D.[4] Of these fatty acids, very-long chain saturated fatty acids (VLCSFAs) attract much research interests recently. VLCSFAs include fatty acids with chain length ≥20, of which the most commonly examined in epidemiological studies are arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0). VLCSFAs are present in low levels in food products such as peanuts and peanut butter, canola oil, macadamia nuts, and dairy fat.[5] However, the main source of these fatty acids in mammalian tissues is endogenous synthesis by elongation in the endoplasmic reticulum from saturated fatty acids with a chain length of 12:0-18:0, which is mediated via a set of seven fatty acid elongases (ELOVL 1-7) embedded in the endoplasmic reticulum.[6–8] In multiple studies conducted recently, VLCSFAs were inversely associated with multiple cardiovascular health outcomes, such as CHD,[9] sudden cardiac arrest,[10] and atrial fibrillation.[11] Several epidemiological studies have examined the

association between circulating levels of VLCSFAs and T2D, although data were still sparse and somewhat mixed. For example, studies from EPIC-InterAct[12] and Cardiovascular Health Study (CHS) have demonstrated consistent inverse associations for all three VLCSFAs in plasma,[13] although the association for erythrocyte VLCSFA levels was unclear.[14] Therefore, it is of interest to further investigate the association between levels of VLCSFAs in both plasma and erythrocytes and T2D risk and also to explore dietary and lifestyle predictors of these fatty acids. We hypothesize that levels of 20:0, 22:0, and 24:0 in plasma and erythrocyte membranes are inversely associated with T2D risk.

METHODS

Study population: The Nurses' Health Study (NHS) was established in 1976 with a recruitment of 121,700 female nurses ages 30 - 55 who responded to a questionnaire inquiring information related to their health, lifestyle practices and occurrence of chronic diseases.[15] The Health Professionals Follow-Up Study (HPFS) started in 1986, when 51,529 male health professionals, who were 40 – 75 years of age at recruitment in 1986.[16] In both cohorts, questionnaires were administered at baseline to gather information on lifestyle practices and occurrence of chronic diseases. The questionnaires were repeated biennially to update information on these variables. The cumulative follow-up of the participants in these cohorts was all >90%.[17] A subset of participants from the NHS and the HPFS, whose lifestyle and dietary variables were similar to the overall cohorts provided blood samples in 1990 and 1994, respectively.[18,19]

Study design: For this analysis, we used previously measured fatty acid levels in plasma and erythrocytes generated through two nested case-control studies of cardiovascular disease (CVD) in the NHS and HPFS studies. Study participants were free of CVD and cancer at the time of blood draw.[9] In the current analysis, we excluded men and women who had diagnoses of diabetes at blood draw. We also excluded participants who had missing fatty acid data and participants with missing diabetes diagnostic date a. We further excluded plasma and erythrocyte VLCSFA outlier measurements (9 for NHS and 8 for HPFS VLCSFA plasma values and 1 for NHS and 2 for HPFS erythrocyte values) identified using the Generalized Extreme Studentized deviate method.[20,21] After exclusions, data from 2,854 participants were available for analysis of plasma samples and 2,831 with erythrocyte fatty acid data. Person-years were calculated from the date of blood sampling to the date when participants developed T2D, death or censoring at return of the last questionnaire through 2012. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. Return of a completed questionnaire was considered as informed consent.

Measurement of circulating biomarkers of VLCSFAs: Participant's blood samples were returned to the laboratory with a cold pack via overnight courier and most the samples arrived within 24 hours. Upon arrival, samples were centrifuged and divided into aliquots for plasma, buffy coat, and erythrocytes, and stored in liquid nitrogen freezers at ≤-130°C.[22,23] Fatty acid levels in plasma and erythrocytes were analyzed by gas-liquid chromatography at the Department of Nutrition, Harvard T.H. Chan School of Public Health. Procedures for fatty acid analyses in NHS and HPFS have been published previously.[23–26] The average intra-assay

coefficients of variation (CV) derived from measurements of quality control samples were 5.3% for 20:0, 6.0% for 22:0, and 7.7% for 24:0 for NHS plasma assessments of fatty acids. Corresponding CVs in NHS erythrocyte measurements were 7.2%, 10.6%, and 11.2%. For the HPFS samples, the average intra-assay CVs for quality-controls in plasma samples were 9.3% for 20:0, 11.2% for 22:0, and 13.4% for 24:0. Corresponding CVs in HPFS erythrocyte measurements were 12.2%, 14.6%, and 18.2%. Levels of individual circulating fatty acids were expressed as a percentage of total fatty acids either in plasma or erythrocyte membranes and we used the circulating biomarkers of 20:0, 22:0, 24:0 and the sum of these three fatty acids as the exposure of interest.[25]

Ascertainment of T2D cases: Incident cases of T2D are identified by self-reports on the mail questionnaires and confirmed by a supplementary questionnaire inquiring diagnosis date, symptoms, blood glucose levels and medication use. Self-reported diagnosis of type 2 diabetes was confirmed using the following criteria from the National Diabetes Data Group (NDDG) up until 1998[27]: (1) manifestation of classic symptoms such as excessive thirst, polyuria, weight loss and hunger, in conjunction with elevated fasting glucose ≥140 mg/dL (7.77 mmol/L) or non-fasting glucose levels ≥200 mg/dL (11.1 mmol/L) (2) asymptomatic but elevated plasma glucose in two separate occasions or abnormal glucose tolerance test results and (3) receiving any hypoglycemic treatment for diabetes. After 1998, a fasting glucose concentration ≥126 mg/dL (6.99 mmol/L) was adopted per the new diagnostic criteria of the American Diabetes Association (ADA). This supplementary questionnaire has been validated in previous studies with >97%

questionnaire-confirmed cases reconfirmed through medical record review by a blinded study physician.[28,29]

Covariate Assessment. In the biennial follow-up questionnaires, we inquired about and updated information on risk factors for chronic diseases, such as body weight, cigarette smoking, physical activity, medication use, as well as history of chronic diseases, including diabetes, hypertension and hypercholesterolemia. Among the NHS participants, we ascertained menopausal status, postmenopausal hormone use, and oral contraceptive use in the questionnaires. Diet information was collected using food frequency questionnaires (FFQ) with 61 items that was first administered in 1980 in the NHS. The FFQ was subsequently expanded to include 131 items and administered in 1984, 1986, and every 4 years thereafter. The expanded FFQs have been administered quadrennially in the HPFS since 1986.[30,31] Participants were asked about consumption frequency of food with a pre-specified portion size over the prior year by selecting from nine frequency categories ranging from "never" to "6 or more per day". The reproducibility and validity of these FFQs have been previously described in detail.[32–34] Total nutrient intake is calculated by multiplying the consumption frequency times the nutrient content of each item and summing the contributions of each food to derive total intake. Nutrient composition data were obtained from the Harvard University Food Composition Database. To measure diet quality, we used a modified version of the 2010 Alternative Healthy Eating Index (AHEI) score which summarizes the intake of 10 dietary components that are most predictive of chronic disease risk: higher intakes of fruits, vegetables, whole grains, nuts, long-chain omega-3 fatty acids, and polyunsaturated fatty acids (PUFAs), and lower intakes of sugar sweetened

beverages, red and processed meats, *trans* fatty acids (TFAs), and sodium.[35] Each component was scored from 0 to 10 where 10 indicated best adherence to recommended servings per day, and 0 for the poorest adherence.

Statistical analyses: For this analysis, we categorized participants into quartiles of 20:0, 22:0, and 24:0 and their sum, respectively. We used a Cox proportional hazards regression to model the association between circulating biomarkers of 20:0, 22:0, and 24:0 and their sum and T2D risk. Fatty acids were also evaluated continuously to estimate associations per one standard deviation (SD) change of fatty acids. To increase statistical power, we used data from both CVD cases and controls. To evaluate potential heterogeneity of associations between CVD cases and controls, we examined the significance of interaction terms of VLCSFAs (per SD change) and case-control status of the original CVD studies, and all the interaction terms were not statistically significant ($P > 0.05$), suggesting that the main associations were unlikely dependent on CVD case-control status. To examine linear trend, we assigned the median intake within each quartile and modeled this variable continuously. Person-time was counted from the time of blood draw until T2D diagnosis, death, or censoring at return of the last questionnaire, whichever came first.

The basic model was adjusted for age and BMI. The multivariate model was additionally adjusted for race/ethnicity (white, nonwhite), physical activity (METs per week), smoking (never, former, current), alcohol use (servings/day), family history of diabetes, parental history of MI, menopausal status, post-menopausal hormone use (in NHS), baseline diagnosis of hypertension, hypercholesterolemia, glycemic load, and total energy intake. The full multivariate model

additionally adjusted for biomarkers of 18:2, biomarkers of dairy intake (15:0, 17:0, and *trans* 16:1n-7), and 18:1 and 18:2 *trans* fatty acids, and AHEI. The associations for each VLCSFA and T2D risk were modeled separately for each cohort, and the composite HRs were estimated by pooling the data from both cohorts and analyzing the data using a Cox model stratified by sex. To assess the nonlinearity of the associations between each plasma and erythrocyte biomarkers and T2D risk, we used restricted cubic splines with 3 knots. Spearman partial correlation coefficients adjusted for age and BMI among CVD controls were used to evaluate correlations between plasma and erythrocyte VLCSFAs and dietary and lifestyle variables averaged for the two FFQ cycles closest to the date of blood draw: 1986 and 1990 for the NHS and 1990 and 1994 for the HPFS. We also conducted a sensitivity analysis to address the potential issue of reverse causation by excluding cases that occurred in the first two or four years after blood sampling. Additionally, we performed an analysis restricted to CVD control participants.

All P values were 2-sided, and 95% confidence intervals (CIs) were calculated for HRs. Data were analyzed with the Statistical Analysis Systems software package, version 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

During 23,757 years of follow-up we documented 243 confirmed cases of T2D in the analysis of plasma VLCSFAs (136 in the NHS, and 107 in the HPFS) and 245 cases in the analysis of erythrocyte VLCSFAs (133 in the NHS, and 112 in the HPFS). Baseline characteristics of NHS and HPFS participants are shown in **Table 3.1**. The mean age (SD) was 64.0 (8.6) among men and

Table 3.1. Baseline characteristics of 1,392 women and 1,462 men with plasma fatty acid measurements in the Nurses' Health Study (1990) and Health Professionals Follow Up Study (1994)[1]

	Women (n =1,392)	Men (n =1,462)
Age, years	60.4 (6.4)	64.0 (8.6)
Age range, years	43 to 70	47 to 81
Race/Ethnicity (%)		
Caucasian	99.3	93.6
African-Americans	0.3	0.1
Asian/Other	0.4	6.3
Weight status (%)		
Normal (BMI <25 kg/m^2)	55.4	42.1
Overweight (BMI 25 to <30 kg/m^2)	31.5	47.1
Obese (BMI ≥ 30 kg/m^2)	13.1	10.8
BMI-kg/m^2	25.3 (4.5)	25.8 (3.3)
Smoking status (%)		
Current smoker	18.3	8.2
Past smoker	40.2	49.3
Never smoker	41.5	42.5
Physical activity, MET-hours/week	16.4 (19.4)	36.4 (39.0)
Medical History		
Hypertension (%)	22.5	25.0
Hypercholesterolemia (%)	35.9	26.8
Family history of diabetes (%)	26.9	22.3
Dietary factors		
Total energy (kcal/day)	1765 (507)	2046 (622)
Peanuts (oz./day)	0.1 (0.2)	0.1 (0.4)
Peanut butter (Tbsp./day)	0.2 (0.4)	0.2 (0.5)
Fruits (servings/day)	1.7 (1.2)	1.84 (1.6)
Vegetables (servings/day)	3.9 (2.2)	4.1 (2.3)
Unprocessed meats (servings/day)	0.9 (0.5)	0.9 (0.6)
Processed meats (servings/day)	0.2 (0.3)	0.3 (0.4)
Coffee (cups/day)	1.6 (1.6)	2.0 (1.8)
Alcohol (g/day)	5.7 (10.3)	12.2 (16.2)
AHEI	39.9 (9.6)	42.4 (9.8)
Plasma fatty acids, % of total fatty acids		
20:0	0.20 (0.1)	0.18 (0.1)
22:0	0.52 (0.2)	0.46 (0.2)
24:0	0.40 (0.2)	0.38 (0.2)
Erythrocyte fatty acids, % of total fatty acids		
20:0	0.44 (0.1)	0.39 (0.06)
22:0	1.51 (0.4)	1.58 (0.29)
24:0	3.12 (0.9)	3.88 (0.82)

[1]Values are mean and (SD) for continuous variables and percent for categorical variables.

60.4 (6.4) among women. The percentages of women with BMI in the 25 to <30 kg/m^2 and BMI ≥ 30 categories were 31.5% and 13.1%, respectively. Among men, 47.1% had BMI in the 25 to <30 kg/m^2 category and 10.8% had BMI ≥ 30 kg/m^2. Prevalence of current smoking was 18.2% in the NHS and 8.2% in the HPFS. The prevalence of hypertension was 22.5% and 25.0% in the NHS and HPFS, respectively, and the prevalence of hypercholesterolemia was 35.9% and 26.8%. In the NHS, the mean plasma levels of 20:0, 22:0, and 24:0 were: 0.20%, 0.52%, and 0.40%, respectively. In the HPFS the mean plasma level of 20:0, 22:0, and 24:0 were: 0.18%, 0.46%, and 0.38%, respectively. The mean erythrocyte membrane levels of 20:0, 22:0, and 24:0 were 0.44%, 1.51%, and 3.12%, respectively. In the HPFS the mean levels were: 0.39%, 1.58%, and 3.88%, respectively. Overall, the erythrocyte VLCSFA levels were higher than their plasma counterparts.

Baseline characteristics according to quartiles of each VLCSFA are shown in **Tables S3.1 (NHS) and S3.2 (HPFS)**. Each VLCSFA and their sum were positively associated with physical activity, and inversely associated with BMI, hypertension, hypercholesterolemia, and family history of diabetes. Within each cohort, partial Spearman correlation coefficients (r_s) ranged between 0.68 and 0.92 among plasma VLCSFA. The correlations among erythrocyte VLCSFAs ranged from 0.31 to 0.83. Each VLCSFA was moderately, negatively correlated with biomarkers of 14:0 and 16:0 with r_s ranging from -0.39 to -0.61 in plasma and from -0.17 to -0.69 in erythrocytes, negatively correlated with biomarkers of 15:0 with r_s in the range of -0.10 to -0.31, in plasma and -0.05 to -0.44 in erythrocytes. VLSCSFAs were positively correlated with 18:0; r_s ranging from 0.07 to 0.45 in plasma and 0.01 to 0.42 in erythrocytes. VLCSFAs were weakly correlated with *trans* 18:1, *trans* 16:1n-7, EPA and DHA (**Tables S3.3 and S3.4). Table 3.2** shows

Table 3.2. Risk of incident diabetes according to circulating plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 among 1,392 women in the Nurses' Health Study (N=136 cases) and 1,462 men in the Health Professionals Follow-Up Study (N=107 cases)

Fatty acid	Cohort-specific fatty acid quartiles				
	1	2	3	4	P for trend[3]
20:0, NHS					
% of total FA, mean (SD)	0.14 (0.02)	0.18 (0.01)	0.22 (0.01)	0.27 (0.03)	
No. of cases	53	38	22	23	
Person-months	74,132	72,255	71,707	66,995	
Age and BMI-adjusted hazard ratio	Reference	0.91 (0.59, 1.39)	0.53 (0.32, 0.88)	0.61 (0.37, 1.00)	0.01
Multivariable hazard ratio 1[1]	Reference	0.88 (0.57, 1.35)	0.57 (0.34, 0.95)	0.59 (0.35, 0.99)	0.02
Multivariable hazard ratio 2[2]	Reference	0.81 (0.52, 1.25)	0.53 (0.32, 0.90)	0.53 (0.31, 0.90)	0.007
20:0, HPFS					
% of total FA, mean, (SD)	0.13 (0.02)	0.17 (0.01)	0.20 (0.01)	0.25 (0.03)	
No. of cases	48	27	16	16	
Person, months	50,153	48,821	46,996	48,179	
Age and BMI-adjusted hazard ratio	Reference	0.65 (0.40, 1.04)	0.44 (0.25, 0.79)	0.44 (0.25, 0.77)	0.001
Multivariable hazard ratio 1[1]	Reference	0.66 (0.40, 1.07)	0.42 (0.23, 0.75)	0.40 (0.23, 0.72)	<0.001
Multivariable hazard ratio 2[2]	Reference	0.66 (0.41, 1.08)	0.43 (0.24, 0.77)	0.44 (0.25, 0.80)	0.001
20:0, pooled[3]	**Reference**	**0.77 (0.56, 1.07)**	**0.57 (0.40, 0.81)**	**0.50 (0.34, 0.73)**	**<0.001**
22:0, NHS					
% of total FA, mean, (SD)	0.32 (0.07)	0.46 (0.03)	0.57 (0.04)	0.76 (0.10)	
No. of cases	50	44	26	16	
Person, months	70,309	75,612	72,219	66,949	
Age and BMI-adjusted hazard ratio	Reference	1.10 (0.72, 1.67)	0.64 (0.39, 1.04)	0.42 (0.24, 0.75)	<0.001
Multivariable hazard ratio 1[1]	Reference	1.10 (0.72, 1.69)	0.69 (0.42, 1.13)	0.43 (0.24, 0.78)	0.002
Multivariable hazard ratio 2[2]	Reference	1.09 (0.71, 1.66)	0.62 (0.38, 1.02)	0.38 (0.21, 0.69)	<0.001
22:0, HPFS					
% of total FA, mean, (SD)	0.27 (0.07)	0.42 (0.04)	0.55 (0.03)	0.73 (0.10)	
No. of cases	43	28	21	15	
Person, months	48,208	50,348	48,119	47,474	
Age and BMI-adjusted hazard ratio	Reference	0.72 (0.44, 1.16)	0.60 (0.35, 1.02)	0.46 (0.25, 0.84)	0.006
Multivariable hazard ratio 1[1]	Reference	0.70 (0.43, 1.14)	0.54 (0.32, 0.93)	0.43 (0.24, 0.80)	0.003
Multivariable hazard ratio 2[2]	Reference	0.77 (0.47, 1.27)	0.62 (0.36, 1.07)	0.50 (0.27, 0.93)	0.018
22:0, pooled[3]	**Reference**	**0.94 (0.69, 1.29)**	**0.59 (0.41, 0.85)**	**0.42 (0.28, 0.64)**	**<0.001**

Table 3.2. - Continued

24:0, NHS					
% of total FA, mean, (SD)	0.22 (0.05)	0.34 (0.03)	0.44 (0.03)	0.63 (0.10)	
No. of cases	57	43	21	15	
Person, months	74,602	70,819	72,111	67,557	
Age and BMI-adjusted hazard ratio	Reference	0.99 (0.66, 1.48)	0.47 (0.29, 0.79)	0.38 (0.22, 0.68)	<0.001
Multivariable hazard ratio 1[1]	Reference	0.99 (0.66, 1.50)	0.51 (0.31, 0.85)	0.40 (0.22, 0.71)	<0.001
Multivariable hazard ratio 2[2]	Reference	0.95 (0.63, 1.43)	0.45 (0.27, 0.76)	0.34 (0.19, 0.62)	<0.001
24:0, HPFS					
% of total FA, mean, (SD)	0.22 (0.05)	0.35 (0.03)	0.46 (0.03)	0.62 (0.09)	
No. of cases	50	21	21	15	
Person, months	48,817	49,796	49,064	46,472	
Age and BMI-adjusted hazard ratio	Reference	0.48 (0.29, 0.80)	0.52 (0.31, 0.87)	0.42 (0.23, 0.75)	0.002
Multivariable hazard ratio 1[1]	Reference	0.52 (0.31, 0.87)	0.51 (0.30, 0.86)	0.43 (0.24, 0.78)	0.002
Multivariable hazard ratio 2[2]	Reference	0.56 (0.33, 0.95)	0.59 (0.34, 1.01)	0.48 (0.26, 0.89)	0.013
24:0, pooled[3]	**Reference**	**0.72 (0.53, 0.99)**	**0.48 (0.33, 0.69)**	**0.40 (0.26, 0.61)**	**<0.001**
Sum (20:0 + 22:0 + 24:0), NHS					
% of total FA, mean, (SD)	0.70 (0.14)	1.00 (0.06)	1.22 (0.08)	1.63 (0.20)	
No. of cases	56	40	26	14	
Person, months	72,556	74,816	70,549	67,168	
Age and BMI-adjusted hazard ratio	Reference	0.89 (0.59, 1.34)	0.59 (0.37, 0.95)	0.35 (0.19, 0.62)	<0.001
Multivariable hazard ratio 1[1]	Reference	0.94 (0.62, 1.43)	0.63 (0.39, 1.02)	0.35 (0.19, 0.65)	<0.001
Multivariable hazard ratio 2[2]	Reference	0.94 (0.61, 1.43)	0.57 (0.35, 0.93)	0.32 (0.17, 0.59)	<0.001
Sum (20:0 + 22:0 + 24:0), HPFS					
% of total FA, mean, (SD)	0.63 (0.13)	0.94 (0.08)	1.20 (0.07)	1.57 (0.20)	
No. of cases	48	23	20	16	
Person, months	48,626	49,820	48,935	46,768	
Age and BMI-adjusted hazard ratio	Reference	0.53 (0.32, 0.88)	0.53 (0.31, 0.90)	0.45 (0.25, 0.81)	0.004
Multivariable hazard ratio 1[1]	Reference	0.57 (0.34, 0.95)	0.52 (0.30, 0.89)	0.45 (0.25, 0.80)	0.003
Multivariable hazard ratio 2[2]	Reference	0.60 (0.36, 1.00)	0.58 (0.33, 1.00)	0.50 (0.28, 0.90)	0.014
Sum (20:0 + 22:0 + 24:0), pooled[3]	**Reference**	**0.72 (0.53, 0.99)**	**0.54 (0.38, 0.78)**	**0.40 (0.26, 0.61)**	**<0.001**

[1]Additionally adjusted for race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), AHEI, glycemic load (continuous), and total energy (kcal/day)

[2] Multivariate Model 1 + sum biomarkers of 15:0, 17:0, *trans* 16:1n-7, and *trans* 18:1, *trans* 18:2

[3] Pooled estimates and p, values were calculated by combining the participant data from both cohorts and further adjusting multivariate model 1 by sex

the hazard ratios of T2D for quartiles of plasma VLCSFA levels. In the fully-adjusted multivariate model, comparing extreme quartiles of plasma VLCSFAs, the pooled HRs (95% CIs) were 0.50 (0.34, 0.73; P-trend <0.001) for 20:0; 0.42 (0.28, 0.64; P-trend <0.001) for 22:0; 0.40 (0.26, 0.61; P-trend <0.001) for 24:0; and 0.40 (0.26, 0.61; P-trend <0.001) for the sum of the three VLCSFAs. We observed similar associations for each VLCSFA when examined as continuous variables per SD (**Table 3.3**). The pooled HRs (95% CIs) of T2D for each SD increase was 0.73 (0.63, 0.84) for 20:0; 0.72 (0.63, 0.83) for 22:0; 0.68 (0.58, 0.79) for 24:0; and 0.70 (0.60, 0.80) for the sum of the three VLCSFA.

Table 3.4 shows the associations for erythrocyte VLCSFAs. In the fully-adjusted multivariate model, comparing extreme quartiles of erythrocyte biomarker levels, the pooled HR (95% CIs) of T2D for each VLCSFA was 0.58 (0.39, 0.87; P-trend = 0.01) for 20:0; 0.51 (0.34, 0.77; P-trend = 0.001) for 22:0; 0.94 (0.61, 1.46; P-trend = 0.98) for 24:0; and 0.79 (0.52, 1.21; P-trend = 0.94) for the sum of the three VLCSFA. The associations were similar for each VLCSFA examined continuously in per standard deviation in **Table 3.5**. The pooled HR (95% CIs) of T2D for each SD increase in erythrocyte VLCSFA was 0.79 (0.68, 0.92) for 20:0; 0.75 (0.65, 0.88) for 22:0; 0.99 (0.84, 1.17) for 24:0; and 0.90 (0.77, 1.05) for the sum of the three VLCSFAs.

The associations between each plasma VLCSFA and their sum and the risk of T2D were examined using spline regressions after pooling the data from both cohorts. All associations for plasma VLCSFA levels were highly significant for test of linearity (all P for linearity ≤ 0.0001) (**Figure 3.1**). For erythrocyte biomarkers, the associations were linear for all four exposures, but

Table 3.3. Risk of incident diabetes according to circulating plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 evaluated continuously (per 1 SD difference), among men and women in the Nurses' Health Study (N=136 cases), Health Professionals Follow-Up Study (N=107 cases), and both cohorts combined.

Fatty acids	NHS	P-value	HPFS	P-value	Pooled Estimate[3]	Pooled P-value[3]
20:0						
Age and BMI-adjusted HR	0.80 (0.67, 0.95)	0.01	0.69 (0.56, 0.84)	<0.001	0.74 (0.64, 0.85)	<0.001
Multivariate HR 1[1]	0.80 (0.67, 0.97)	0.02	0.65 (0.53, 0.81)	<0.001	0.73 (0.64, 0.84)	<0.001
Multivariate HR 2[2]	0.79 (0.65, 0.95)	0.01	0.66 (0.53, 0.82)	<0.001	0.73 (0.63, 0.84)	<0.001
22:0						
Age and BMI-adjusted HR	0.73 (0.61, 0.88)	<0.001	0.73 (0.59, 0.89)	0.002	0.72 (0.62, 0.82)	<0.001
Multivariate HR 1[1]	0.74 (0.61, 0.89)	0.002	0.71 (0.57, 0.87)	0.001	0.71 (0.61, 0.82)	<0.001
Multivariate HR 2[2]	0.72 (0.60, 0.87)	<0.0001	0.73 (0.59, 0.91)	0.004	0.72 (0.63, 0.83)	<0.001
24:0						
Age and BMI-adjusted HR	0.68 (0.56, 0.83)	<0.001	0.66 (0.52, 0.82)	<0.001	0.67 (0.57, 0.77)	<0.001
Multivariate HR 1[1]	0.70 (0.57, 0.85)	<0.001	0.67 (0.54, 0.84)	<0.001	0.69 (0.59, 0.80)	<0.001
Multivariate HR 2[2]	0.67 (0.55, 0.81)	<0.001	0.71 (0.57, 0.90)	0.004	0.68 (0.58, 0.79)	<0.001
20:0+22:0+24:0						
Age and BMI-adjusted HR	0.71 (0.59, 0.85)	<0.001	0.67 (0.54, 0.84)	<0.001	0.68 (0.59, 0.79)	<0.001
Multivariate HR 1[1]	0.72 (0.60, 0.87)	<0.001	0.67 (0.54, 0.83)	<0.001	0.70 (0.60, 0.80)	<0.001
Multivariate HR 2[1]	0.70 (0.58, 0.84)	<0.001	0.70 (0.56, 0.87)	0.002	0.70 (0.60, 0.80)	<0.001

[1]Additionally adjusted for race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), AHEI, glycemic load (continuous), and total energy (kcal/day)

[2] Multivariate Model 1 + sum biomarkers of 15:0, 17:0, *trans* 16:1n-7, and *trans* 18:1, *trans* 18:2

[3] Pooled estimates and p-values were calculated by combining the participant data from both cohorts and further adjusting multivariate model 1 by sex

Table 3.4. Risk of incident diabetes according to circulating erythrocyte fatty acid biomarkers of 20:0, 22:0, and 24:0 among 1,314 women in the Nurses' Health Study (N=133 cases) and 1,517 men in the Health Professionals Follow-Up Study (N=112 cases)

Fatty acid	Cohort, specific fatty acid quartiles				
	1	2	3	4	P for trend[3]
20:0, NHS					
% of total FA, mean (SD)	0.35 (0.03)	0.42 (0.01)	0.47 (0.01)	0.55 (0.05)	
No. of cases	53	39	28	13	
Person, months	70,178	68,167	63,648	66,511	
Age and BMI-adjusted hazard ratio	Reference	0.72 (0.47, 1.09)	0.56 (0.36, 0.89)	0.30 (0.16, 0.56)	<0.001
Multivariable hazard ratio 1[1]	Reference	0.80 (0.52, 1.22)	0.61 (0.38, 0.97)	0.33 (0.18, 0.60)	<0.001
Multivariable hazard ratio 2[2]	Reference	0.84 (0.55, 1.30)	0.61 (0.38, 0.98)	0.31 (0.17, 0.58)	<0.001
20:0, HPFS					
% of total FA, mean (SD)	0.32 (0.03)	0.36 (0.01)	0.39 (0.01)	0.45 (0.04)	
No. of cases	32	24	27	29	
Person, months	52,709	50,780	49,281	49,278	
Age and BMI-adjusted hazard ratio	Reference	0.90 (0.53, 1.54)	1.00 (0.60, 1.67)	1.11 (0.67, 1.85)	0.66
Multivariable hazard ratio 1[1]	Reference	0.80 (0.46, 1.37)	0.87 (0.52, 1.48)	1.07 (0.63, 1.48)	0.82
Multivariable hazard ratio 2[2]	Reference	0.81 (0.47, 1.40)	0.90 (0.53, 1.54)	1.04 (0.60, 1.79)	0.89
20:0, pooled[1]	**Reference**	**0.86 (0.61, 1.21)**	**0.94 (0.66, 1.33)**	**0.58 (0.39, 0.87)**	**0.01**
22:0, NHS					
% of total FA, mean, (SD)	1.06 (0.18)	1.42 (0.07)	1.65 (0.07)	1.98 (0.18)	
No. of cases	46	33	31	23	
Person, months	69,890	65,861	67,322	65,431	
Age and BMI-adjusted hazard ratio	Reference	0.93 (0.59, 1.46)	0.88 (0.55, 1.40)	0.62 (0.38, 1.03)	0.08
Multivariable hazard ratio 1[1]	Reference	1.06 (0.67, 1.68)	0.95 (0.59, 1.53)	0.65 (0.39, 1.10)	0.14
Multivariable hazard ratio 2[2]	Reference	0.68 (0.41, 1.13)	0.59 (0.34, 1.00)	0.36 (0.20, 0.65)	<0.001
22:0, HPFS					
% of total FA, mean, (SD)	1.22 (0.14)	1.50 (0.06)	1.67 (0.05)	1.94 (0.16)	
No. of cases	39	26	25	22	
Person, months	49,175	52,248	48,997	51,628	
Age and BMI-adjusted hazard ratio	Reference	0.65 (0.39, 1.06)	0.71 (0.43, 1.18)	0.57 (0.34, 0.97)	0.05
Multivariable hazard ratio 1[1]	Reference	0.61 (0.37, 1.05)	0.63 (0.38, 1.04)	0.50 (0.29, 0.87)	0.01
Multivariable hazard ratio 2[2]	Reference	0.68 (0.41, 1.14)	0.71 (0.42, 1.22)	0.61 (0.34, 1.11)	0.11
22:0, pooled[1]	**Reference**	**0.83 (0.59, 1.18)**	**0.72 (0.49, 1.04)**	**0.51 (0.34, 0.77)**	**0.001**

Table 3.4. - Continued

24:0. NHS					
% of total FA, mean, (SD)	2.03 (0.36)	2.81 (0.16)	3.38 (0.18)	4.31 (0.52)	
No. of cases	38	22	37	36	
Person, months	70,523	67,677	64,749	65,555	
Age and BMI-adjusted hazard ratio	Reference	0.58 (0.34, 0.98)	1.25 (0.79, 1.98)	0.99 (0.63, 1.57)	0.50
Multivariable hazard ratio 1[1]	Reference	0.62 (0.36, 1.06)	1.39 (0.87, 2.21)	1.02 (0.64, 1.63)	0.48
Multivariable hazard ratio 2[2]	Reference	0.47 (0.26, 0.84)	0.97 (0.57, 1.65)	0.66 (0.37, 1.17)	0.46
24:0, HPFS					
% of total FA, mean, (SD)	2.84 (0.42)	3.72 (0.16)	4.21 (0.14)	4.87 (0.33)	
No. of cases	31	23	35	23	
Person, months	50,512	47,819	52,438	51,279	
Age and BMI-adjusted hazard ratio	Reference	0.85 (0.49, 1.45)	1.11 (0.68, 1.81)	0.73 (0.42, 1.25)	0.44
Multivariable hazard ratio 1[1]	Reference	0.87 (0.50, 1.50)	1.04 (0.64, 1.71)	0.64 (0.37, 1.11)	0.21
Multivariable hazard ratio 2[2]	Reference	1.00 (0.57, 1.75)	1.40 (0.82, 2.41)	0.95 (0.51, 1.79)	0.80
24:0, pooled[1]	**Reference**	**1.09 (0.75, 1.57)**	**1.22 (0.82, 1.81)**	**0.94 (0.61, 1.46)**	**0.98**
Sum (20:0 + 22:0 + 24:0), NHS					
% of total FA, mean, (SD)	3.52 (0.54)	4.68 (0.23)	5.48 (0.25)	6.71 (0.65)	
No. of cases	40	27	32	34	
Person, months	68,189	68,454	66,405	65,456	
Age and BMI-adjusted hazard ratio	Reference	0.68 (0.42, 1.12)	0.99 (0.62, 1.58)	0.90 (0.57, 1.42)	0.92
Multivariable hazard ratio 1[1]	Reference	0.77 (0.47, 1.27)	1.09 (0.68, 1.76)	0.93 (0.58, 1.49)	0.98
Multivariable hazard ratio 2[2]	Reference	0.54 (0.31, 0.93)	0.71 (0.41, 1.23)	0.55 (0.31, 0.97)	0.12
Sum (20:0 + 22:0 + 24:0), HPFS					
% of total FA, mean, (SD)	4.47 (0.56)	5.59 (0.21)	6.25 (0.19)	7.15 (0.46)	
No. of cases	31	28	29	24	
Person, months	50,085	48,375	51,717	51,871	
Age and BMI-adjusted hazard ratio	Reference	1.02 (0.61, 1.70)	0.96 (0.57, 1.59)	0.77 (0.45, 1.32)	0.37
Multivariable hazard ratio 1[1]	Reference	1.04 (0.62, 1.75)	0.88 (0.52, 1.47)	0.69 (0.40, 1.19)	0.17
Multivariable hazard ratio 2[2]	Reference	1.22 (0.71, 2.10)	1.16 (0.66, 2.04)	1.02 (0.55, 1.89)	0.90
Sum (20:0 + 22:0 + 24:0), pooled[1]	**Reference**	**1.12 (0.78, 1.60)**	**1.12 (0.77, 1.65)**	**0.79 (0.52, 1.21)**	**0.94**

[1]Additionally adjusted for race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), AHEI, glycemic load (continuous), and total energy (kcal/day)

[2] Multivariate Model 1 + sum biomarkers of 15:0, 17:0, *trans* 16:1n-7, and *trans* 18:1, *trans* 18:2

[3] Pooled estimates and p-values were calculated by combining the participant data from both cohorts and further adjusting multivariate model 1 by sex

Table 3.5. Risk of incident diabetes according to circulating erythrocyte fatty acid biomarkers of 20:0, 22:0, and 24:0 evaluated continuously (per 1 SD difference), among men and women in the Nurses' Health Study (N=133 cases), Health Professionals Follow-Up Study (N=112 cases), and both cohorts combined

	NHS	P-value	HPFS	P-value	Pooled Estimate[3]	Pooled P-value[3]
20:0						
Age and BMI-adjusted HR	0.67 (0.56, 0.81)	<0.001	1.08 (0.88, 1.33)	0.45	0.80 (0.70, 0.93)	0.003
Multivariate HR 1[1]	0.69 (0.57, 0.83)	<0.001	1.04 (0.84, 1.29)	0.71	0.80 (0.69, 0.93)	0.003
Multivariate HR 2[2]	0.68 (0.57, 0.83)	<0.001	1.04 (0.83, 1.30)	0.73	0.79 (0.68, 0.92)	0.002
22:0						
Age and BMI-adjusted HR	0.87 (0.74, 1.02)	0.09	0.85 (0.70, 1.03)	0.09	0.86 (0.76, 0.97)	0.013
Multivariate HR 1[1]	0.87 (0.74, 1.03)	0.11	0.79 (0.65, 0.97)	0.02	0.86 (0.76, 0.97)	0.016
Multivariate HR 2[2]	0.64 (0.51, 0.80)	<0.001	0.85 (0.68, 1.06)	0.16	0.75 (0.65, 0.88)	<0.001
24:0						
Age and BMI-adjusted HR	1.06 (0.90, 1.25)	0.48	0.96 (0.80, 1.16)	0.70	1.01 (0.88, 1.15)	0.92
Multivariate HR 1[1]	1.06 (0.89, 1.25)	0.53	0.92 (0.76, 1.11)	0.39	0.98 (0.85, 1.12)	0.74
Multivariate HR 2[2]	0.88 (0.71, 1.10)	0.28	1.09 (0.87, 1.37)	0.45	0.99 (0.84, 1.17)	0.90
20:0+22:0+24:0						
Age and BMI-adjusted HR	0.98 (0.83, 1.16)	0.81	0.93 (0.77, 1.13)	0.48	0.95 (0.83, 1.08)	0.42
Multivariate HR 1[1]	0.98 (0.83, 1.16)	0.80	0.89 (0.73, 1.07)	0.21	0.92 (0.81, 1.05)	0.21
Multivariate HR 2[1]	0.78 (0.63, 0.97)	0.03	1.03 (0.82, 1.29)	0.83	0.90 (0.77, 1.05)	0.18

[1]Additionally adjusted for race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), AHEI, glycemic load (continuous), and total energy (kcal/day)

[2] Multivariate Model 1 + sum biomarkers of 15:0, 17:0, *trans* 16:1n-7, and *trans* 18:1, *trans* 18:2

[3] Pooled estimates and p-values were calculated by combining the participant data from both cohorts and further adjusting multivariate model 1 by sex

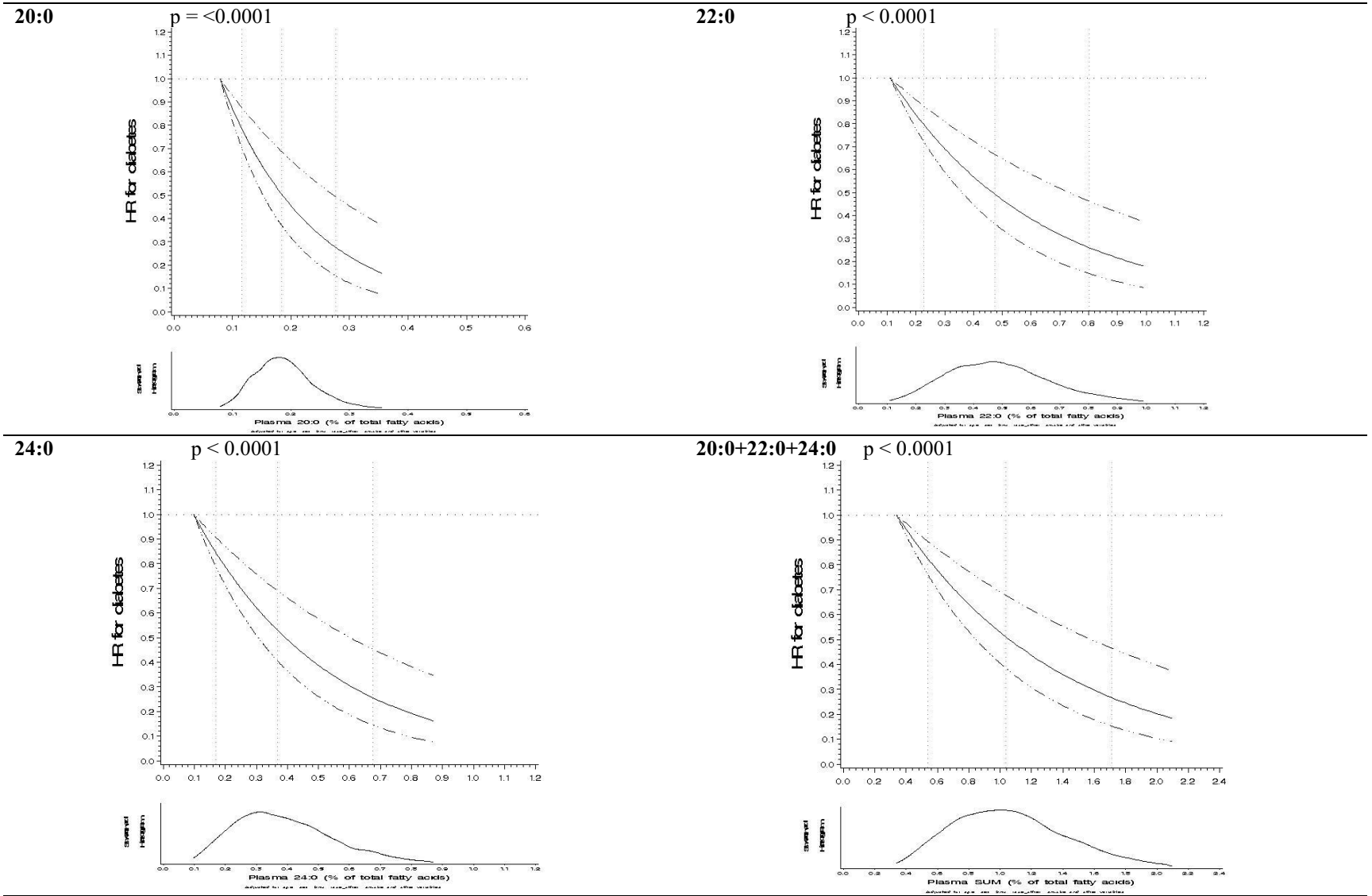


Figure 3.1. Semi, parametric multivariable, adjusted associations of plasma fatty acid biomarkers of 20:0, 22:0, and 24:0, with incident diabetes among 2,854 men and women in the Nurses' Health Study (N=136 cases), Health Professionals Follow-Up Study (N=107 cases), evaluated using restricted cubic splines with covariate adjustments as in Table 2. Solid and dashed lines represent hazard ratios (HRs) and 95% confidence intervals, respectively; dotted vertical lines represent 3 knots. P-values for linear association are shown

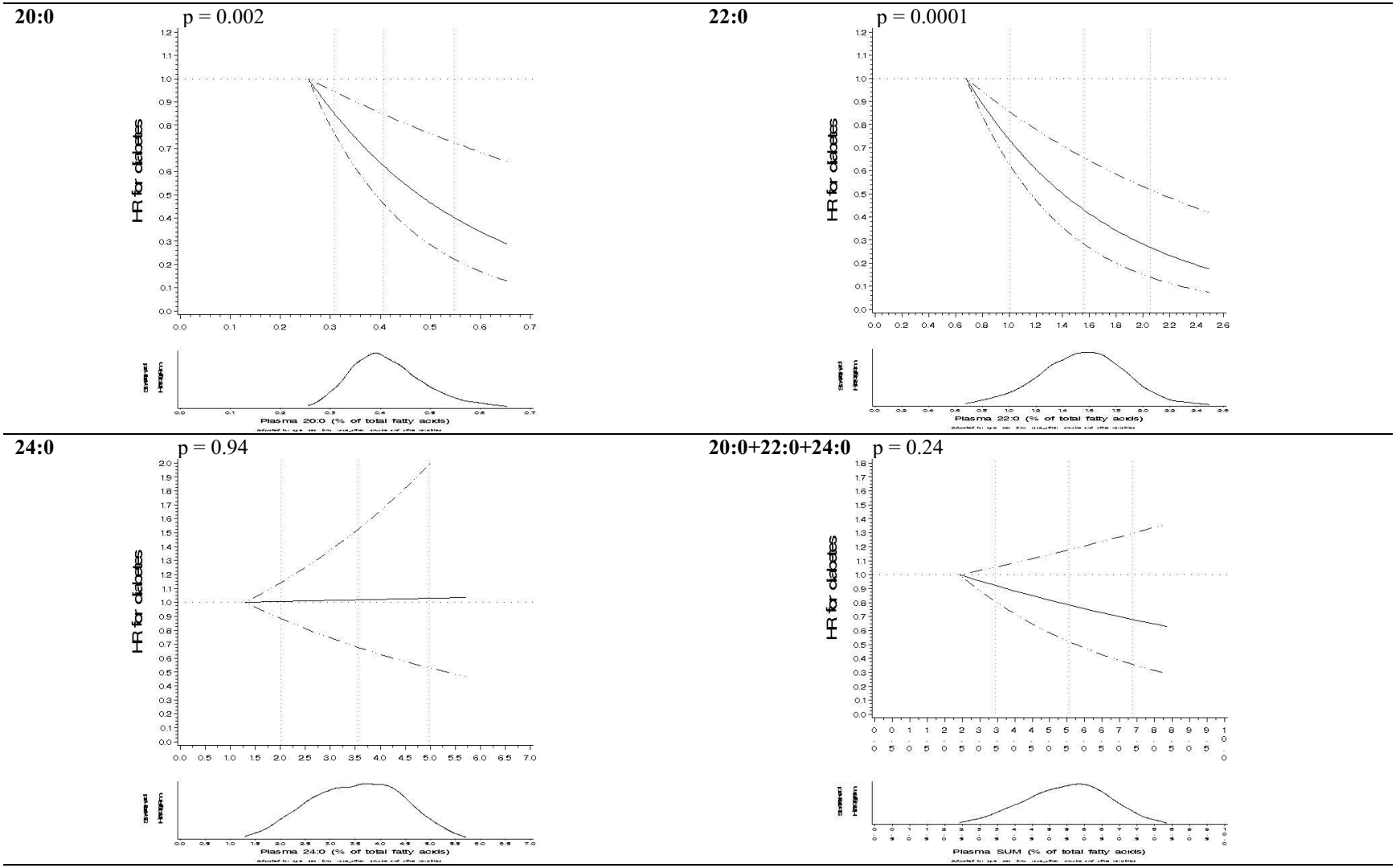


Figure 3.2. Semi, parametric multivariable, adjusted associations of erythrocyte fatty acid biomarkers of 20:0, 22:0, and 24:0, with incident diabetes among 2,831 men and women in the Nurses' Health Study (N=134 cases), Health Professionals Follow-Up Study (N=112 cases), evaluated using restricted cubic splines with covariate adjustments as in Table 2. Solid and dashed lines represent hazard ratios (HRs) and 95% confidence intervals, respectively; dotted vertical lines represent 3 knots. P-values for linear association are shown

only the linear association for 20:0 and 22:0 reached statistical significance whereas the associations for 24:0 and the sum of VLCSFAs were not statistically significant (**Figure 3.2**). Cross-sectional partial Spearman correlations between each plasma VLCSFA biomarker and dietary and lifestyle variables were calculated in both the NHS (**Table 3.6**) and the HPFS (**Table 3.7**). In the NHS, biomarkers of 22:0 and 24:0 and the sum of VLCSFAs were positively correlated with peanut, peanut butter, coffee, total fat, PUFAs, and vegetable fat intake. 22:0 was positively correlated with dietary intakes of 16:0-18:0. 24:0 was positively correlated with dietary 16:0. In the HPFS, 22:0, 24:0 and the sum of VLCSFAs were positively correlated with intake of peanut butter and potato chips and with physical activity. Each fatty acid and their sum were positively correlated with, intakes of total fat, dairy fat, vegetable fat, PUFAs, and dietary 16:0 and 18:0. 22:0 was inversely correlated with fish and vegetable intake and positively correlated with intake of French fries and processed meat. Similar results were observed for cross-sectional partial Spearman correlations between each erythrocyte VLCSFA and intakes of peanut butter, total dairy, coffee, and correlations between 22:0 and dietary 16:0-20:0, total fat, dairy fat and animal fat in the NHS (**Table 3.8**) and the HPFS (**Table 3.9**). Despite the statistical significance, all correlations were weak ($r_s \leq 0.19$)

In the sensitivity analysis, we excluded T2D cases diagnosed within the first 2 or 4 years after blood draw and the original associations between each VLCSFA and T2D risk largely persisted with wider confidence intervals because of fewer cases included (**Table S3.5**). In another sensitivity analysis conducted among CVD controls only, the inverse associations for each plasma VLCSFA biomarker and T2D risk persisted but they lost statistical significance due to the

Table 3.6. Partial Spearman correlations between circulating plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 and dietary factors among 674 participants in the Nurses' Health Study at baseline

	Plasma fatty acids[1]			
	20:0	**22:0**	**24:0**	**20:0+22:0+24:0**
Dietary factors[2]				
Peanuts	0.04	**0.11****	**0.12***	**0.11***
Peanut butter	0.02	**0.15*****	**0.14*****	**0.13*****
Other nuts	0.08	0.03	0.06	0.06
Fish	-0.04	-0.07	-0.05	-0.06
Total dairy	0.02	0.01	-0.01	0.02
Sugar-sweetened beverages	-0.06	**-0.09***	**-0.09***	**-0.09***
Coffee	0.06	**0.10***	**0.10*****	**0.10****
Alcohol	-0.06	-0.05	0.03	-0.02
French fries	<0.01	0.03	0.01	< -0.01
Potato chips	-0.06	-0.03	-0.02	-0.02
Fruits	0.08	< -0.01	<0.01	0.01
Vegetables	0.05	-0.01	0.02	0.01
Processed meat	-0.04	0.04	0.02	0.03
Unprocessed meat	-0.02	< -0.01	-0.02	-0.01
Chocolate	0.01	< -0.01	-0.01	< -0.01
Whole grains	0.01	-0.01	-0.01	< -0.01
Nutrients[2]				
Total fat	0.04	**0.11****	**0.09***	**0.10****
Vegetable fat	0.04	**0.12****	**0.10****	**0.11****
Animal fat	0.01	0.03	0.02	0.02
Dairy fat	0.06	0.06	0.06	0.06
16:0 – diet	0.04	**0.09***	**0.08***	0.08
18:0 – diet	0.04	**0.09***	0.06	0.07
20:0 - diet	0.05	**0.11****	0.07	**0.09***
Polyunsaturated fat	0.04	**0.11****	**0.10****	**0.10****
Carbohydrate	0.07	0.03	0.01	0.03
Total fiber	**0.10***	0.04	0.05	0.05
Lifestyle factors[2]				
Physical activity	0.06	0.04	0.06	0.05

Correlations were adjusted for age, total energy intake, and BMI

[1]Fatty acid concentrations were assessed from the plasma fraction of NHS participants

[2]Dietary and nutrient factors were assessed using the average of self-reported responses in 1986 and 1990 in the NHS

*p-value for correlation coefficient < 0.05; ** p-value <0.01; *** p-value <0.001

Table 3.7. Partial Spearman correlations between plasma fatty acids and dietary factors among 691 participants in the Health Professionals Follow up Study at baseline

	Plasma fatty acids[1]			
	20:0	**22:0**	**24:0**	**20:0+22:0+24:0**
Dietary factors[2]				
Peanuts	-0.08	0.04	0.04	0.03
Peanut butter	-0.01	**0.15****	**0.11****	**0.10****
Other nuts	0.01	0.01	0.04	0.02
Fish	-0.03	**-0.08***	<0.01	-0.04
Total dairy	**0.14*****	**0.11****	**0.09***	**0.11****
Sugar-sweetened beverages	0.01	0.01	-0.05	-0.02
Coffee	0.05	0.07	0.05	0.06
Alcohol	**-0.09***	-0.06	0.03	-0.03
Fruits	-0.05	**-0.04**	-0.04	-0.05
Vegetables	-0.04	**-0.08***	-0.03	-0.06
Potato chips	0.06	**0.09***	**0.09****	**0.09****
French fries	0.05	**0.07***	0.03	0.07
Processed meat	0.02	**0.09***	0.04	0.06
Unprocessed meat	0.01	< -0.01	-0.04	-0.02
Chocolate	**0.12****	0.03	< -0.01	0.03
Whole grains	**-0.11****	**-0.09***	-0.07	-0.09
Nutrients[2]				
Total fat	**0.13*****	**0.16*****	**0.12****	**0.15*****
Dairy fat	**0.15*****	**0.14*****	**0.10****	**0.13*****
Vegetable fat	**0.09***	**0.15*****	**0.14*****	**0.15*****
Animal fat	**0.10****	**0.10****	0.05	**0.08***
16:0 – diet	**0.13*****	**0.15*****	**0.09***	**0.14*****
18:0 – diet	**0.14*****	**0.16*****	**0.09***	**0.14*****
20:0 - diet	**0.09****	**0.12****	0.05	**0.10***
Polyunsaturated fat	**0.09***	**0.14*****	**0.13*****	**0.14*****
Carbohydrate	-0.03	-0.02	**-0.10****	-0.07
Total fiber	-0.07	-0.02	0.01	-0.07
Lifestyle factors[2]				
Physical activity	-0.04	**0.08****	**0.11*****	**0.09***

Correlations were adjusted for age, total energy intake, and BMI

[1]Fatty acid concentrations were assessed from the plasma fraction of HPFS participants

[2]Dietary and nutrient factors were assessed using the average of self-reported responses in 1990 and 1994 in the HPFS

*p-value for correlation coefficient < 0.05; ** p-value <0.01; *** p-value <0.001

Table 3.8. Partial Spearman correlations between circulating erythrocyte fatty acid biomarkers of 20:0, 22:0, and 24:0 and dietary factors among 639 participants in the Nurses' Health Study at baseline

	Plasma fatty acids[1]			
	20:0	**22:0**	**24:0**	**20:0+22:0+24:0**
Dietary factors[2]				
Peanuts	0.04	0.04	**0.10***	**0.08***
Peanut butter	0.06	**0.08***	0.08	**0.08***
Other nuts	0.01	-0.03	0.02	0.01
Fish	**-0.12***	-0.06	0.01	-0.02
Total dairy	**0.09***	**0.13****	**0.09***	**0.11****
Sugar-sweetened beverages	-0.04	-0.04	-0.02	-0.03
Coffee	0.04	0.04	-0.01	<0.01
Alcohol	**-0.12****	-0.08	0.04	<0.01
French fries	0.03	0.03	0.03	<0.01
Potato chips	0.02	0.05	0.02	0.01
Fruits	0.07	<0.01	0.03	0.03
Vegetables	-0.01	-0.04	-0.03	-0.03
Processed meat	0.04	0.05	<0.01	0.01
Unprocessed meat	-0.05	0.02	< -0.01	< -0.01
Chocolate	0.02	0.06	0.05	0.06
Whole grains	-0.01	-0.02	-0.02	-0.02
Nutrients[2]				
Total fat	0.07	**0.09***	0.02	0.04
Vegetable fat	0.06	0.01	-0.04	-0.02
Animal fat	0.04	**0.08***	0.04	0.05
Dairy fat	**0.12*****	**0.12****	0.06	**0.09***
16:0 – diet	**0.08***	**0.11****	0.04	0.07
18:0 – diet	**0.08***	**0.11****	0.02	0.05
20:0 - diet	0.06	**0.11****	0.05	0.07
Polyunsaturated fat	0.05	0.01	< -0.02	< -0.01
Carbohydrate	0.04	0.02	-0.05	-0.03
Total fiber	0.06	< -0.01	-0.01	<0.01
Lifestyle factors[2]				
Physical activity	**0.06***	**0.11***	0.07	**0.09***

Correlations were adjusted for age, total energy intake, and BMI

[1]Fatty acid concentrations were assessed from the erythrocyte fraction of NHS participants

[2]Dietary and nutrient factors were assessed using the average of self-reported responses in 1986 and 1990 in the NHS

*p-value for correlation coefficient < 0.05; ** p-value <0.01; *** p-value <0.001

Table 3.9. Partial Spearman correlations between erythrocyte fatty acids and dietary factors among 731 participants in the Health Professionals Follow-Up Study at baseline

	Erythrocyte fatty acids[1]			
	20:0	**22:0**	**24:0**	**20:0+22:0+24:0**
Dietary factors[2]				
Peanuts	-0.03	-0.03	0.06	0.03
Peanut butter	-0.03	**0.09***	**0.11****	**0.11****
Other nuts	0.04	-0.04	0.04	0.02
Fish	-0.02	**-0.09***	-0.01	-0.03
Total dairy	**0.19*****	**0.16*****	0.07	**0.11****
Sugar-sweetened beverages	<0.01	0.02	-0.02	-0.01
Coffee	0.04	0.04	-0.02	-0.02
Alcohol	**-0.13****	**-0.21*****	-0.01	-0.07
Fruits	0.03	0.05	0.06	0.06
Vegetables	-0.01	-0.06	0.02	-0.01
Potato chips	0.01	-0.03	-0.03	-0.03
French fries	0.06	**0.09***	< -0.01	0.01
Processed meat	0.04	0.09	0.01	0.03
Unprocessed meat	0.07	0.03	-0.05	-0.03
Chocolate	**0.13*****	**0.15*****	**0.10****	**0.12****
Whole grains	**-0.12****	**-0.07***	-0.03	-0.05
Nutrients[2]				
Total fat	**0.17*****	**0.16*****	0.06	**0.09***
Dairy fat	**0.21*****	**0.17*****	0.05	**0.09***
Vegetable fat	0.05	**0.09***	**0.10****	**0.10****
Animal fat	**0.17*****	**0.13*****	< -0.01	0.07
16:0 – diet	**0.18*****	**0.15*****	0.03	**0.11****
18:0 – diet	**0.15*****	**0.20*****	0.06	**0.20****
20:0 - diet	**0.15*****	**0.18*****	**0.08***	**0.08***
Polyunsaturated fat	**0.07***	**0.08***	0.07	**0.08***
Carbohydrate	< -0.01	**0.08***	0.05	0.06
Total fiber	-0.06	-0.02	0.05	0.03
Lifestyle factors[2]				
Physical activity	-0.01	0.02	-0.05	-0.03

Correlations were adjusted for age, total energy intake, and BMI

[1]Fatty acid concentrations were assessed from the erythrocyte fraction of HPFS participants

[2]Dietary and nutrient factors were assessed using the average of self-reported responses in 1990 and 1994 in the HPFS

*p-value for correlation coefficient < 0.05; ** p-value <0.01; *** p-value <0.001

loss of statistical power (**Table S3.6**). Comparing extreme quartiles, the pooled HR (95% CIs) of T2D were: 0.75 (0.41, 1.41; P-trend = 0.30) for 20:0; 0.58 (0.30, 1.13; P-trend = 0.08) for 22:0; 0.63 (0.34, 1.19; P-trend = 0.10 for 24:0); and 0.66 (0.35, 1.27; P-trend = 0.13) for the sum of the three VLCSFAs.

DISCUSSION

Plasma levels of 20:0, 22:0, 24:0 and the sum of these VLCSFAs were significantly associated with a lower risk of T2D after multivariate adjustment of covariates in two cohorts of U.S. men and women. For erythrocyte biomarkers, only 20:0 and 22:0 were significantly associated with a lower risk of T2D. Cross-sectionally, we observed weak yet significant positive correlations between plasma and erythrocyte levels of VLCSFA and intakes of peanut and peanut butter, coffee, vegetable fat, alcohol and dietary 20:0 in the NHS. In the HPFS, VLCSFAs were positively correlated with intakes of peanut butter, potato chips, PUFAs, dairy fat, dietary 16:0-20:0. Plasma VLCSFAs were also positively correlated with physical activity levels.

Multiple studies have been conducted to examine the relationship between VLCSFAs in various blood compartments and T2D risk. Our findings of inverse associations between plasma levels of VLCSFAs and diabetes risk are in line with previous studies. In the EPIC-InterAct study, inverse associations with T2D risk were observed for all three plasma VLCSFA biomarkers: HRs (95% CIs) of T2D per SD were 0.78 (0.68, 0.88) for 20:0, 0.81 (0.71, 0.93]) for 22:0, and 0.75 (0.64, 0.88) for 24:0.[12] In the CHS, inverse associations were also found between plasma levels of VLCSFAs and T2D risk; the HRs (95% CIs) comparing extreme quartiles were : 0.53 (0.37, 0.77) for 20:0; 0.67 (0.47, 0.94) for 22:0, and 0.63 (0.45, 0.89) for 24:0.[13] In contrast, findings regarding erythrocyte VLCSFAs are mixed. Similar to our findings, in the EPIC-Potsdam study, erythrocyte levels of 20:0 were

inversely associated with T2D risk (RR for extreme quintiles: 0.66; 95% CI:0.47, 0.94; P-trend = 0.04], and a significant, positive association was observed for 24:0 [RR for extreme quintiles: 1.56; 95%CI: 1.11, 2.21; P-trend = 0.02].[14] Lastly, in a case-control study in nested in the Hunter Community Study (HCS), levels of 24:0 in whole blood were associated with a reduced T2D risk but other VLCSFAs were not measured.[36] Taken together, these data suggest that VLCSFAs, especially those in plasma, were robustly associated with a lower risk of T2D. Of note, the VLCSFAs in plasma were also inversely associated with coronary heart disease (CHD) risk in the case-control studies in the NHS and HPFS,[9] sudden cardiac arrest in a population- based case-control study in the Seattle, Washington area ,[10] and atrial fibrillation risk in the CHS.[11] Thus, the inverse associations seem to be consistent not only for T2D but for broader cardiometabolic diseases.

In our study, the associations for plasma VLCSFAs are stronger than those in erythrocyte membranes. This finding is consistent with associations with T2D in other cohorts and with findings from our cohorts examining the associations with CHD. The reason underlying the weaker associations for erythrocyte VLCSFAs is unknown, although this may be ascribed to factors such as the uptake and release of these fatty acids from erythrocyte membranes that can influence their availability to be utilized in metabolic processes. Evidence from feeding trials where patients were randomized to supplementation with long-chain PUFA, palmitic and linoleic acids showed that their uptake into erythrocyte cell membranes or other compartments depends on fatty acid chain length and duration of supplementation.[37–39] Fatty acid chain length also affects their rate of release cellular phospholipids into plasma through phospholipase A2 (PLA2).[40] Shorter chain lengths are hydrolyzed more readily which may increase the availability of 20:0 and 22:0 in plasma and in turn be able to interact with target tissues which may explain their inverse association of these two

erythrocyte membrane fatty acids with T2D in our study. In addition, it should be also noted that CVs for erythrocyte measurements were higher than for plasma (especially for 24:0), and thus measurement error may contribute to the non-significant associations observed for 24:0 and the sum of VLCSFAs, which is largely driven by 24:0.

The current study is among the first to explore dietary and lifestyle predictors of VLCSFAs. We observed significant correlations between plasma 22:0 and 24:0 and intakes of peanuts and peanut butter, probably because these foods contain 20:0 and 22:0.[5] These data are consistent with those from feeding trials, where peanut intake led to higher plasma levels of 22:0, 24:0, and 26:0 after 2-8 hours of intake.[41] Positive associations between nut and seed intake and VLCSFA levels in plasma were also found in the EPIC-InterAct,[12] although whether such an observation was driven by peanut intake is unclear. In the current study, we also observed significant positive correlations for vegetable fat and potato chips, which may be explained by the fact that canola oil contains 20:0 and 22:0 and this oil is commonly used in the manufacture of potato chips.[5] The positive correlations between dairy fat intake and 20:0 and 22:0 in the HPFS could be possibly explained by the fact that dairy fat is among food sources of all three VLCSFA.[5] In the EPIC-InterAct, intake of dairy products, milk, and butter was also positively correlated with the levels of VLCSFAs in plasma.[12] The positive correlation with coffee in the NHS is unexpected because coffee does not contain these fatty acids, although we cannot exclude the possibility that milk or cream added to coffee may partially explain the associations. Of other factors, alcohol intake was inversely associated with VLCSFAs and physical activities were positively associated with these fatty acids. More research is needed to help understand these observations.

The mechanistic pathways underlying the observed associations for 20:0-24:0 and T2D risk are not well established. VLSCFAs may modulate insulin sensitivity through multiple mechanisms. VLCSFA are constituents of sphingolipids and form part of cell membrane lipids as ceramides when they are attached to sphingosine.[6,42] Ceramides that incorporate saturated fatty acids such as 16:0 and 18:0 are associated with increased insulin resistance[43] inhibition of β cell function and[44] inhibition of glucose uptake by decreasing the translocation of GLUT1 and GLUT4 glucose transporters to plasma membranes[45,46] However, ceramides with very long-chain sphingolipid species are inversely associated with hepatic insulin resistance in rodent models.[47,48] Ceramide chain length may also be relevant for other outcomes such as cardiac function, where increasing ceramides rich in 16:0 or decreasing ceramides with 24:0 side chains may contribute to apoptosis and cardiac dysfunction in rodent models.[49] Epidemiological evidence showed that the ratio of ceramides with side groups 18:0 to 16:0 is a strong predictor of T2D whereas the ratio of ceramides with acyl chains 18:0 to 24:0 or 24:1 was not associated with T2D risk.[50]

In addition, VLCSFAs undergo β-oxidation in peroxisomes because mitochondria lacks the very-long-chain acyl-CoA synthetase.[51–53] The resulting medium-chain acyl-CoA β-oxidation products can act as precursors in the synthesis of plasmalogens in peroxisomes and undergo further modifications in the endoplasmic reticulum.[53,54] Plasmalogens are constituents of cell membrane phospholipids and maybe preferentially oxidized to spare the oxidation of PUFAs and other membrane components, and stop the propagation of lipid peroxidation.[54] Oxidative stress has been implicated in the development of T2D and CVD and evidence of PUFA downregulation in LDL particles of T2D patients has been reported.[55]

Moreover, the regulation of β-oxidation of VLCSFAs is modulated by PPARδ as opposed to other fatty acids, which are modulated by PPARα.[56] Interactions between VLCSFAs and PPARD gene variants have been reported in the NHS and HPFS.[9] PPARδ is widely expressed in many tissues including muscle, liver and fat where it can regulate energy balance,[56] and stimulate β-oxidation, triglyceride, and glucose utilization in adipose tissue.[56–58] PPARδ agonists have shown improvements in insulin sensitivity in OGTTs in mouse models[59] and reverse metabolic abnormalities in obese men, including fasting TG, insulin and LDL levels but the mechanisms are still unclear.[60] PPARδ can upregulate transport of glucose transporter‐4 (GLUT4) to the cell membrane in myocytes[61] and its activation reduces the production of pro-inflammatory cytokines involved in insulin resistance and it improves the functions of pancreatic β-cells.[62] The relationship between VLCSFAs and glucose metabolism, PPARδ activation, and interaction with lifestyle and dietary determinants is an area that needs to be further investigated.

This study has several strengths, including long duration of follow-up, examination of both plasma and erythrocyte VLCSFA levels, and the examination of diet/lifestyle associations with VLCSFAs. Our study also has some limitations. First, relatively large laboratory measurement errors for erythrocyte VLCSFA assessments may lead to attenuation of true associations for erythrocyte VLCSFAs in this prospective study. Second, the study participants were exclusively middle-aged and older health professionals, and > 95% of them are of European ancestry. These may limit the generalizability of the results to other populations. Third, due to the observational nature of these studies, the reported associations will not necessarily entail causal interpretation. While the adjustment of a multitude of covariates may help better control for confounding, we cannot exclude the role of residual and/or unmeasured confounding in our findings. Finally, although we largely

observed consistent associations for VLCSFAs in both NHS and HPFS, we cannot exclude the role of chance in this study.

CONCLUSIONS

In summary, we found that in US men and women, plasma levels of VLCSFAs were associated with a lower risk of T2D. These associations were independent of established and potential dietary and lifestyle risk factors of T2D and robust in various sensitivity analyses. The inverse associations with T2D and other cardiometabolic diseases observed in the current and previous studies call for more research to understand the mechanistic pathways behind these associations.

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Table S3.1. Baseline characteristics of 1,392 women in the Nurses' Health Study (1990) according to quartiles of plasma fatty acids of VLCSFA

	Quartiles of 20:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	60.5 (6.1)	61.1 (6.1)	60.4 (6.3)	59.3 (6.9)
Race, Caucasian (%)	99.7	99.2	98.9	99.4
BMI (kg/m^2)	26.1 (4.7)	25.4 (4.4)	24.9 (4.5)	24.6 (3.8)
Current smoker (%)	16.5	17.4	19.6	19.9
Physical activity, MET, hours/week	14.1 (18.1)	18.2 (22.2)	16.2 (17.9)	16.6 (18.4)
Hypertension (%)	33.7	21.7	18.1	14.8
Hypercholesterolemia (%)	40.9	39.9	34.3	27.3
Family history of diabetes (%)	31.0	26.8	23.0	26.4
Total energy (kcal/day)	1715 (462)	1797 (503)	1788 (532)	1760 (492)
AHEI	39.0 (9.3)	40.9 (9.3)	40.1 (9.4)	39.4 (10.0)
Peanuts (oz./day)	0.08 (0.15)	0.08 (0.15)	0.08 (0.17)	0.08 (0.23)
Peanut butter (Tbsp./day)	0.20 (0.36)	0.20 (0.36)	0.21 (0.28)	0.24 (0.50)
Coffee (cups/day)	1.4 (1.5)	1.5 (1.5)	1.7 (1.6)	1.7 (1.7)
Alcohol (g/day)	5.7 (10.3)	5.5 (10.1)	5.6 (9.3)	3.8 (6.0)
Vegetable fat (g/day)	27.4 (11.5)	29.3 (13.5)	28.9 (12.8)	30.0 (14.3)
Dairy fat (g/day)	10.9 (6.2)	11.1 (6.5)	12.3 (7.8)	12.2 (7.6)

	Quartiles of 22:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	60.6 (6.3)	60.8 (5.8)	60.4 (6.6)	59.5 (6.7)
Race, Caucasian (%)	99.2	100	98.8	99.0
BMI (kg/m^2)	26.3 (5.2)	24.9 (4.1)	25.1 (4.2)	24.8 (3.9)
Current smoker (%)	17.1	18.9	17.6	19.6
Physical activity, MET, hours/week	15.4 (20.2)	16.8 (19.8)	16.2 (18.2)	16.6 (18.7)
Hypertension (%)	32.6	22.7	16.1	17.3
Hypercholesterolemia (%)	39.7	36.8	34.0	32.6
Family history of diabetes (%)	31.3	26.5	23.8	25.6
Total energy (kcal/day)	1746 (491)	1774 (504)	1805 (480)	1730 (515)
AHEI	40.1 (9.8)	39.9 (8.9)	40.2 (9.4)	39.1 (9.9)
Peanuts (oz/day)	0.07 (0.15)	0.08 (0.15)	0.08 (0.15)	0.1 (0.31)
Peanut butter (Tbsp./day)	0.15 (0.27)	0.21 (0.35)	0.24 (0.39)	0.30 (0.55)
Coffee (cups/day)	1.4 (1.5)	1.5 (1.5)	1.7 (1.6)	1.8 (1.7)
Alcohol (g/day)	6.9 (12.8)	5.6 (9.6.)	5.1 (9.1)	4.1 (7.5)
Vegetable fat (g/day)	27.2 (12.1)	27.9 (12.3)	29.9 (12.6)	29.7 (14.9)
Dairy fat (g/day)	10.8 (6.0)	12.0 (7.2)	11.9 (7.5)	11.7 (7.4)

Table S3.1. - Continued

	Quartiles of 24:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	60.4 (6.4)	60.9 (5.9)	60.1 (6.6)	59.9 (6.7)
Race, Caucasian (%)	99.5	99.4	99.1	99.1
BMI (kg/m^2)	26.4 (4.9)	25.2 (4.5)	25.0 (4.2)	24.4 (3.7)
Current smoker (%)	14.9	21.1	20.4	16.9
Physical activity, MET, hours/week	14.7 (19.9)	16.3 (19.3)	17.2 (19.2)	17.0 (18.4)
Hypertension (%)	30.6	22.5	18.6	16.9
Hypercholesterolemia (%)	38.4	37.3	34.0	33.4
Family history of diabetes (%)	30.6	26.2	24.0	26.3
Total energy (kcal/day)	1765 (470)	1757 (523)	1788 (485)	1746 (515)
AHEI	40.0 (9.7)	39.8 (8.8)	39.9 (9.6)	39.6 (9.9)
Peanuts (oz/day)	0.07 (0.14)	0.07 (0.20)	0.09 (0.16)	0.10 (0.31)
Peanut butter (Tbsp./day)	0.15 (0.26)	0.19 (0.32)	0.27 (0.48)	0.29 (0.50)
Coffee (cups/day)	1.3 (1.5)	1.5 (1.5)	1.73 (1.61)	1.8 (1.7)
Alcohol (g/day)	5.5 (11.1)	5.5 (10.2)	6.0 (10.2)	5.1 (8.4)
Vegetable fat (g/day)	28.0 (12.2)	27.2 (12.3)	29.8 (12.7)	29.6 (14.7)
Dairy fat (g/day)	11.1 (6.0)	11.7 (7.0)	11.9 (7.8)	11.7 (7.3)

	Quartiles of 20:0+22:0+24:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	60.5 (6.4)	61.0 (5.8)	60.2 (6.6)	59.7 (6.7)
Race, Caucasian (%)	99.5	99.7	99.1	98.8
BMI (kg/m^2)	26.3 (4.9)	25.0 (4.4)	25.1 (4.3)	24.6 (3.8)
Current smoker (%)	16.3	18.7	19.8	18.4
Physical activity, MET, hours/week	14.5 (19.6)	17.1 (19.7)	16.7 (19.0)	16.8 (18.7)
Hypertension (%)	31.3	22.8	17.6	16.9
Hypercholesterolemia (%)	39.3	39.3	31	33.1
Family history of diabetes (%)	31.3	25.2	24.3	26.3
Total energy (kcal/day)	1763 (479)	1744 (506)	1804 (489)	1748 (518)
AHEI	39.8 (9.5)	40.1 (9.0)	40.0 (9.7)	39.4 (9.8)
Peanuts (oz/day)	0.07 (0.15)	0.07 (0.20)	0.09 (0.16)	0.10 (0.30)
Peanut butter (Tbsp./day)	0.16 (0.28)	0.21 (0.35)	0.24 (0.41)	0.29 (0.53)
Coffee (cups/day)	1.4 (1.5)	1.4 (1.5)	1.8 (1.6)	1.8 (1.8)
Alcohol (g/day)	6.2 (12.3)	5.4 (9.4)	5.9 (10.1)	4.4 (7.7)
Vegetable fat (g/day)	27.7 (12.0)	27.4 (12.1)	29.7 (12.7)	29.9 (15.1)
Dairy fat (g/day)	11.0 (5.9)	11.6 (7.3)	12.0 (7.6)	11.8 (7.3)

Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; AHEI, alternative healthy eating index. Values are mean and (SD) for continuous variables and percent for categorical variables.

Table S3.2. Baseline characteristics of 1,462 men in the Health Professionals Follow-Up Study (1994) according to quartiles of plasma fatty acids of VLCSFA

	Quartiles of 20:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	66.1 (8.3)	64.9 (8.6)	63.2	63.2 (8.8)
Race, Caucasian (%)	94.6	93.1	91.7	94.7
BMI (kg/m^2)	26.3 (3.3)	25.6 (3.3)	25.4 (3.0)	25.5 (3.0)
Current smoker (%)	8.5	5.9	7.0	9.9
Physical activity, MET, hours/week	35.8 (42.2)	37.3 (40.7)	36.2 (36.9)	34.9 (32.5)
Hypertension (%)	30.1	26.9	22.9	17.1
Hypercholesterolemia (%)	32.5	23.8	25.5	23.4
Family history of diabetes (%)	25.7	21.8	23.0	19.4
Total energy (kcal/day)	2054 (598)	2010 (598)	2033 (633)	2075 (595)
AHEI	43.0 (9.8)	42.0 (9.6)	42.4 (9.6)	41.8 (8.9)
Peanuts (oz/day)	1.3 (1.51)	1.1 (1.4)	1.0 (1.2)	1.2 (1.5)
Peanut butter (Tbsp./day)	1.7 (1.8)	1.4 (1.6)	1.7 (1.8)	1.5 (1.7)
Coffee (cups/day)	2.0 (1.6)	1.9 (1.8)	2.0 (1.8)	2.1 (1.74)
Alcohol (g/day)	14.2 (18.9)	11.3 (15.2)	10.9 (14.0)	9.5 (12.2)
Vegetable fat (g/day)	33.3 (16.7)	31.7 (14.7)	33.1 (16.5)	35.7 (17.3)
Dairy fat (g/day)	11.0 (8.3)	11.0 (8.6)	11.4 (7.9)	12.4 (9.4)

	Quartiles of 22:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	66.5 (8.3)	64.4 (8.4)	63.4 (8.6)	62.9 (8.7)
Race, Caucasian (%)	93.6	94.5	92.8	93.4
BMI (kg/m^2)	26.3 (3.4)	25.6 (3.1)	25.6 (3.1)	25.4 (3.0)
Current smoker (%)	7.5	8.3	8.2	7.3
Physical activity, MET, hours/week	33.5 (36.9)	37.0 (42.3)	36.0 (38.0)	38.9 (38.0)
Hypertension (%)	32.3	24.0	23.5	16.6
Hypercholesterolemia (%)	32.3	21.1	29.5	22.5
Family history of diabetes (%)	24.2	21.3	25.2	20.3
Total energy (kcal/day)	2041 (619)	2047 (621)	2015 (579)	2073 (590)
AHEI	42.5 (9.9)	43.2 (9.8)	41.9 (8.9)	41.4 (8.9)
Peanuts (oz/day)	1.1 (1.4)	1.2 (1.6)	1.2 (1.4)	1.2 (1.4)
Peanut butter (Tbsp./day)	1.5 (1.7)	1.5 (1.7)	1.6 (1.7)	1.9 (1.8)
Coffee (cups/day)	1.9 (1.7)	1.9 (1.7)	2.0 (1.8)	2.1 (1.8)
Alcohol (g/day)	13.4 (18.5)	12.1 (16.2)	10.9 (13.2)	9.6 (12.9)
Vegetable fat (g/day)	32.1 (16.1)	33.3 (17.3)	33.1 (15.8)	35.8 (16.0)
Dairy fat (g/day)	10.8 (7.8)	11.4 (9.6)	11.0 (7.7)	12.5 (8.9)

Table S3.2. - continued

	Quartiles of 24:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	66.6 (8.2)	64.7 (8.5)	63.0 (8.7)	62.5 (8.6)
Race, Caucasian (%)	92.3	95.3	92.3	94.2
BMI (kg/m^2)	26.3 (3.3)	25.7 (3.2)	25.6 (3.2)	25.3 (3.0)
Current smoker (%)	8.0	6.4	9.6	7.2
Physical activity, MET, hours/week	32.6 (37.6)	38.6 (42.4)	34.9 (37.4)	40.4 (37.5)
Hypertension (%)	33.1	22.8	22.4	16.3
Hypercholesterolemia (%)	30.2	25.1	24.9	16.3
Family history of diabetes (%)	24.0	22.3	22.9	24.9
Total energy (kcal/day)	2054 (617)	2033 (617)	2020 (592)	2062 (581)
AHEI	42.0 (9.9)	43.3 (9.7)	42.2 (9.0)	42.1 (9.0)
Peanuts (oz/day)	1.2 (1.5)	1.2 (1.5)	1.2 (1.3)	1.2 (1.4)
Peanut butter (Tbsp./day)	1.5 (1.7)	1.6 (1.7)	1.5 (1.6)	1.9 (1.9)
Coffee (cups/day)	1.9 (1.6)	1.9 (1.6)	2.0 (1.9)	2.2 (1.8)
Alcohol (g/day)	12.3 (17.6)	12.0 (15.7)	11.2 (14.5)	11.3 (13.8)
Vegetable fat (g/day)	32.5 (16.0)	33.0 (17.4)	33.2 (15.8)	35.6 (16.3)
Dairy fat (g/day)	1.9 (1.6)	1.9 (1.6)	11.3 (8.3)	2.2 (1.8)

	Quartiles of 20:0+22:0+24:0			
	Q1	**Q2**	**Q3**	**Q4**
Age, years	66.4 (8.2)	64.8 (8.6)	62.8 (8.5)	63.1 (8.7)
Race, Caucasian (%)	94.1	93.8	92.8	93.6
BMI (kg/m^2)	26.4 (3.3)	25.6 (3.2)	25.5 (3.0)	25.3 (3.0)
Current smoker (%)	7.9	7.3	8.2	7.8
Physical activity, MET, hours/week	32.8 (36.9)	38.6 (44.2)	35.3 (36.9)	39.3 (36.4)
Hypertension (%)	33.3	22.8	22.3	17.0
Hypercholesterolemia (%)	32.7	21.1	25.8	25.1
Family history of diabetes (%)	25.2	21.2	23.9	20.1
Total energy (kcal/day)	2059 (623)	2021 (603)	2016 (580)	2074 (604)
AHEI	42.4 (9.9)	43.0 (9.8)	42.0 (9.2)	42.0 (8.9)
Peanuts (oz/day)	1.1 (1.4)	1.2 (1.5)	1.2 (1.4)	1.2 (1.4)
Peanut butter (Tbsp./day)	1.6 (1.7)	1.5 (1.8)	1.5 (1.6)	1.9 (1.9)
Coffee (cups/day)	1.9 (1.7)	1.9 (1.7)	2.0 (1.9)	2.2 (1.8)
Alcohol (g/day)	13.2 (18.5)	12.1 (15.8)	10.7 (13.3)	10.2 (13.2)
Vegetable fat (g/day)	32.5 (16.3)	32.9 (16.9)	33.0 (16.2)	35.9 (15.8)
Dairy fat (g/day)	11.2 (8.7)	11.0 (8.7)	11.0 (7.6)	12.3 (9.1)

Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; AHEI, alternative healthy eating index. Values are mean and (SD) for continuous variables and percent for categorical variables.

Table S3.3. Partial Spearman correlations between circulating plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 among 708 participants in the Nurses' Health Study and 808 participants in the Health Professionals Follow-Up Study

	Plasma fatty acids[1]		
	20:0	**22:0**	**24:0**
NHS			
20:0	1.0	**0.77*** **	**0.69*** **
22:0	**0.77*** **	1.0	**0.92*** **
24:0	**0.69*** **	**0.92*** **	1.0
14:0	**-0.42*** **	**-048*** **	**-0.45*** **
16:0	**-0.48*** **	**-0.62*** **	**-0.61*** **
18:0	**0.45*** **	**0.23*** **	**0.22*** **
15:0	**-0.20*** **	**-0.25*** **	**-0.31*** **
17:0	**0.26*** **	**0.12*** **	**0.08** **
trans 16:1n-7	**-0.06***	**-0.12*** **	**-0.23*** **
trans 18:1	**0.09***	-0.03	**-0.17*** **
trans 18:2	<-0.01	**-0.07***	**-0.22*** **
18:2	**0.24*** **	**0.41*** **	**0.38*** **
20:4	**0.26*** **	**0.39*** **	**0.45*** **
EPA	**0.11** **	**0.11** **	**0.20*** **
DHA	**0.21*** **	**0.24*** **	**0.34*** **
HPFS			
20:0	1.0	**0.71*** **	**0.68*** **
22:0	**0.71*** **	1.0	**0.89*** **
24:0	**0.68*** **	**0.89*** **	1.0
14:0	**-0.39*** **	**-0.46*** **	**-0.49*** **
16:0	**-0.51*** **	**-0.60*** **	**-0.59*** **
18:0	**0.36*** **	**0.07***	**0.09***
15:0	**-0.10*** **	**-0.20*** **	**-0.29*** **
17:0	**0.21*** **	0.03	-0.03
trans 16:1n-7	**0.07***	**-0.07***	**-0.10*** **
trans 18:1	<0.01	-0.06	**-0.13*** **
trans 18:2	**-0.08*** **	**-0.17*** **	**-0.20*** **
18:2	**0.27*** **	**0.42*** **	**0.43*** **
20:4	**0.23*** **	**0.40*** **	**0.41*** **
EPA	0.07	0.05	**0.14*** **
DHA	**0.16*** **	**0.15*** **	**0.24*** **

[1]Fatty acid concentrations were assessed from the plasma fraction of NHS and HPFS participants
*p-value for correlation coefficient < 0.05; ** p-value <0.01; *** p-value <0.001

Table S3.4. Partial Spearman correlations between circulating erythrocyte fatty acid biomarkers of 20:0, 22:0, and 24:0 among 669 participants in the Nurses' Health Study and 852 participants in the Health Professionals Follow-Up Study

	Erythrocyte fatty acids[1]		
	20:0	**22:0**	**24:0**
NHS			
20:0	1.0	**0.53*** **	**0.32*** **
22:0	**0.53*** **	1.0	**0.83*** **
24:0	**0.32*** **	**0.83*** **	1.0
14:0	**-0.29*** **	**-0.52*** **	**-0.48*** **
16:0	**-0.27*** **	**-0.63*** **	**-0.69*** **
18:0	**0.21*** **	**0.40*** **	**-0.42*** **
15:0	-0.05	**-0.36*** **	**-0.44*** **
17:0	**0.08***	**-0.48*** **	**-0.54*** **
trans 16:1n-7	-0.03	**-0.44*** **	**-0.54*** **
trans 18:1	**0.16*** **	**-0.33*** **	**-0.49*** **
trans 18:2	**0.31*** **	**0.25*** **	0.02
18:2	-0.05	**0.28*** **	**0.19*** **
20:4	0.05	**0.52*** **	**0.50*** **
EPA	**-0.11****	**0.12****	**0.23*** **
DHA	-0.01	**0.32*** **	**0.44*** **
HPFS			
20:0	1.0	**0.53*** **	**0.31*** **
22:0	**0.53*** **	1.0	**0.79*** **
24:0	**0.31*** **	**0.79*** **	1.0
14:0	**-0.17*** **	**-0.39*** **	**-0.38*** **
16:0	**-0.31*** **	**-0.64*** **	**-0.60*** **
18:0	**0.35*** **	0.01	-0.04
15:0	0.06	**-0.13*** **	**-0.21*** **
17:0	**0.26*** **	0.12	**-0.16*** **
trans 16:1n-7	**0.14*** **	**-0.07***	**-0.23*** **
trans 18:1	**0.10*** **	0.06	**-0.19*** **
trans 18:2	**-0.09**	**-0.26*** **	**-0.28*** **
18:2	**-0.19****	**-0.14*** **	**-0.35*** **
20:4	**0.17*** **	**0.43*** **	**0.38*** **
EPA	<0.01	-0.06	**0.09***
DHA	**0.09***	**0.11*** **	**0.25*** **

[1]Fatty acid concentrations were assessed from the plasma fraction of NHS and HPFS participants
*p-value for correlation coefficient < 0.05; ** p-value <0.01; *** p-value <0.001

Table S3.5. Risk of incident diabetes according to plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 in sensitivity analyses excluding cases in the first 2 and 4 years of follow-up in the NHS (N=129, and 113 cases, respectively) and HPFS (N=86, 71 cases, respectively)

Fatty acid	Cohort, specific fatty acid quartiles				
	1	2	3	4	P for trend
20:0, NHS					
No. of cases, entire follow-up period	53	38	22	23	
Multivariable hazard ratio 2[1]	Reference	0.81 (0.52, 1.25)	0.53 (0.32, 0.90)	0.53 (0.31, 0.90)	0.007
No. of cases, excluding first 2 years	50	34	22	23	
Multivariable hazard ratio 2[1]	Reference	0.78 (0.49, 1.23)	0.55 (0.33, 0.93)	0.54 (0.32, 0.93)	0.01
No. of cases, excluding first 4 years	43	31	17	22	
Multivariable hazard ratio 2[1]	Reference	0.83 (0.51, 1.35)	0.50 (0.28, 0.90)	0.63 (0.36, 1.10)	0.04
20:0, HPFS					
No. of cases, entire follow-up period	48	27	16	16	
Multivariable hazard ratio 2[1]	Reference	0.66 (0.41, 1.08)	0.43 (0.24, 0.77)	0.44 (0.25, 0.80)	0.001
No. of cases, excluding first 2 years	31	23	16	16	
Multivariable hazard ratio 2[1]	Reference	0.80 (0.46, 1.41)	0.58 (0.31, 1.08)	0.62 (0.33, 1.16)	0.08
No. of cases, excluding first 4 years	22	18	16	15	
Multivariable hazard ratio 2[1]	Reference	0.93 (0.49, 1.79)	0.84 (0.43, 1.64)	0.84 (0.42, 1.68)	0.58
22:0, NHS					
No. of cases, entire follow-up period	50	44	26	16	
Multivariable hazard ratio 2[1]	Reference	1.09 (0.71, 1.66)	0.62 (0.38, 1.02)	0.38 (0.21, 0.69)	<0.001
No. of cases, excluding first 2 years	29	24	18	15	
Multivariable hazard ratio 2[1]	Reference	1.02 (0.66, 1.57)	0.61 (0.37, 1.01)	0.39 (0.21, 0.70)	<0.001
No. of cases, excluding first 4 years	40	36	22	15	
Multivariable hazard ratio 2[1]	Reference	1.09 (0.68, 1.75)	0.64 (0.37, 1.11)	0.44 (0.23, 0.82)	0.004
22:0, HPFS					
No. of cases, entire follow-up period	43	28	21	15	
Multivariable hazard ratio 2[1]	Reference	0.77 (0.47, 1.27)	0.62 (0.36, 1.08)	0.50 (0.27, 0.94)	0.018
No. of cases, excluding first 2 years	40	29	19	15	
Multivariable hazard ratio 2[1]	Reference	0.85 (0.48, 1.49)	0.66 (0.36, 1.23)	0.62 (0.32, 1.20)	0.11
No. of cases, excluding first 4 years	22	18	16	15	
Multivariable hazard ratio 2[1]	Reference	0.84 (0.44, 1.60)	0.77 (0.39, 1.53)	0.84 (0.41, 1.70)	0.58
24:0, NHS					
No. of cases, entire follow-up period	57	43	21	15	
Multivariable hazard ratio 2[1]	Reference	0.95 (0.63, 1.43)	0.45 (0.27, 0.76)	0.34 (0.19, 0.62)	<0.001
No. of cases, excluding first 2 years	54	39	21	15	
Multivariable hazard ratio 2[1]	Reference	0.89 (0.58, 1.37)	0.48 (0.28, 0.80)	0.36 (0.20, 0.65)	<0.001
No. of cases, excluding first 4 years	46	35	18	14	
Multivariable hazard ratio 2[1]	Reference	0.93 (0.59, 1.48)	0.46 (0.26, 0.81)	0.39 (0.21, 0.74)	<0.001

Table S3.5. - Continued

24:0, HPFS					
No. of cases, entire follow-up period	50	21	21	15	
Multivariable hazard ratio 2[1]	Reference	0.56 (0.33, 0.95)	0.59 (0.34, 1.01)	0.48 (0.26, 0.89)	0.013
No. of cases, excluding first 2 years	32	19	20	15	
Multivariable hazard ratio 2[1]	Reference	0.72 (0.40, 1.29)	0.79 (0.44, 1.43)	0.66 (0.34, 1.27)	0.24
No. of cases, excluding first 4 years	23	13	20	15	
Multivariable hazard ratio 2[1]	Reference	0.72 (0.35, 1.45)	1.14 (0.60, 2.16)	0.98 (0.49, 1.99)	0.09
Sum (20:0 + 22:0 + 24:0), NHS					
No. of cases, entire follow-up period	56	40	26	14	
Multivariable hazard ratio 2[1]	Reference	0.94 (0.61, 1.43)	0.57 (0.35, 0.93)	0.32 (0.17, 0.59)	<0.001
No. of cases, excluding first 2 years	53	36	26	14	
Multivariable hazard ratio 2[1]	Reference	0.88 (0.57, 1.36)	0.60 (0.37, 0.97)	0.33 (0.18, 0.61)	<0.001
No. of cases, excluding first 4 years	45	32	23	13	
Multivariable hazard ratio 2[1]	Reference	0.91 (0.57, 1.46)	0.62 (0.36, 1.04)	0.36 (0.19, 0.69)	<0.001
Sum (20:0 + 22:0 + 24:0), HPFS					
No. of cases, entire follow-up period	48	23	20	16	
Multivariable hazard ratio 2[1]	Reference	0.60 (0.36, 1.00)	0.58 (0.33, 1.00)	0.50 (0.28, 0.90)	0.014
No. of cases, excluding first 2 years	31	22	17	16	
Multivariable hazard ratio 2[1]	Reference	0.78 (0.45, 1.37)	0.66 (0.35, 1.22)	0.66 (0.35, 1.24)	0.16
No. of cases, excluding first 4 years	24	15	16	16	
Multivariable hazard ratio 2[1]	Reference	0.71 (0.37, 1.38)	0.81 (0.42, 1.59)	0.88 (0.45, 1.74)	0.77

[1]Adjusted for age (years), BMI (continuous), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), total energy (kcal/day) sum of biomarkers of 15:0, 17:0, *trans 16:1n, 7,* and trans, 18:1, trans, 18:2, glycemic load, and AHEI

Table S3.6. Risk of incident diabetes according to circulating plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 among 707 women in the Nurses' Health Study (N=55 cases) and 820 men who were selected as CVD controls in the Health Professionals Follow-Up Study (N=38 cases)

Fatty acid	Cohort, specific fatty acid quartiles				
	1	2	3	4	P for trend
20:0					
NHS % of total FA, mean (SD)	0.14 (0.02)	0.18 (0.01)	0.22 (0.010)	0.27 (0.03)	
NHS cases / person, months	22 / 36,411	12 / 38,378	10 / 39,023	11 / 40,196	
HPFS % of total FA, mean (SD)	0.13 (0.02)	0.17 (0.01)	0.20 (0.01)	0.24 (0.03)	
HPFS cases / person, months	7 / 33,522	13 / 33,888	8 / 34,811	8 / 34,287	
Multivariate HR, pooled – controls only[1]	Reference	0.92 (0.52, 1.60)	0.76 (0.43, 1.35)	0.75 (0.41, 1.41)	0.30
22:0					
NHS % of total FA, mean (SD)	0.32 (0.06)	0.46 (0.03)	0.57 (0.04)	0.76 (0.10)	
NHS cases / person, months	17 / 36,110	19 / 38,309	11 / 39,143	8 / 40,446	
HPFS % of total FA, mean (SD)	0.26 (0.07)	0.42 (0.04)	0.55 (0.03)	0.73 (0.10)	
HPFS cases / person, months	10 / 33,295	9 / 34,039	9 / 34,809	8 / 34,365	
Multivariate HR, pooled – controls only[1]	Reference	1.01 (0.64, 1.92)	0.81 (0.45, 1.46)	0.58 (0.30, 1.13)	0.08
24:0					
NHS % of total FA, mean (SD)	0.22 (0.05)	0.34 (0.03)	0.44 (0.03)	0.64 (0.10)	
NHS cases / person, months	19 / 36,086	18 / 37,987	11 / 39,723	7 / 40,212	
HPFS % of total FA, mean (SD)	0.23 (0.05)	0.35 (0.03)	0.46 (0.03)	0.62 (0.09)	
HPFS cases / person, months	12 / 32,704	6 / 34,223	9 / 34,255	9 / 35,326	
Multivariate HR, pooled – controls only[1]	Reference	0.94 (0.55, 1.61)	0.65 (0.36, 1.20)	0.63 (0.34, 1.19)	0.10
Sum (20:0 + 22:0 + 24:0)					
NHS % of total FA, mean (SD)	0.70 (0.13)	1.00 (0.06)	1.22 (0.08)	1.65 (0.20)	
NHS cases / person, months	18 / 36,284	19 / 37,945	12 / 39,761	6 / 40,018	
HPFS % of total FA, mean (SD)	0.65 (0.12)	0.94 (0.08)	1.20 (0.07)	1.57 (0.19)	
HPFS cases / person, months	11 / 33,027	8 / 34,063	8 / 34,957	9 / 34,461	
Multivariate HR, pooled – controls only[1]	Reference	1.15 (0.67, 1.97)	0.76 (0.41, 1.39)	0.66 (0.35, 1.27)	0.13

Model adjusted for age (years), BMI (continuous), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), total energy (kcal/day) sum of biomarkers of 15:0, 17:0, *trans 16:1n, 7,* and trans, 18:1, trans, 18:2, glycemic load, and AHEI. Pooled estimates and p-values were calculated by combining the participant data from both cohorts and further adjusting multivariate model 1 by sex

[1] Pooled estimates and p-values were calculated by combining the participant data from both cohorts and stratifying the model by sex

Table S3.7. Risk of diabetes according to circulating plasma fatty acid biomarkers of 20:0, 22:0, and 24:0 among 1,392 women in the Nurses' Health Study (N=136 cases) and 1,462 men in the Health Professionals Follow-Up Study (N=107 cases) mutually adjusting for other VLCSFA

Fatty acid	Cohort-specific fatty acid quartiles				
	1	2	3	4	P for trend
20:0, NHS					
No. of cases	53	38	22	23	
Person-months	74,132	72,255	71,707	66,995	
Multivariable hazard ratio 2[1]	Reference	0.97 (0.60-1.56)	0.76 (0.41-1.75)	0.85 (0.41-1.75)	0.55
20:0, HPFS					
No. of cases	48	27	16	16	
Person-months	50,153	48,821	46,996	48,179	
Multivariable hazard ratio 2[1]	Reference	0.74 (0.43-1.26)	0.51 (0.26-1.02)	0.58 (0.26-1.33)	0.12
20:0, pooled[2]	**Reference**	**0.95 (0.67-1.37)**	**0.81 (0.53-1.23)**	**0.86 (0.51-1.44)**	**0.45**
22:0, NHS					
No. of cases	50	44	26	16	
Person-months	70,309	75,612	72,219	66,949	
Multivariable hazard ratio 2[1]	Reference	1.38 (0.81-2.36)	0.97 (0.46-2.04)	0.89 (0.29-2.75)	0.74
22:0, HPFS					
No. of cases	43	28	21	15	
Person-months	48,208	50,348	48,119	47,474	
Multivariable hazard ratio 2[1]	Reference	1.16 (0.64-2.12)	1.29 (0.57-2.94)	1.60 (0.50-5.21)	0.44
22:0, pooled[2]	**Reference**	**1.28 (0.87-1.89)**	**1.04 (0.61-1.78)**	**1.10 (0.50-2.45)**	**0.81**
24:0, NHS					
No. of cases	57	43	21	15	
Person-months	74,602	70,819	72,111	67,557	
Multivariable hazard ratio 2[1]	Reference	0.90 (0.55-1.49)	0.41 (0.20-0.87)	0.29 (0.10-0.91)	0.02
24:0, HPFS					
No. of cases	50	21	21	15	
Person-months	48,817	49,796	49,064	46,472	
Multivariable hazard ratio 2[1]	Reference	0.76 (0.41-1.40)	0.99 (0.45-2.15)	1.11 (0.38-3.28)	0.81
24:0, pooled[2]	**Reference**	**0.80 (0.55-1.16)**	**0.56 (0.33-0.95)**	**0.51 (0.24-1.09)**	**0.05**

[1]Adjusted for age (continuous), BMI (continuous), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), AHEI, glycemic load (continuous), and total energy (kcal/day), + sum biomarkers (15:0, 17:0, *trans* 16:1n-7), *trans* 18:1, *trans* 18:2, and mutual adjustment for other two fatty acids

[2] Pooled estimates and p-values were calculated by combining the participant data from both cohorts and stratifying the model by sex

CONCLUDING REMARKS

Type 2 diabetes and its health consequences continue to be significant public health issues in the U.S. and globally. Dietary variables remain important modifiable risk factors in the development of T2D and its complications such as CVD health outcomes. Dairy products account for almost 10% of calories consumed in the U.S. and several national dietary guidelines include dairy products, recommending low-fat dairy products as part of healthy diet.

In chapter 1, we explored the association between dairy fat intake and risk of T2D. We concluded that dairy fat intake was not associated with T2D risk when compared to calories from carbohydrate. However, replacement of dairy fat with carbohydrates from whole grains was associated with lower T2D risk, whereas replacement of dairy fat with other animal fats or refined carbohydrates was associated with higher risk of T2D. These findings provide evidence that specific macronutrient comparisons are important, and that dairy fat intake is not associated with T2D risk reduction. Future research can explore specific replacements of dairy fat for other oils and fats in both observational studies and randomized trials.

In chapter 2, we examined the association between dairy products and risk of CVD in a T2D patient population. We concluded that total dairy product intake was not associated with risk of CVD among participants with diabetes and neither were dairy products stratified by their fat content. We found a surprising inverse association between ice-cream intake and CVD risk that warrants further research. Replacing dairy products with red and processed read meat is associated with higher CVD risk, whereas replacing dairy product intake with nus is associated

with lower CVD risk. In conclusion, the overall pattern of associations with dairy intake and CVD is similar to heathy populations at baseline. These data provide initial evidence regarding the health consequences of consuming dairy product intake among patients of diabetes and need replications in future studies.

In chapter 3, we explored the association between plasma and erythrocyte biomarkers of VLCSFA and T2D risk. Our findings suggest that in US men and women, plasma levels VLCSFAs are associated with a lower risk of T2D and erythrocyte biomarkers of 20:0 and 22:0 are also inversely associated with T2D risk. More research is needed to understand the mechanistic pathways underlying these associations and determine whether VLCSFA play a causal role in the development of T2D.