Appendix 3:

Evidence reviewed but did not directly inform GRADE evidence summary

Contents

Evidence on saturated fat that was reviewed, but that did not directly inform the GRADE evidence summary

Results from Prospective Cohort Studies

Polyunsaturated:Saturated Fat and all-cause and CVD mortality

The Kuopio Ischaemic Heart Disease Risk Factor Study (IHD), a prospective population-based study including 1551 men followed for a median of 14.6 years (NOS quality=9), observed 78 deaths from cardiovascular disease and 225 total deaths[1]. Dietary fatty acid intake was assessed at baseline using a 4-day diet record, and serum fatty acids were measured by gas chromatography. A dietary polyunsaturated:saturated fat (P:S) ratio in the upper third was associated with a 29% reduction in risk of all-cause mortality in age-adjusted models (RR: 0.71; 95% CI: 0.51 to 0.98), the association was not significant after adjustment for other risk factors. A serum P:S ratio in the upper third was associated with a 31% reduction in all-cause mortality (OR: 0.69; 95% CI: 0.41 to 1.03). A dietary P:S ratio in the upper third was associated with a 56% reduction in all-cardiovascular mortality (fully MV adjusted OR: 0.44; 95% CI: 0.20 to 1.00). A serum P: S ratio in the upper third was associated with a similar 60% reduction in risk of CVD mortality (OR: 0.40; 95% CI: 0.18 to 0.87), but was no longer significant when adjusted for measures of adiposity. *This study was not included in our quantitative synthesis because it did not present relative risk for higher vs. lower saturated fat intake; the P: S ratio is affected both by polyunsaturated and saturated fat.*

Saturated Fatty Acid Isomers and CHD

The strongest epidemiologic evidence to date for the effects of specific food sources of saturated fatty acids on cardiovascular health comes from the Nurses' Health Study, a prospective cohort study of 80,082 women aged 34-59 y[2]. During 14 years of follow-up, the investigators documented 939 new cases of major CHD events. Food intake was measured by a validated semiquantitative food-frequency-questionnaire administered in 1980, 1986, and 1990. The major sources of saturated fat in this population were beef (13%), and hard cheese (11%). Five groups of fatty acids were examined: 4:0 to 10:0 (found mainly in hard cheese, butter, and milk), 12:0 (found mainly in coffee whitener, hard cheese, and low-fat milk), 14:0 (found mainly in hard cheese, beef, low-fat milk, and butter), 16:0 (found mainly in beef, and hard cheese), and 18:0 (found mainly in beef and hard cheese). In multivariable-adjusted models, no significant associations between individual saturated fatty acids and CHD risk were found (**Appendix D, Table D1**). The authors additionally estimated the MVRR for a 1% increase in each fatty acid class, substituted for carbohydrate, and the effect of replacement of 12:0 to 18:0 saturated fatty acids with carbohydrate, monounsaturated fat, or polyunsaturated fat (**Table D2**), and found that substitution of longer-chain saturated fatty acids (12:0 to 18:0) with carbohydrate were associated with reduced CHD risk; stronger associations were found when unsaturated fats replaced these isomers of saturated fatty acids. *This study was not included in our quantitative synthesis because it was the lone prospective cohort study to assess individual isomers of saturated fat with CHD risk.*

Saturated Fatty Acid Isomers and Ischemic Stroke

In a Swedish prospective cohort study of 2,313 middle-aged men aged ≥50 recruited in 1970-73, 421 cases of stroke or transient ischemic attack were observed over a 32-year follow-up[3]. At the baseline visit, cholesterol ester proportions of fatty acids were measured by gas chromatography. The authors found no association between baseline proportions of 14:0 (OR: 1.07; 95% CI: 0.97 to 1.18) or 16:0 (RR: 1.07; 95% CI: 0.96 to 1.20) and risk of ischemic stroke.

A case-control study, nested within the Nurses' Health (n=371 cases and 371 controls; mean age 61.0 y; matched on age, race/ethnicity, smoking, blood-collection date, and length of follow-up) and Health Professionals' Follow-up (n=80 cases and 80 controls; mean age 67.6 y) Studies[4] found no association of 14:0 (pooled RR: 0.89; 95% CI: 0.47 to 1.66), 15:0 (pooled RR: 0.78; 95% CI: 0.45 to 1.34), or 17:0 (pooled RR: 0.99; 95% CI: 0.62 to 1.58) with incident ischemic stroke over 8.3 y of follow-up.

These studies were not included in our primary analysis of total SFA and ischemic stroke because they only presented associations of individual isomers of saturated fat with ischemic stroke risk.

Polyunsaturated:Saturated Fat Ratio and type 2 diabetes

The European Prospective Investigation of Cancer (EPIC), a population-based cohort study investigators examined the association between the ratio of polyunsaturated:saturated fat in the diet and risk of type 2 diabetes[5]. Diet was measured once at baseline using a validated semiquantitative food-frequency questionnaire. During 3-7 years of follow-up, 414 incident cases of diabetes were reported among 23,631 men and women. In this study, a 1-standard deviation increase in the energy-adjusted P:S ratio (0.22-units) was associated with a 16% reduction in type 2 diabetes (OR: 0.84; 95% CI: 0.75 to 0.94), however when adjusted for measures of adiposity, this association was attenuated (OR: 0.91; 95% CI: 0.81 to 1.03). *This study was not included in our quantitative synthesis because it did not present relative risk for higher vs. lower saturated fat intake; the P:S ratio is affected both by polyunsaturated and saturated fat.*

Saturated Fatty Acid Food Sources and CVD

A recent publication from the Multi-Ethnic Study of Atherosclerosis (MESA), a prospective cohort study of 5,209 participants across the United States, aged 45-84, provides insight into the effects on cardiovascular health of different food sources of saturated fat[6]. Diet was measured with a modified Block FFQ, administered once at baseline, to identify dairy, meat, butter, plant, and mixed animal and plant sources of saturated fat. Over 10 years of follow-up, between 2000 and 2010, 316 new cases of CVD were observed. The mean saturated fat intake in the sample was 10.2±3.2%. Associations between CVD and saturated fat from each source were calculated per 5 g or 5% energy increments (**Table D3**). The authors further investigated the effect on CVD risk of substitution of one food source of saturated fat for another, and found that the only substitution likely to have cardiovascular benefit was replacement of saturated fat from meat with saturated fat from dairy (**Table D4**).

In the Zutphen Elderly Study (ZES)[7], a prospective study of 686 elderly men from the Netherlands, aged 65-85 at baseline, with a range of 14-22% intake of energy from saturated fat, 132 incident CHD events were observed over 15y of follow-up. In this study, no single food source of SFA (butter, dairy, or meat) were significantly associated with CHD, comparing the top to bottom tertiles. *Individual FA risk estimates from these studies were not included in our quantitative synthesis because estimates for total SFA were also provided; these were included.*

Saturated fat and CVD and mortality in high-risk subgroups

Saturated Fat and Total and Cardiovascular Mortality in diabetes

In a subset of 5,672 participants in the Nurses' Health Study, Tanasescu et al.[8] examined the association between saturated fat intake and CVD risk in women with type 2 diabetes. Over 18 years of follow-up, between 1980 and 1998, they observed 619 new cases of CVD, defined as non-fatal myocardial infarction, fatal CHD, or stroke. Diet was assessed at baseline, and at 4 follow-up occasions using a validated semiquantitative food frequency questionnaire. Replacement of 5% of energy from carbohydrate with saturated fat was associated with a 29% increased risk of CVD (RR: 1.29; 95% CI: 1.02, 1.63). This effect was stronger than what was seen in the entire NHS cohort, which may reflect the adverse effects of saturated fat on lipoproteins, or other metabolic sequelae of insulin resistance, such as blunting of insulin sensitivity. *This study was not included in our main quantitative synthesis because it examined the effect of SFA on CVD mortality in people with type 2 diabetes, a sample which would not be generalizable to the target population for guidelines (generally healthy individuals).*

Trichopoulou et al.[9] followed 1,013 participants with type 2 diabetes enrolled in the Greek EPIC cohort from 1993 to mid-2004. Diet was assessed at baseline with a validated 150-item food-frequency questionnaire. Over a median of 4.5 years of follow-up, 80 deaths were observed, 46 of which were from cardiovascular causes. In this study, a 10-g increase in saturated fat intake was associated with an 82% increased risk of all-cause mortality (HR: 1.82; 95% CI: 1.14, 2.90), adjusted for gender, age, education, smoking, waist-to-height, hip circumference, total energy, insulin use, hypertension, and hypercholesterolemia. *This study was not included in our main quantitative synthesis because it examined the effect of SFA on CVD mortality in people with type 2 diabetes, a sample from which results would not be generalizable to the target population for guidelines (generally healthy individuals).*

In the EURODIAB prospective study of complications of diabetes[10], 2,108 people with type 1 diabetes were followed for 7 years, during which time 148 incident cases of fatal and non-fatal CVD, and 46 all-cause deaths were documented. Diet was assessed once at baseline using a 3-day diet record. In multivariable-adjusted models, a high (45.5 g) intake of saturated fat was not associated with increased risk of CVD in this high risk cohort (RR: 0.84; 95% CI: 0.53 to 1.32; P for trend=0.43). A 10-g increase in saturated fat was not associated with increased CVD risk (RR: 0.85; 95% CI: 0.69 to 1.05) or all-cause mortality (HR: 0.70; 95% CI: 0.48 to 1.04). Substitution models were also explored, and failed to find a significant impact on CVD risk of exchanging saturated fat for other nutrients in type 1 diabetes (**Table D5**). *This study was not included in our main quantitative synthesis because it examined the effect of SFA*

on non-fatal CVD in people with type 1 diabetes, a sample from which results would not be generalizable to the target population for guidelines (generally healthy individuals).

Saturated Fat and Total and Cardiovascular Mortality in Secondary Prevention

In 285 men and 130 women participating in the EUROASPIRE (European Action on Secondary Prevention through Intervention to Reduce Events) study[11], diet was measured by 4-d food records, and validated against fatty acid composition of serum cholesteryl esters. During 5y of follow-up, 36 participants died, 21 had an MI, and 12 had strokes. For a 1-SD increase in saturated fat (as measured by a 4-d food record), a 57% increase risk of death was observed (RR: 1.57; 95% CI: 1.13 to 2.17), but not specifically due to coronary disease (RR for coronary death: 1.01; 95% CI: 0.61 to 1.69), and there was no increased risk of any coronary (RR: 1.00; 95% CI: 0.68 to 1.46) or cardiovascular events (RR: 1.23; 95% CI: 0.89 to 1.68; for a composite of CVD death, acute MI, and stroke).

In an analysis of the Western Norway B-Vitamin Intervention Trial, which enrolled 2412 participants, 292 of which experienced one coronary event during a mean of 4.8 y follow-up, saturated fat was not associated with risk of coronary events (HR: 0.83; 95% CI: 0.59 to 1.16). *A sensitivity analysis including these alongside prospective studies in generally healthy individuals did not alter the main findings of the study. This sensitivity analysis appears in* **Figures D1** *(all-cause mortality);* **D2** *(coronary mortality; and* **D3** *(total CHD).*

Results from Retrospective Case-Control Studies

Case-Control Studies

Case-control studies were not included in our GRADE assessment of the evidence because higher-quality levels of observational evidence, specifically prospective cohort and prospective nested case-control or case-cohort designs are available.

Saturated Fat and All-Cause Mortality

We did not identify any case-control studies that examined the association between saturated fat intake and all-cause mortality.

Saturated Fat and CHD Mortality

In a nested case-control study conducted within the Whitehall study[12] (NOS quality=6), Clarke et al. compared phospholipid fatty acid concentrations, measured by gas chromatography, between 116 cases of CHD death (aged≈80) and 239 controls frequency matched on age and employment grade. In analyses adjusted only for the matching factors, being in the top quartile of phospholipid saturated fatty acids was associated with twofold higher risk of CHD death (OR: 2.12; 95% CI: 1.13, 3.99; P_{trend}=0.02); further adjustment for biomarkers of CVD risk, however, attenuated this association (OR: 1.77; 95% CI: 0.89 to 3.51; P=0.10), suggesting that the adverse effects of SFA on risk are, at least in part, mediated by the traditional CHD risk factors. *This study was included in a sensitivity analysis including nested-case control with prospective cohort studies of saturated fat and CHD death (eFigure 51).*

Polyunsaturated:Saturated Fat and all-cause and CVD mortality

A case-control study conducted within the Eastern Finland Heart Survey (92 cases and 92 controls; NOS quality=6) reported the association between the serum P:S ratio (fatty acids measured by gas chromotography) and coronary artery disease mortality[13]. In a multivariable model adjusting for matching factors (smoking, sex, age, serum cholesterol, MAP, and history of CVD), alcohol, work absenteeism, diabetes, family history of MI, medication use, a serum P:S ≤0.28 was associated with a 5.7 (95% CI: 2.0 to 16.4) increased odds of coronary death, independent of serum lipid levels, which may relate to an effect of the balance of fatty acids on prostacyclin/thromboxane A2 synthesis.

Saturated Fat and Total CHD

Total Saturated Fat

We identified 4 retrospective case-control studies or nested-case control studies (n=2,700 cases and 5,306 controls; NOS≥6) reporting the association between total saturated fat, as measured by food frequency questionnaires[14 15] or biomarkers[16 17], and total CHD. The summary OR of high versus low saturated fat is 1.36 (95% CI: 0.96, 1.92; P=0.35; P=0.09; P_{het}=0.35; I^2=9%) (**Figure D4**). The majority of the weight (69%) was carried by one study; 2 studies reported borderline positive associations (prospective, nested case-control biomarker studies); and 2 reported no association with wide confidence limits (retrospective case-control FFQ studies). In a fixed-effect model (**Figure D5**), the summary OR of high versus low saturated fat is 1.36 (95% CI: 1.02 to 1.82; P=0.04; P_{het}=0.35; (I^2=9%).

Odd Chained Saturated Fatty Acids (Dairy Saturated Fatty Acids)

Three nested case-control[17-19] and two retrospective case-control[20 21] studies including 4,650 cases and 9,861 controls and each with a NOS≥6 measured the association between dairy saturated fatty acids (15:0 and 17:0), measured in adipose tissue or plasma phospholipid, and risk of CHD. The individual study multivariable-adjusted odds ratios for 15:0, pentadecanoic acid, ranged from 0.41 to 2.36, and the summary OR for 15:0 was 0.94 (95% CI: 0.65 to 1.37; P=0.76; P_{het}=0.003; I^2=75%) (**Figures D6** and **D7 f**or fixed-effect). The individual study multivariable-adjusted odds ratios for 17:0, heptadecanoic acid, ranged from 0.91 to 1.25, and the summary OR for 17:0 fatty acid isomers of dairy fat was 0.97 (95% CI: 0.85, 1.11; P=0.69; P_{het}=0.33; I^2=12%) (**Figures D8** and **D9** for fixed-effect). *These nested case-control studies were not included in the main quantitative synthesis because they did not address total saturated fatty acids.*

Even chained Saturated Fatty Acids

Three case-control[20 22 23] and four nested case-control studies[17-19 24] including 3,276 cases and 8,500 controls, and exposures measured in serum, plasma, and adipose tissue sites reported the association between 14:0 (myristic acid) and CHD. The individual OR ranged from 0.55 (95% CI: 0.17 to 1.78) to 2.36 (95% CI: 1.16 to 4.79), with a summary OR of 1.11 (95% CI: 0.89 to 1.39; P_{het}=0.07; I^2=48%) (**Figures D10 and D11** (fixed effect)). *The nested case-control studies were not included in the main quantitative synthesis because they did not address total saturated fatty acids.*

One case-control study in Portugal[22] and one nested case-control study in the MRFIT cohort[24], including 391 cases and 404 controls, measured the association between 16:0 (stearic acid) and CHD, with FA levels measured in adipose and serum tissues. Simon et al. found that high concentrations of stearic acid in serum was associated with 35% increased risk of CHD (OR: 1.35; 95% CI: 1.06, 1.72), while Lopes found a protective effect (OR: 0.58; 95% CI: 0.33 to 1.01) (**Figure D12**). In these same studies, the summary OR estimate of the association between 18:0 (stearic acid) and CHD risk was 1.13 (95% CI: 0.90 to 1.42; P=0.41; P_{het}=0.41; I^2=0%) (**Figure D13**). *The nested case-control studies were not included in the main quantitative synthesis because they did not address total saturated fatty acids.*

Saturated Fatty Acid Isomers and CHD Mortality

Case Control Studies

Sun et al.[25] measured serum free palmitic and oleic acids using gas chromatography, and calculated the palmitic:oleic ratio in the serum of 108 cases of MI in Nanjing, China from 2006 to 2008, and 108 age-matched controls attending local hospitals for a routine check-up. They found an OR of fatal myocardial infarct of 50.6 (95% CI: 8.3 to 310.4) comparing those in the 4th quartile of the ratio to those in the first.

Saturated Fat Isomers and Ischemic Stroke

Case Control Studies

Even Chained Saturated Fatty Acids

A case-control study in Portgual (297 cases, 968 controls; NOS quality score=8)[26] found that higher intakes of lauric acid (12:0; OR: 0.34 in men and 0.09 in women), myristic (14:0; OR: 0.41 in men and 0.08 in women), palmitic (16:0; OR: 0.40 in men and 0.07 in women), and stearic (18:0; OR 0.47 in men and 0.05 in women) acids, as assessed by a validated semi-quantitative food-frequency questionnaire were each associated with reduced risk of ischemic stroke.

Saturated Fatty Acid Isomers and type 2 Diabetes

Nested Case-Control and Prospective Cohort Studies

Odd chained Saturated Fatty Acids

Nested case-control studies within the Melbourne Collaborative Studies[27] (346 cases and 3388 controls; NOS=8) and EPIC-Potsdam[28] (673 cases and 26,875 controls; NOS=8), and the EPIC-InterAct (12,403 cases and 16,154 from the subcohort; NOS=8)[29] study, measured the association between odd-chained saturated fatty acids and development of type 2 diabetes. In Hodge et al., intake of 15:0 (pentadecyclic) acid measured by food-frequency questionnaire was not associated with development of type 2 diabetes (OR: 0.82, 95% CI: 0.54, 1.24 for high vs. low intake), but when measured in erythrocyte phospholipid, they were protective (OR: 0.40; 95% CI: 0.26, 0.62 for high vs. low concentration). The EPIC-Potsdam group found no association between erythrocyte phospholipid 15:0

(OR: 0.79; 95% CI: 0.54, 1.16) or 17:0 (OR: 0.74; 95% CI: 0.52, 1.06) and odds of type 2 diabetes. EPIC Interact found that plasma phospholipid 15:0 (HR: 0.79; 95% CI: 0.74 to 0.85) and 17:0 (HR: 0.67 to 0.73) were both protective against T2DM. In the prospective Insulin Resistance Atherosclerosis Study (IRAS), which enrolled 659 participants (age ≈55), 103 cases of incident T2DM were observed during 5 y of followup; in this study, high, compared with low (tertile 3 vs. tertile 1) serum 15:0 was inversely related with T2DM (OR: 0.53; 95% CI: 0.29 to 0.90). In EPIC InterAct, a pooled analysis of serum 15:0 and 17:0 found a protective association of these odd-chained FA with type 2 diabetes (HR: 0.71; 95% CI: 0.67 to 0.75). (**Figures D14** and **D15** present quantitative synthesis of these results).

Even chained saturated fatty acids

Kroger et al. conducted a nested-case cohort study within the European Prospective Investigation in to Cancer and Nutrition (EPIC-Potsdam), including 673 cases and 26,875 controls (NOS quality=8)[28]. Erythrocyte membrane fatty acids were measured by gas chromotography, and cases of diabetes were confirmed by a physician. During a mean follow-up of 7 years, erythrocyte phospholipid 14:0 concentration was not associated with risk of type 2 diabetes (OR: 0.78; 95% CI: 0.55, 1.10).

Ma et al. reported the association of circulating 14:0, 16:0, and 18:0 saturated fatty acids in plasma phospholipid with incident diabetes in 3004 people free of diabetes enrolled in the Cardiovascular Health Study[30] in whom 297 incident diabetes cases were observed over a median of 9y of follow-up (NOS=9). In this study, 16:0 (HR: 1.89; 95% CI: 1.27 to 2.83) and 18:0 (HR: 1.62; 95% CI: 1.09 to 2.41); but not 14:0 (HR: 0.98; 95% CI: 0.65 to 1.47) were associated with incident type 2 diabetes.

Mahendran et al. reported associations of 16:0 and 18:0 erythrocyte membrane fatty acids followed 1346 Finnish men, aged 45-73, and observed 30 incident cases over 5 y. of follow-up in the METSIM study[31] (NOS=7). In this study, neither 16:0 (OR: 0.74; 95% CI: 0.48 to 1.12) nor 18:0 (OR: 1.25; 95% CI: 0.63 to 2.50) were associated with development of incident T2DM over 5 y of follow-up.

Nested case-control studies within the Melbourne Collaborative Studies[27] (346 cases and 3388 controls; NOS=8) and EPIC-Potsdam[28] (673 caes and 26,875 controls; NOS=8) measured the association between 18:0 (stearic acid) and development of type 2 diabetes. In Hodge et al., intake of stearic acid measured by food-frequency questionnaire was not associated with development of type 2 diabetes (RR: 1.23, 95% CI: 0.81, 1.86 for high vs. low intake), but when measured in erythrocyte phospholipid, a positive association was found (RR: 2.25; 95% CI: 1.39 to 3.63 for high vs. low concentration). The EPIC-Potsdam group found no association between erythrocyte phospholipid 18:0 (RR: 1.38; 95% CI: 0.96 to 1.99) and odds of type 2 diabetes.

In the EPIC-InterAct (12,403 cases and 16,154 from the subcohort; NOS=8)[29] study, positive associations with type 2 diabetes were reported for serum 14:0 myristic (HR: 1.17; 95% CI: 1.11 to 1.22), 16:0 palmitic (HR: 1.19; 95% CI: 1.10 to 1.28); and 18:0 stearic acids (HR: 1.12; 95% CI: 1.08 to 1.16). The pooled estimate for all 3 even-chained FA was 1.39 (95% CI: 1.27 to 1.52).

Pooled estimates of these associations appear in **Figures D16** to **D18**.

Dose-Respose association of Saturated Fat With Health Outcomes and Substitution effects

Dose-response effects are nuanced when discussing energy-yielding macronutrients, such as saturated fat, as the aim of this type of analysis is to assess the effects of increasing the nutrient, without increasing total food energy. Controlling for total energy in multivariable regression models typically accomplishes this; however, this raises the simple question of which nutrient is exchanged for saturated fat. The most common approach is to construct models such that the dose coefficient is interpretable as a 1% increase in energy from the nutrient of interest, and an equal 1% reduction in energy from another nutrient, usually carbohydrate; but other models may be interpreted differently, as described below.

Prospective Cohort Studies

The continuous association of saturated fat with risk of CHD events and death was directly measured in 4 publications, none of which found significant dose-response effects of increasing saturated fat intake (as a percent of energy) at the expense of total carbohydrate (**Tables D6** and **D7**).

Overall, the strongest epidemiological evidence to-date for the effects of isocaloric substitution of saturated fat for other macronutrients comes from the pooled analysis of 11 prospective cohort studies from the United States of America and Europe in the *Pooling Project of Cohort Studies on Diet and Coronary Disease*, including 344,696 people followed for 4-10 y, with 5,429 coronary events and 2,155 coronary deaths[32]. This project allowed the investigators to study the associations between major types of dietary fat and CHD risk in different populations, with differing diets and a broad range of nutrient intakes. Study-specific natural logarithms of the individual study HRs were weighted by the inverse of their variances, and a pooled estimate of the HRs was obtained with a random-effects model. The possibility of effect modification by age at study entry (<60 vs. ≥60 y.o.) and sex were also examined, but no evidence was found. **Tables D8** and **D9** list the HR for coronary events and deaths for the replacement of 5% of energy from total saturated fat with 5% of another macronutrient.

Saturated Fat for Carbohydrate on CVD: The Role of Carbohydrate Quality

Hu et al.[33] first examined the impact of substitution of saturated fat for carbohydrate on CHD risk, in a 14y. prospective cohort study of 80,082 women aged 34-59 years old at entry, with no previous CHD, diabetes, or cancer. Dietary information was collected using a validated food-frequency-questionnaire, and updated in 1986 and 1990. Using updated dietary information, the multivariable adjusted RR for the substitution of 5% of energy from saturated fat with carbohydrate was 0.85 (95% CI: 0.71, 1.03; P=0.10), which was similar to the association observed when only baseline dietary data were used (RR: 0.88; 95% CI: 0.75, 1.03; P=0.12).

While the effect of replacing saturated fat with polyunsaturated fat was found to be the most protective in both the pooling project, replacement of saturated fat with carbohydrate was less consistently associated with benefit, suggesting that there may be important differences in the effects of carbohydrate not captured by the concept of total carbohydrate. The dietary glycemic index, a tool to classify carbohydrate-containing foods on their ability to raise post-prandial glycemia was developed in 1981 by Jenkins et al. is one method of determining the “quality” of a carbohydrate. In a prospective

cohort design, Jakobsen et al. followed 57,063 men and women for a median of 12 y. to determine the risk of first myocardial infarction associated with replacement of saturated fat with either low, medium, or high-glycemic index carbohydrates[34]. Diet was assessed using a validated 192-item semiquantitative food frequency questionnaire at baseline. In this study, no benefit was seen of replacing saturated fat with total carbohydrate (RR: 1.04; 95% CI: 0.92, 1.17). However, important differences were seen when the substitutions were stratified by glycemic index of carbohydrate (**Table D10**), indicating that replacement of saturated fat with high-glycemic carbohydrates was associated with icnreased risk of CVD, but replacement with low-glycemic index carboyhdrate was associated with reduced risk.

Saturated Fat for Carbohydrate or Protein on ischemic stroke

In the Framingham Heart Study, a prospective cohort of 832 middle-aged U.S. men, followed for 20 years, Gillman et al.[35] estimated the effect of increasing saturated fat, at the expense of energy from other energy-contributing nutrients, on stroke risk. Diet was measured once at baseline using a 24-hour recall. In this study, a 5% increase in energy from saturated fat (substitution for protein and carbohydrate) was associated with a 42% reduced risk of stroke (RR per 5% increase: 0.58; 95% CI: 0.39, 0.82). Two later studies, however, failed to support this finding. In the Women's Health Initiative Observational Study, Yaemsiri et al. [36]report on 87,025 generally healthy post-menopausal women followed for 7 years. Diet was measured using a validated semi-quantitative FFQ at baseline and 3 years later. The authors noted a non-significant increase in risk of total ischemic stroke with increasing saturated fat intake (substituted for energy-contributing nutrients; RR per 10 g/d: 1.04; 95% CI: 0.96, 1.13). Similarly, He et al.[37] followed-up 43,732 male health professionals for 14 years, measuring diet using a validated FFQ at baseline and 4 and 8 years later. These authors reported no dose-response for a 10% increase in energy from saturated fat (substituted for other energy-containing nutrients) on total ischemic stroke using baseline diet (RR: 1.24; 95% CI: 0.85 to 1.82), updated diet (RR: 1.01; 95% CI: 0.68 to 1.52), or cumulative average diet (RR: 1.10; 95% CI: 0.72 to 1.68).

Saturated Fat for Carbohydrate on type 2 diabetes

Schulze[38] prospectively examined the impact of substitution of saturated fat for carbohydrate in 9,702 men and 15,365 women aged 35-65 and free of diabetes as baseline. Diet was measured with a validated food-frequency questionnaire. Over 10 years of follow-up, 844 incident cases of type 2 diabetes were observed (491 in men and 353 in women). The estimated multivariate RR of type 2 diabetes associated with replacement of 5% of saturated fat with carbohydrate was 0.99 (95% CI: 0.72 to 1.35) for men; 1.12 (95% CI: 0.78 to 1.61) for women; and 1.07 (95% CI: 0.85 to 1.35) for both sexes combined.

Appended Figures (D)

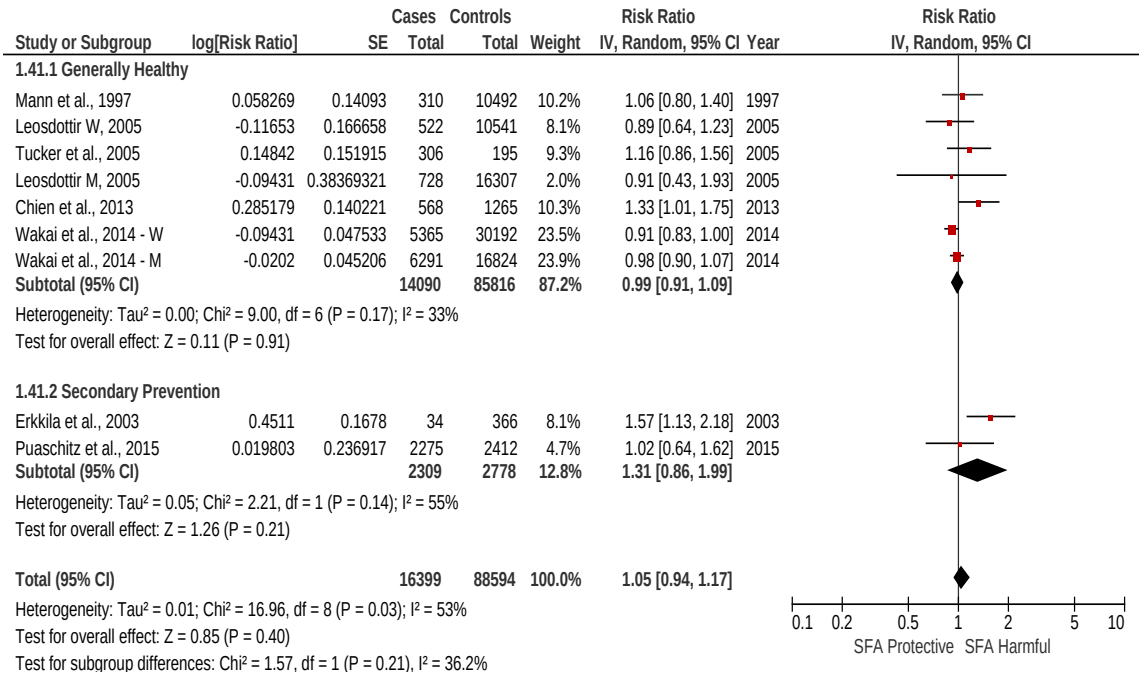

Figure D1. Pooled most-adjusted (random effects) risk ratios of total saturated fatty acids and all-cause mortality in primary prevention (n=7 comparisons) and secondary prevention (n=2 comparisons).

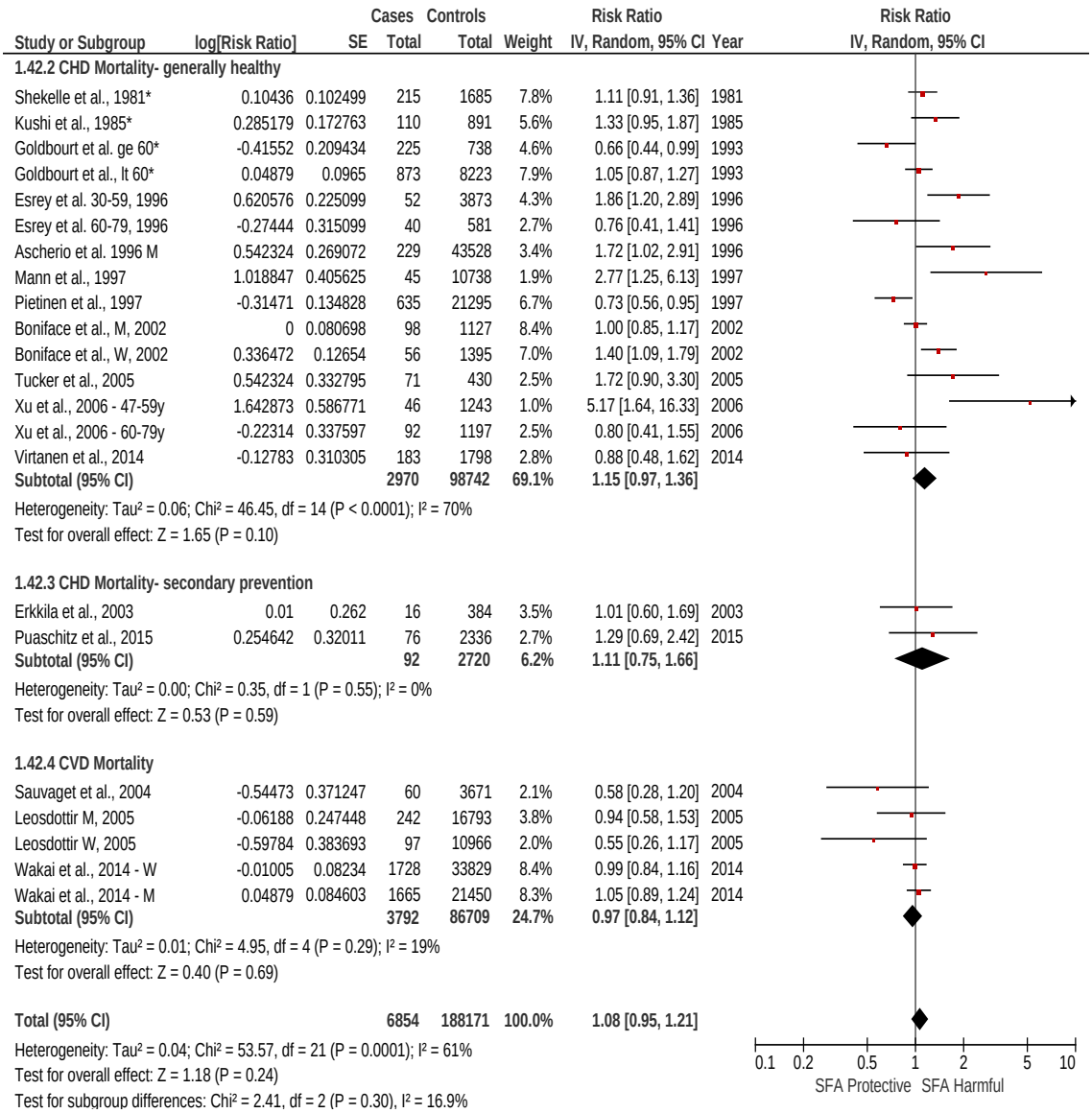

Figure D2. Pooled most-adjusted (random effects) risk ratios of total saturated fatty acids and CHD and CVD mortality in primary prevention and secondary prevention.

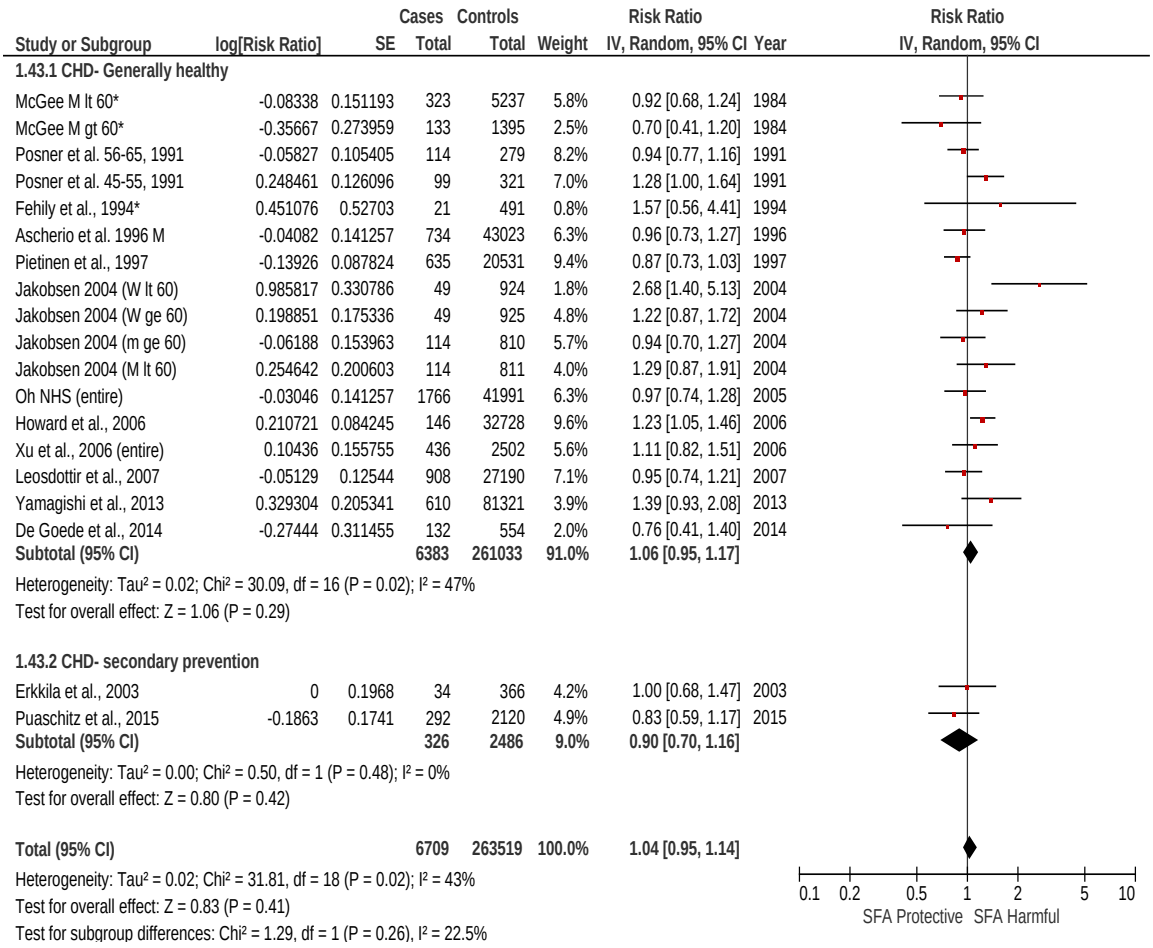

Figure D3. Pooled most-adjusted (random effects) risk ratios of total saturated fatty acids and total CHD in primary prevention and secondary prevention.

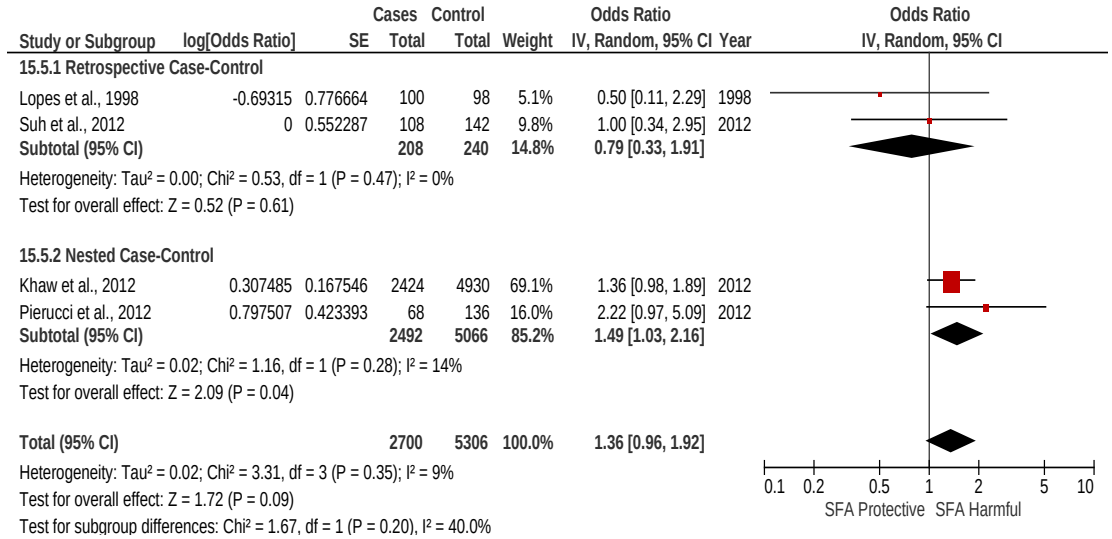

Figure D4. Pooled most-adjusted (random effects) odds ratios of total saturated fatty acids and CHD in 2 retrospective case-control studies and 2 prospective nested case-control studies.

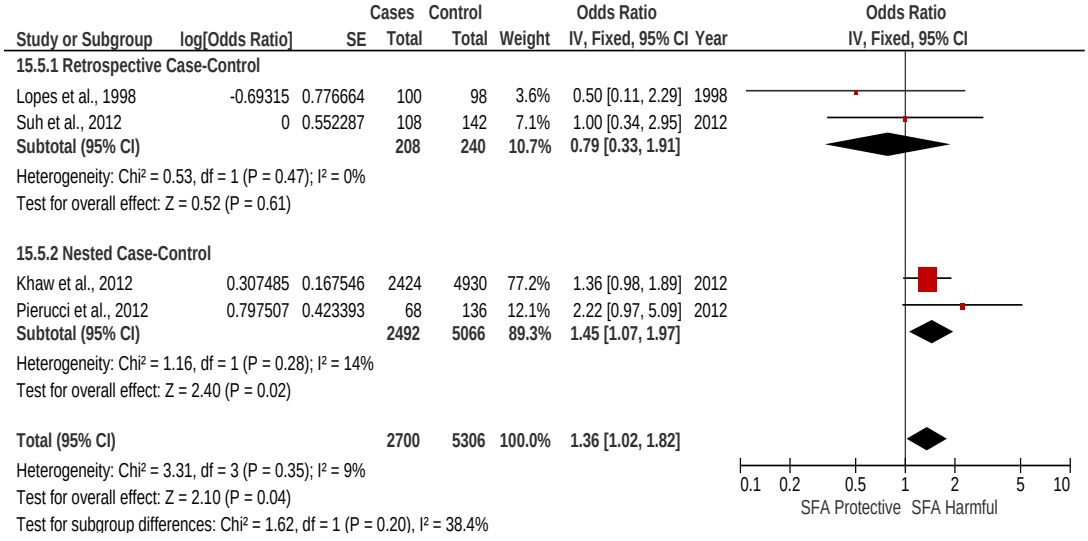

Figure D5. Pooled most-adjusted (fixed effect) odds ratios of total saturated fatty acids and CHD in 2 retrospective case-control studies and 2 prospective nested case-control studies.

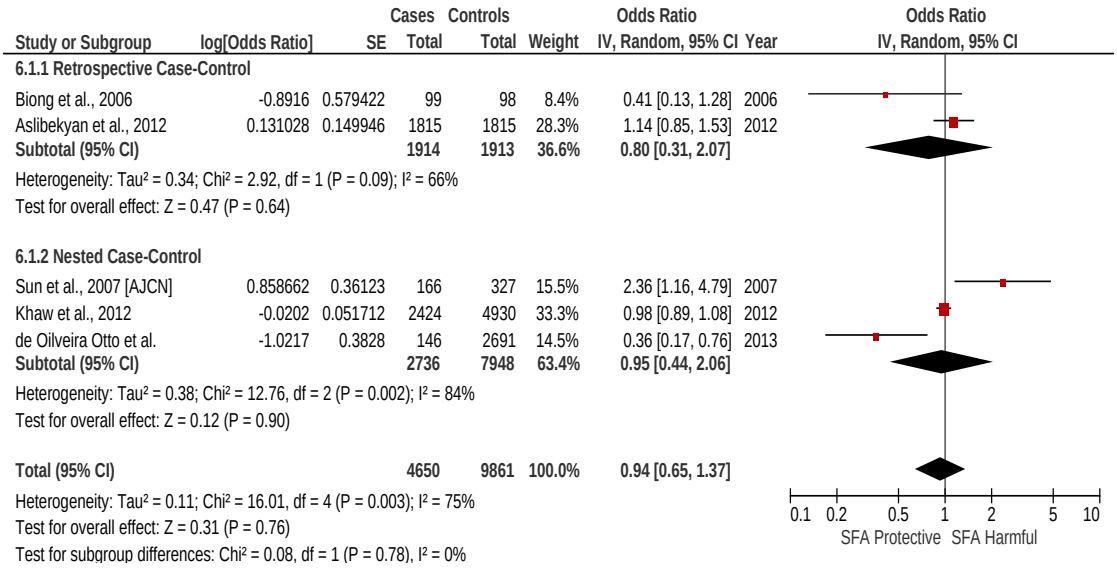

Figure D6. Pooled most-adjusted (random effects) odds ratios of pentadecanoic acid (15:0) and CHD in 2 retrospective case-control studies and 3 prospective nested case-control studies.

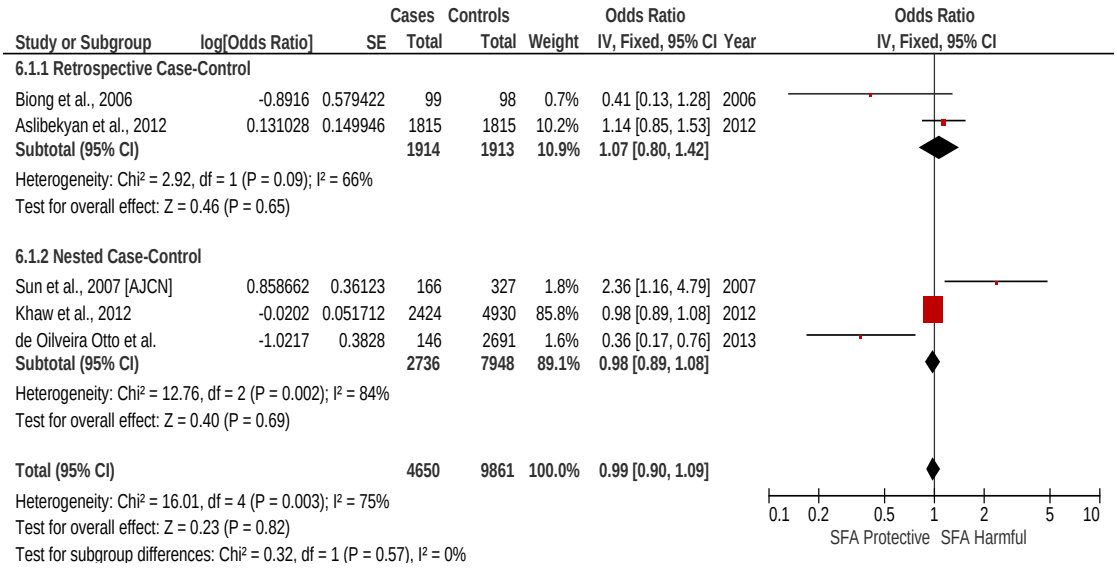

Figure D7. Pooled most-adjusted (fixed effect) odds ratios of pentadecanoic acid (15:0) and CHD in 2 retrospective case-control studies and 3 prospective nested case-control studies.

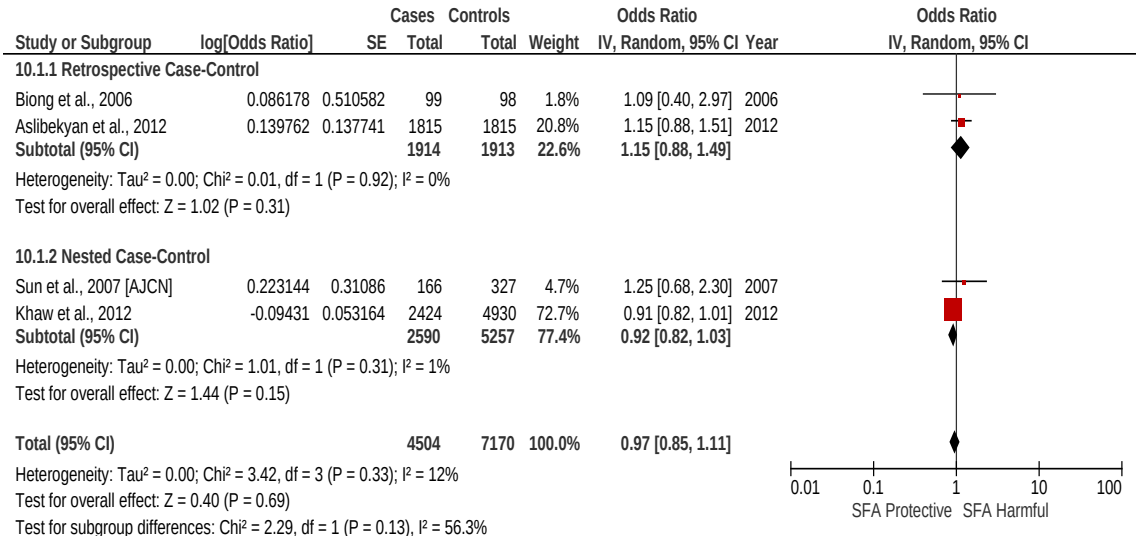

Figure D8. Pooled most-adjusted (random effects) odds ratios of heptadecanoic acid (17:0) and CHD in 2 retrospective case-control studies and 2 prospective nested case-control studies.

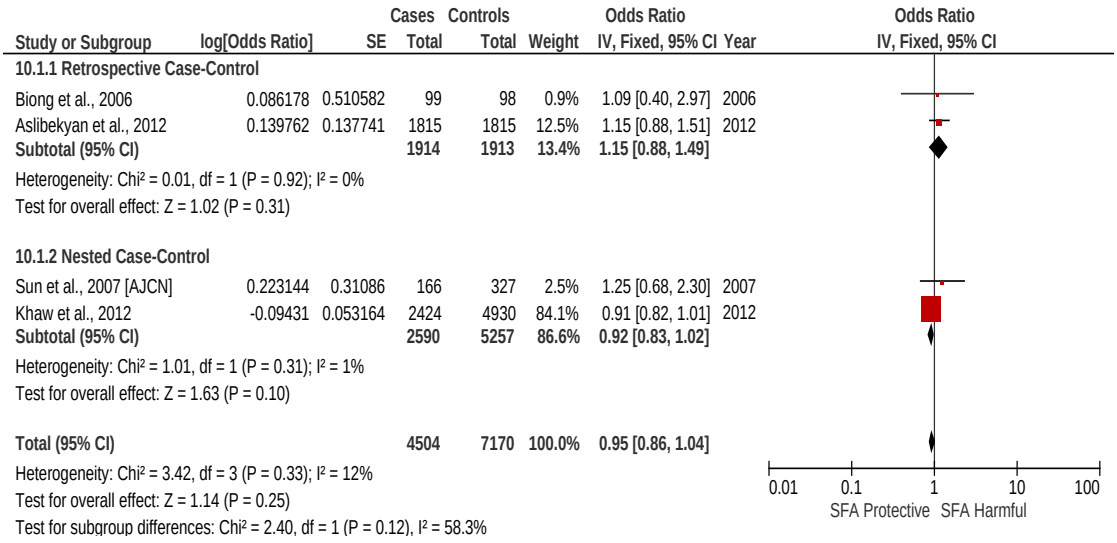

Figure D9. Pooled most-adjusted (fixed effect) odds ratios of heptadecanoic acid (17:0) and CHD in 4 retrospective case-control studies.

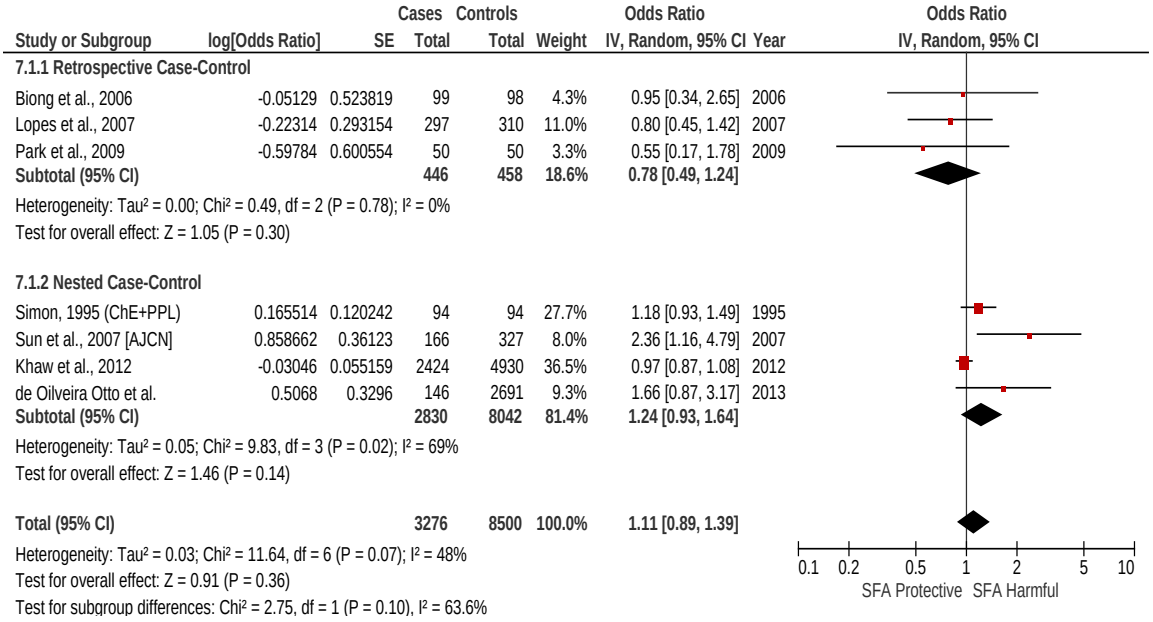

Figure D10. Pooled most-adjusted (random effects) odds ratios of myristic acid (14:0) and CHD in 3 retrospective case-control studies and 4 prospective nested case-control or case-cohort studies.

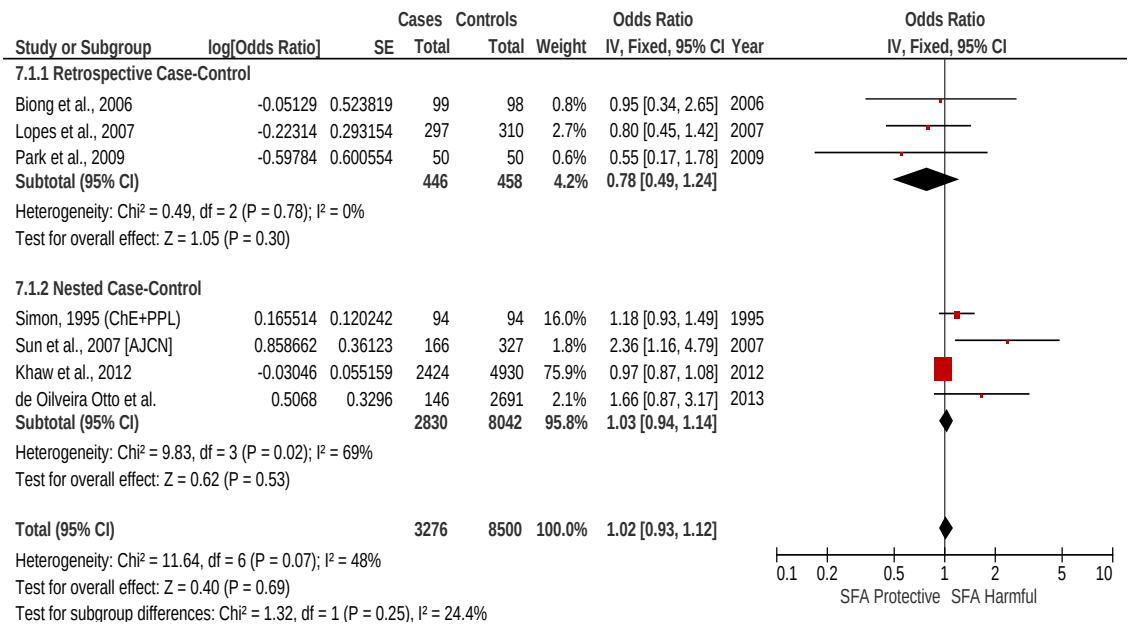

Figure D11. Pooled most-adjusted (fixed effect) odds ratio of myristic acid (14:0) and CHD in 3 retrospective case-control studies and 4 prospective nested case-control or case-cohort studies.

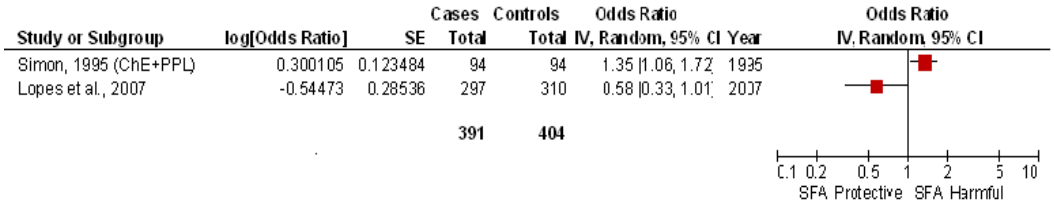

Figure D12. multivariable odds ratio of 16:0 (stearic acid) and CHD

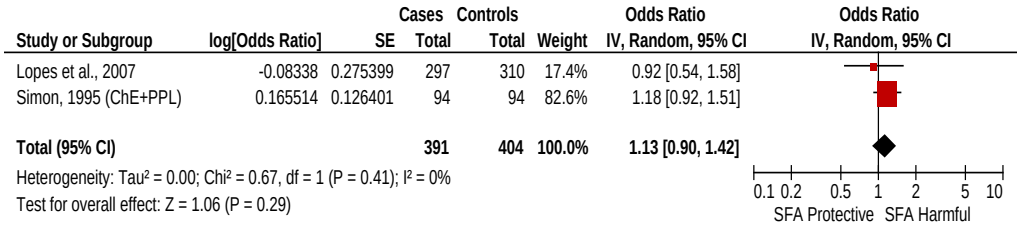

Figure D13. Summary multivariable OR of 18:0 (stearic acid) and CHD

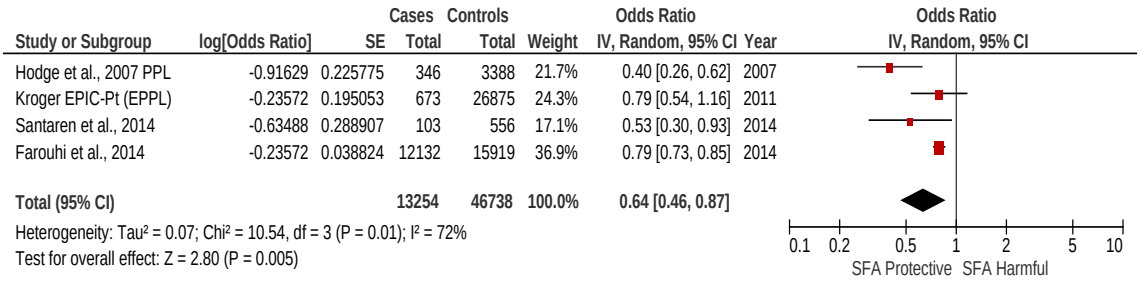

Figure D14. Pooled association between 15:0 (pentadecanoic acid) and type 2 diabetes in 4 nested case-control or case-cohort studies.

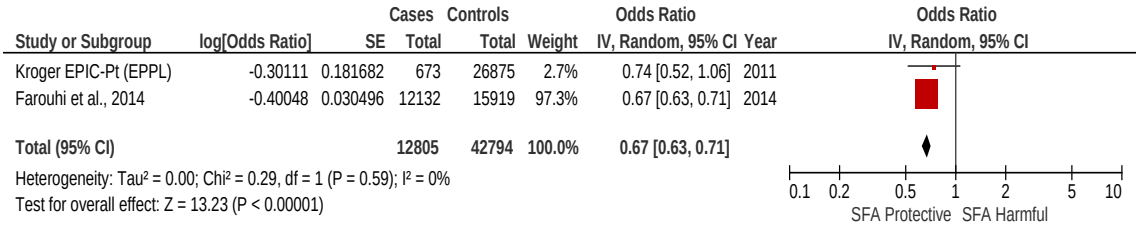

Figure D15. Pooled association between 17:0 (heptadecanoic acid) and type 2 diabetes in 2 case-cohort studies.

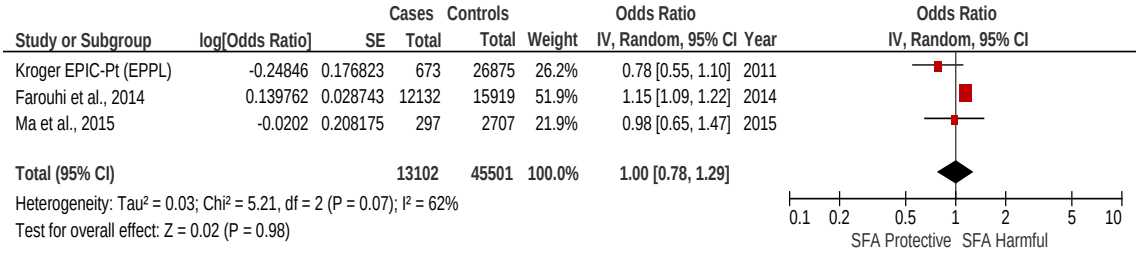

Figure D16. Pooled association between 14:0 (myristic acid) and type 2 diabetes in 2 case-cohort studies.

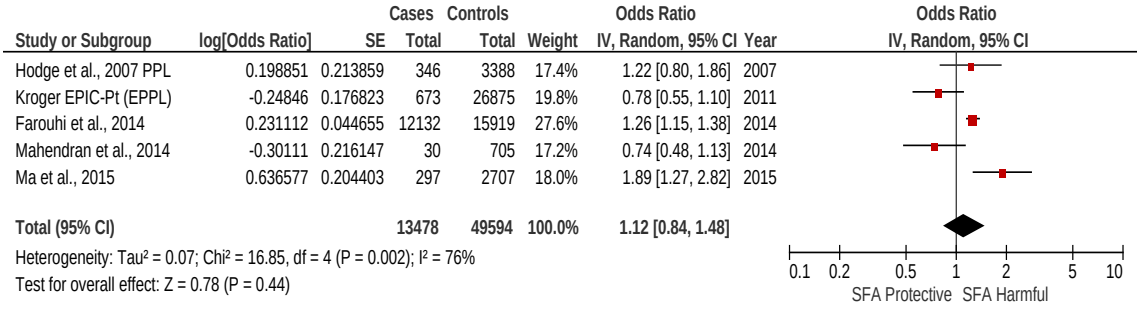

Figure D17. Pooled association between 16:0 (palmitic acid) and type 2 diabetes in 5 prospective nested case-control or case-cohort studies.

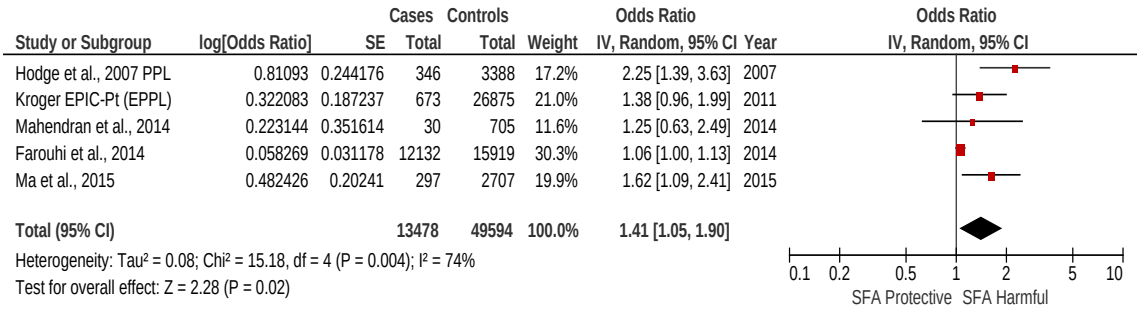

Figure D18. Pooled association between 18:0 (stearic acid) and type 2 diabetes in 5 prospective nested case-control or case-cohort studies.

Appended Tables (D)

Table D1. Associations between individual saturated fatty acid isomers and CHD risk *(adapted)*[2]

Fatty acid group	Intake Range (%E)	MVRR (Q5 v Q1)[a]	P for trend
4:0 to 10:0	<1.05% to >1.77%	1.00 (0.82, 1.21)	0.60
12:0 + 14:0	<1.13% to >1.87%	1.05 (0.83, 1.32)	0.46
16:0	<6.46% to 9.33 to 20.45%	1.03 (0.71, 1.50)	0.45
18:0	<2.93% to >4.42%	1.16 (0.81, 1.66)	0.30
Sum of 12:0 to 18:0	<10.6% to >15.5%	1.04 (0.72, 1.48)	0.47

[a]Adjusted for age, time period, BMI, cigarette smoking, menopausal status, parental history of MI before age 60, vitamin E supplement use, alcohol consumption, history of hypertension, aspirin use, vigorous exercise, intakes of monounsaturated fat, polyunsaturated fat, trans fat, protein, dietary cholesterol, dietary fibre, and total energy

Table D2. Multivariate relative risks of CHD associated with substitution of different isomers of saturated fatty acids with carbohydrate, monounsaturated fat, or polyunsaturated fat *(adapted)*[2]

Substitution of 1% carbohydrate with	RR [a]	P	Substitution of 5% 12:0 to 18:0 SFA with = energy from	RR
4:0 to 10:0 SFA	0.97 (0.90, 1.05)	0.45	Carbohydrate	0.78 (0.60 to 1.00)
12:0 + 14:0 SFA	1.12 (0.97, 1.31)	0.13	Monounsaturated	0.58 (0.35 to 0.94)
16:0 SFA	1.07 (0.98, 1.17)	0.16	Polyunsaturated	0.50 (0.36 to 0.70)
18:0 SFA	1.19 (1.02, 1.37)	0.02		
12:0 to 18:0 SFA	1.29 (1.00, 1.66)	0.05		

[a]Adjusted for age, time period, BMI, cigarette smoking, menopausal status, parental history of MI before age 60, vitamin E supplement use, alcohol consumption, history of hypertension, aspirin use, vigorous exercise, intakes of monounsaturated fat, polyunsaturated fat, trans fat, protein, dietary cholesterol, dietary fibre, and total energy

Table D3. Associations between cardiovascular disease and saturated fat from various food sources *(adapted)*[6]

Source	Per 5 g/d[a]	P	Per 5% energy/d[a]	P
Dairy	0.79 (0.68, 0.92)	<0.01	0.62 (0.47, 0.82)	<0.01
Meat	1.26 (1.02, 1.54)	0.03	1.48 (0.98, 2.23)	0.06
Butter	0.87 (0.66, 1.15)	0.33	0.83 (0.50, 1.37)	0.47
Plant sources	1.00 (0.50, 2.01)	0.99	0.62 (0.18, 2.11)	0.44
Mixed sources	1.01 (0.77, 1.32)	0.96	0.83 (0.51, 1.36)	0.51

[a] adjusted for age, sex, race-ethnicity, study center, energy intake, education, alcohol, physical activity, BMI, cigarette smoking, dietary supplement use, use of cholesterol-lowering medication, fruits+vegetables intake, energy-adjusted fiber, vitamin E, trans fat, PUFA

Table D4. Effect on CVD risk of exchanging one food source of saturated fat for another *(adapted)*[6 7]

Replace saturated fat from	***With saturated fat from***	***MVHR (95% CI)***
MESA[a]		
Meat	Dairy	0.75 (0.63, 0.91)
Meat	Butter	0.81 (0.64, 1.03)
Meat	Plant	0.63 (0.38, 1.03)
Dairy	Butter	1.08 (0.88, 1.33)
Dairy	Plant	0.83 (0.52, 1.33)
Butter	Plant	0.77 (0.48, 1.25)
Zutphen Elderly Study[b]		
Dairy	Plant or butter	0.98 (0.79, 1.20)
Meat	Plant or butter	1.12 (0.75, 1.66)
Dairy	Meat	1.15 (0.80, 1.66)

[a] adjusted for age, sex, race-ethnicity, study center, energy intake, education, alcohol, physical activity, BMI, cigarette smoking, dietary supplement use, use of cholesterol-lowering medication, fruits+vegetables intake, energy-adjusted fiber, vitamin E, trans fat, PUFA

[b] adjusted for age, lifestyle (smoking, BMI, physical activity, socioeconomic status, alcohol), diet (total energy, carbohydrates, protein, monounsaturated fat, trans fatty acids, and fibre)

Table D5. Effect of replacing saturated fat with other macronutrients on CVD in type 1 diabetes (*adapted*)[10]

Replace 5% E SFA with…	***HR (95% CI) of CVD***
MUFA	0.98 (0.89, 1.07)
PUFA	0.99 (0.89, 1.09)
Carbohydrate	0.96 (0.89, 1.05)

Table D6. Direct dose-response associations between total saturated fat and CHD/CVD risk in 3 prospective cohort studies

Study	***Unit increase***	***MVRR (95% CI)***
Health Professionals' Follow up study (men)[39]	5% energy	0.86 (0.66, 1.12)
MONICA I and II (women)[40]	5% energy	1.36 (0.98, 1.89)
MONICA I and II (men)[40]	5% energy	1.03 (0.78, 1.36)
Nurses' Health Study (women)[41]	5% energy	1.01 (0.81, 1.26)

Table D7. Direct dose-response associations between total saturated fat and fatal CHD/CVD risk in 3 prospective cohort studies

Study	***Unit increase***	***MVRR (95% CI)***
Strong Heart Study (age 47-59)[42]	5% energy	1.45 (0.84, 2.50)

Strong Heart Study (age 60-79)[42]	5% energy	0.82 (0.62, 1.08)
Health Professionals Follow-Up Study[39]	5% energy	1.34 (0.86, 2.09)

Table D8. Effect of replacement of saturated fat with other macronutrients on risk of coronary events (n=5,249) *(adapted)*[32]

Substitution of 5%E SFA with	***MV HR***	***Heterogeneity***
MUFA	1.19 (1.00, 1.42)	0.32
PUFA	0.87 (0.77, 0.97)	0.70
Carbohydrate	1.07 (1.01, 1.14)	0.51

Table D9. Effect of replacement of saturated fat with other macronutrients on risk of coronary death (n=2,155)(*adapted*)[32]

Substitution of 5%E SFA with	***MV HR***	***Heterogeneity***
MUFA	1.01 (0.73, 1.41)	0.18
PUFA	0.74 (0.61, 0.89)	0.40
Carbohydrate	0.96 (0.82, 1.13)	0.05

Table D10. Effects of replacement of saturated fat with carbohydrates of low, medium, and high-glycemic index (*adapted*)[34]

Substitute 5%E from saturated fat with	***MV HR***	***Median dietary GI***
Low-GI Carbohydrate	0.88 (0.72, 1.07)	82
Medium-GI Carbohydrate	0.98 (0.80, 1.21)	88
High-GI Carbohydrate	1.33 (1.08, 1.64)	93

Evidence on *trans*–unsaturated fatty acids that was reviewed, but that did not directly inform the GRADE evidence summary

Results from Prospective Cohort Studies

We included all prospective cohort studies of total TFA and the health outcomes which met our inclusion criteria. Additional data from some of these prospective cohorts was used to characterize dose-response relationships, effects of specific TFA isomers, and substitution effects.

Prospective Cohort Studies of specific trans-unsaturated fatty acid classes and health outcomes

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention study (n=21,930 men aged 50-69; Finland) assessed the association between elaidic acid (18:1n9*t*), the major 18:1 *trans*-unsaturated fatty acid component of hydrogenated vegetable oils, and fatal CHD events over 6 years of follow-up in middle-aged men[43]. In this prospective study with 635 deaths, those with the highest intake of eliadic acid (Q5; 4.3 g/d) were at 37% increased risk of coronary death (RR: 1.37; 95% CI: 1.07, 1.75; P-trend = 0.002) compared with those with the lowest (Q1; 1.3 g/d). *This study was not included in our quantitative synthesis because it was the lone prospective cohort study to assess individual isomers of TFA with CHD death.*

The Zutphen Elderly Study

The Zutphen Elderly Study (n=667 men aged 64-84; the Netherlands) examined the association between industrially produced 18:1 *trans*-unsaturated fatty acids and CHD events[44]. In this prospective study with 98 incident cases in 667 men followed for 10 y., a 0.5% increase in energy from industrially-manufactured 18:1 trans fatty acids was not associated with increased risk of CHD (RR: 1.05; 95% CI: 0.94, 1.17), however the comparatively small sample size makes it difficult to reliably estimate differences in the effect of specific isomers.

Cardiovascular Health Study

A prospective analysis in the Cardiovascular Health Study (n=2,742 adults aged 74±5 y)[45] assessed the association of plasma phospholipid 18:2, 18:1, and 16:1n9 *trans* fatty acids with all-cause mortality; cardiovascular and non-cardiovascular mortality; and total, fatal, and non-fatal CHD. In this study, 1739 deaths and 639 total CHD events occurred over 31 494 person-years of follow-up. Some major trans isomers found in hydrogenated vegetable oils were associated with all-cause mortality [*trans*/*cis*-18:2 (HR: 1.19; 95% CI: 0.96 to 1.49), and *trans*/*trans* 18:2 (HR: 1.23; 95% CI: 1.04 to 1.44)]; but others were not [*cis*/*trans*-18:2 (HR: 0.92; 95% CI: 0.72 to 1.17) and trans 16:1n9 (HR: 1.05; 95% CI: 0.85 to 1.30)]. *Trans/trans* 18:2 was strongly associated with cardiovascular mortality (HR: 1.40; 95% CI: 1.05 to 1.86), but others were not. *Trans/cis* 18:2 (HR: 1.67; 95% CI: 1.17 to 2.40) and *trans/trans* 18:2 (HR: 1.39; 95% CI: 1.17 to 2.40) were associated with total CHD but not significantly with CHD mortality (HR: 1.38; 95% CI: 0.86 to 2.23 for c/t 18:2 and HR: 1.36; 95% CI: 0.94 to 1.96 for t/t 18:2). The authors posit that heterogeneity of effects across the 18:2 TFA may relate to the manner in which the TFA are incorporated into phospholipids (t/t into the sn-1 position, similar to saturated fatty acids; c/t or t/c into the sn-2 position, similar to linoleic acid). Total trans 18:1, a major contributor to total TFA consumption

from partially-hydrogenated oils were not associated with all-cause mortality, cardiovascular or CHD mortality, or total CHD. The authors posit that this may result from a "healthy survivor" bias in this older cohort; or unmeasured metabolic processes that may result in differential associations across fatty acid subtypes.

The Women's Health Observational Study

The Women's Health Observational Study (87,025 women aged 50-79; U.S.), with 1,049 incident ischemic strokes observed over 7.6y of follow-up, reported positive associations with ischemic stroke of palmitelaidic [16:t1] (P-trend = 0.04), elaidic [18:t1] (P-trend = 0.09), and linolelaidic acids [18:t2] (P-trend = 0.06)[36].

Prospective Cohort Studies of specific trans-unsaturated fatty acid classes and type 2 diabetes

Cardiovascular Health Study

A prospective analysis from the Cardiovascular Health Study (U.S.A.)[46] examined both biomarkers of and self-reported (validated semi-quantitative FFQ) intakes of trans-fatty acids with respect to type 2 diabetes risk over 18 y of follow-up in 2,919 adults aged 74±5 y at baseline. Major phospholipid industrially-derived trans-fatty acids (*trans*-16:1n9, *cis*/*trans*-18:2, *trans*/*cis*-18:2, *trans*/*trans* 18:2) and those shared by both industrial and ruminant sources (*trans* 18:1 and *trans*-16:1n7) were measured. Self-reported FFQ measurements were available for total TFA, 18:1, 18:2, and 16:1 classes. In the most-adjusted models which accounted for traditional risk factors and fatty acids along the de novo lipogenesis pathway, phospholipid t-16:1n9 (HR: 1.59; 95% CI: 1.04 to 2.42) and total t-18:1 (HR: 1.91; 95% CI: 1.20 to 3.03) were associated with incident type 2 diabetes, but none of the other classes were. Trans-palmitoleic acid was nominally inversely associated with type 2 diabetes (HR: 0.73; 95% CI: 0.50 to 1.06). Associations between dietary total TFA (see main paper and **eFigure 23**), 18:1 TFA (HR: 1.32; 95% CI: 0.86 to 1.73), 18:2 TFA (HR: 1.34; 95% CI: 0.94 to 1.90), and 16:1 TFA (HR: 1.16; 95% CI: 0.84 to 1.60) did not reach statistical significance after adjustment for clinical, demographic, and lifestyle risk factors as well as fatty acids on the *de novo* lipogenesis pathway.

Dose-Response and Substitution Models

Dose-response from prospective cohorts

The strongest evidence of a continuous dose-response effect was seen for CHD mortality (31% increase) and total CHD (25% increase) (**eTables 7** and **8**). The U.S. cohort studies were the only studies that provided sufficient continuous data to pool associations for ischemic stroke and type 2 diabetes, and in these studies a 2% increase in TFA was associated with a 41% increase in risk of type 2 diabetes (RR: 1.41; 95% CI: 1.20 to 1.67; **eTable 9**), but not of ischemic stroke (RR: 1.00; 95% CI: 0.76 to 1.30 per 2% energy; **eTable 10**). To enable comparison across studies which did not provide continuous dose-response data, we also pooled most-adjusted relative risks across non-referent quantiles. The extreme quintile comparison showed the strongest association between TFA and CHD mortality, with risks across Q2 through Q4 relatively stable (**Table E1**). For total CHD, all non-referent quantiles of TFA showed an

increased risk, with evidence of a graded increase across quintiles (ranging from 1.11 to 1.21). The pooled across-quantile associations between TFA and ischemic stroke and type 2 diabetes, however, demonstrated no clear dose-response effect (**Tables E4** and **E5**).

Effect of substitution of trans fats for other nutrients Total CHD

Using data from 2 of the largest prospective cohort studies, Mozaffarian and Clarke[47] reported the adjusted RR of CHD for isocaloric replacement of 2% of energy with saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids. They found that replacement of 2% of energy from *trans* fats with saturated fat would reduce CHD risk by 17% (RR: 0.83; 95% CI: 0.75, 0.93), with monounsaturated fat by 21% (RR: 0.79, 95% CI: 0.70-0.88), and polyunsaturated fat by 24% (RR: 0.76; 95% CI: 0.67-0.85). In the present analysis, we found no new evidence that would substantially alter these risks.

Type 2 Diabetes

In the Nurses' Health Study prospective cohort[48], isoenergetic substitution of 2% of energy from trans fats with carbohydrate was associated with a 28% reduction in risk of type 2 diabetes (RR: 0.72; 95% CI: 0.60, 0.87), and replacing 2% of trans with polyunsaturated fat was associated with a 40% lower risk of diabetes (RR: 0.60; 95% CI: 0.48, 0.75). The effect of replacing *trans* fat with monounsaturated fat was not directly assessed, but by subtraction of the published coefficients, we estimated that replacement of 2% energy from trans fats with monounsaturated fat to be associated with a $\approx$27% lower risk. The one study that provided substitution effect estimates for high, medium, and low-glycemic index carbohydrates[49] provided imprecise estimates, likely due to low power for comparisons by glycemic index rating.

Ischemic Stroke

We did not identify any prospective cohort which directly assessed the effect of substitution of trans fat for other macronutrients on risk of ischemic stroke, so we were unable to assess the effect of substitution of trans unsaturated fat with other nutrients on risk.

Results from Retrospective Case-Control Studies

Total *trans*-unsaturated fatty acids and cardiovascular outcomes

In total, twelve retrospective case-control studies provided data on CHD outcomes for quantitative synthesis of *trans* fats and CHD events (**Table 2**). We did not identify any case-control studies specifically examining the association between total *trans* fats and ischemic stroke, all-cause mortality, or CHD mortality. The most-adjusted multivariable models in retrospective case-control studies adjusted for a median of 5 covariates (range: 3 to 12). Least-adjusted models produced weaker and non-significant associations, with slightly increased heterogeneity (eTable 4; **eFigures 45-47**).

Total CHD

The pooled most-adjusted multivariable odds ratio of high versus low total *trans-unsaturated fatty* acid exposure estimated from 11 published reports including 3,945 CHD cases and 3,970 generally age, sex, and neighbourhood-matched controls was 1.51 (95% CI: 1.08 to 2.09; P=.01) with highly significant heterogeneity (I^2=75%; P_{het}<.001) (**eFigure 45**). In a sensitivity analysis, removal of the Colon- Ramos data from 1994-1999, when *trans* fat consumption was high, reduced the pooled OR to 1.39 (95% CI: 1.00 to 1.93; P=.05). When we limited the analysis to 6 high quality studies including 2,876 cases and 2,982 controls, heterogeneity remained high but the effect was weakened, and no longer significant (RR: 1.37; 95% CI: 0.78 to 2.41; P=.28; I^2=78%; P_{het} <.001; **eFigure 46**).

Between-studies heterogeneity was not explained by median age of participants, sex distribution (% men); recruitment year of study (ranged from 1982-2006); study risk of bias (NOS≥7 vs. <7); continent of conduct, method of exposure assessment; exposure level (% total fatty acids or g/d) or adjustment for serum lipids (at least 1 of cholesterol, LDL-C, or triglycerides), total energy, saturated fat, alpha-linoleic acid , alpha-linolenic acid, or polyunsaturated fatty acids.

Retrospective Case-Control Studies of 18:1 *trans*-unsaturated fatty acid isomers and cardiovascular outcomes

Total CHD

The pooled adjusted odds ratio of high versus low total 18:1 trans-fatty acid exposure estimated from 7 published reports (8 comparisons) including 3,919 CHD cases and 3,993 generally age, sex, and neighbourhood-matched controls was 1.19 (95% CI: 0.93, 1.51; P=0.16) with significant heterogeneity across cohorts (I^2=59%; P=0.02) (**eFigure 44**). In one of the included studies, the multi-centre European Community Multicentre Study on Antioxidants, Myocardial Infarction, and Cancer (EURAMIC)[50], conducted in nine European countries between 1991 and 1992, an analysis that excluded the two Spanish centers, which had much lower consumption of 18:1 trans than the other centers was conducted. We also recalculated our effects without these data, and under this condition, the odds ratio is slightly stronger and of borderline significance (1.26; 95% CI: 1.00, 1.58; P=0.05), however heterogeneity still remains high (I^2=57%; P=0.02). Limiting the analysis to the 3 highest-quality studies (4 comparisons, 2353 cases) did not appreciably change the observed association, but did reduce heterogeneity (RR: 1.06; 95% CI: 0.77 to 1.47; P=0.72; P_{het}= 0.25; I^2=28%). No single study met our criteria for an influential outlier. There was no evidence of publication bias on inspection of funnel plot, or detected using Egger's (P=0.642) or Begg's tests (P=0.902). Given the high degree of heterogeneity (59%) and a reasonably large number of included studies (n=8), we proceeded with univariate meta-regression to identify study-level characteristics which might explain heterogeneity. We did not identify any evidence of effect modification by any of our pre-specified potential effect modifiers: baseline year of study, continent of conduct, length of follow-up, median age of participants, proportion of smokers in the sample, amount of SFA in reference category, mean saturated or trans fat intake of the population, sex, α-linoleic acid, total polyunsaturated fat, adjustment for total energy, method and frequency of exposure assessment, risk of bias score, and adjustment for lipids or blood pressure (i.e. causal intermediates).

Retrospective Case-Control Studies of 18:2 *trans*-unsaturated fatty acid isomers and cardiovascular outcomes

Total CHD

The pooled adjusted odds ratio of high versus low total 18:2 *trans*-fatty acid exposure estimated from 6 published reports (7 comparisons) including 3,428 CHD cases and 3,276 generally age, sex, and neighbourhood-matched controls was 1.82 (95% CI: 1.14, 2.90; P=0.01) with significant heterogeneity across cohorts (I^2=77%; P=0.0002) (**eFigure 43**), and some degree of sensitivity to outlier studies. Removal of any one of 4 studies either decreased or increased the effect estimate by >10%. Limiting the analysis to the 4 highest quality studies (5 comparisons) slightly strengthened the association (RR: 2.27; 95% CI: 1.03, 5.02; P = 0.04; P_{het} = 0.0004; I^2=80%). There was no evidence of publication bias on inspection of funnel plot, or detected using Egger's (P=0.086) or Begg's tests (P=0.368). Given the high degree of heterogeneity (77%) and a reasonably large number of included studies (n=7), we used univariate meta-regression to identify study-level characteristics which might explain heterogeneity. We did not identify any evidence of effect modification by any of our pre- specified potential effect modifiers: baseline year of study, continent of conduct, length of follow-up, median age of participants, proportion of smokers in the sample, amount of SFA in reference category, mean saturated or trans fat intake of the population, sex, α-linoleic acid, total polyunsaturated fat, adjustment for total energy, method and frequency of exposure assessment, risk of bias score, and adjustment for lipids or blood pressure (i.e. causal intermediates).

Retrospective Case-Control Studies comparing industrially produced trans-unsaturated fatty acids with ruminant-derived trans-unsaturated fatty acids and cardiovascular outcomes

Total CHD

Industrially-produced vs. Ruminant-derived TFA

Ascherio et al.[51] measured the associations between both dietary vegetable and animal trans fatty acid intake and CHD in a retrospective study of 239 cases and 282 controls matched for age, sex, and cardiac history. In this analysis, increased vegetable trans fatty acid intake was associated with an almost twofold increase in CHD risk (multivariable OR: 1.94 for 5th [5.04 g/d] vs. 1st [0.84 g/d] quintile; 95% CI: 0.93 to 4.04; P-trend <.001); but increased animal *trans*-unsaturated fatty acid intake was not associated with CHD (multivariable OR: 1.02 for 5th [1.79 g/d] vs. 1st [0.45 g/d] quintile; 95% CI: 0.43 to 2.41; P- trend =.57).

Ischemic stroke

We did not identify any retrospective case-control studies specifically examining the association between industrially-produced or ruminant-derived *trans-unsaturated* fatty acids and risk of stroke.

Appended Tables (E)

Outcome	Comparison	# Studies	Summary effect (95% CI)	P-value	I^2	P_{het}
CHD mortality	Reference	6	1.00	.	.	.
	Q2 vs. reference	6	1.11 (0.84, 1.47)	0.45	55%	0.05
	Q3 vs. reference	6	1.09 (0.90, 1.32)	0.36	12%	0.34
	Q4 vs. reference	6	1.02 (0.87, 1.21)	0.80	33%	0.17
	Q5 vs. reference	4	1.29 (0.97, 1.72)	0.08	35%	0.20

Table E1. Pooled most-adjusted (random effects) relative risks of CHD mortality across corresponding quantiles of intake of trans fatty acids in prospective cohort studies.

Outcome	Comparison	# Studies	Summary effect (95% CI)	P-value	I^2	P_{het}
CHD	Reference	6	1.00	.	.	.
	Q2 vs. reference	5	1.11 (1.01, 1.22)	0.04	0%	0.68
	Q3 vs. reference	5	1.16 (0.98, 1.37)	0.08	53%	0.08
	Q4 vs. reference	4	1.12 (1.00, 1.44)	0.05	0%	0.78
	Q5 vs. reference	3	1.21 (1.07, 1.66)	0.002	0%	0.55

Table E2. Pooled most-adjusted (random effects) relative risks of CHD across corresponding quantiles of intake of *trans* fatty acids in prospective cohort studies.

Outcome	Comparison	# Studies	Summary effect (95% CI)	P-value	I^2	P_{het}
Ischemic stroke	Reference	2	1.00	.	.	.
	Q2 vs. reference	2	1.12 (0.86, 1.46)	0.39	43%	0.19
	Q3 vs. reference	2	1.05 (0.64, 1.72)	0.86	82%	0.02
	Q4 vs. reference	2	1.06 (0.82, 1.36)	0.66	26%	0.24
	Q5 vs. reference	2	1.08 (0.63, 1.85)	0.79	82%	0.02

Table E4. Pooled associations between trans-fatty acid intake and ischemic stroke

Outcome	Comparison	# Studies	Summary effect (95% CI)	P-value	I^2	P_{het}
Type 2 diabetes	Reference	4	1.00	.	.	.
	Q2 vs. reference	4	1.07 (0.97, 1.18)	0.18	26%	0.26
	Q3 vs. reference	4	1.04 (0.92, 1.17)	0.57	47%	0.13
	Q4 vs. reference	4	0.96 (0.85, 1.09)	0.55	47%	0.13
	Q5 vs. reference	4	1.04 (0.86, 1.25)	0.72	75%	0.007

Table E5. Pooled associations between trans-fatty acid intake and type 2 diabetes

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