Association of Types of Dietary Fats and All-Cause and Cause-Specific Mortality: A Prospective Cohort Study and Meta-Analysis of Prospective Studies with 1,148,117 Participants

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Running Title: *Dietary fats and Risk of Mortality*

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ABSTRACT:

Background: Associations between dietary fats and mortality are unclear.

Methods: We evaluated the relationship between quartiles of total fat, mono-unsaturated (MUFA), polyunsaturated (PUFA) and saturated fatty acid (SFA) consumption, and all-cause, coronary heart disease (CHD), stroke, and type 2 diabetes (T2D)-associated mortality in 24,144 participants from the National Health and Nutrition Examination Surveys (NHANES) 1999-2010.

We added our results to a meta-analysis based on searches until November 2018.

Results: In fully adjusted Cox-proportional hazard models in our prospective study, there was an inverse association between total fat (HR: 0.90, 95% confidence interval 0.82, 0.99, Q4 *vs* Q1) and PUFA (0.81, 0.78-0.84) consumption and all-cause mortality, whereas SFA were associated with the increased mortality (1.08, 1.04-1.11). In the meta-analysis of 29 prospective cohorts (n=1,148,117) we found a significant inverse association between total fat (0.89, 0.82-0.97), MUFA (0.93, 0.87-0.99) and PUFA (0.86, 0.80-0.93) consumption and all-cause mortality. No association was observed between total fat and CVD (0.92, 0.79-1.08) or CHD mortality (1.03 0.99-1.09). A significant association between SFA intake and CHD mortality (1.10, 1.01-1.20*)* was observed. Neither MUFA nor PUFA were associated with CVD or CHD mortality. Inverse associations were observed between MUFA (0.80, 0.67-0.96) and PUFA (0.84, 0.80-0.90) intakes and stroke mortality.

Conclusions: We showed differential associations of total fat, MUFA and PUFA with all-cause mortality, but not CVD or CHD mortalities. SFA was associated with higher all-cause mortality in NHANES and with CHD mortality in our meta-analysis. The type of fat intake appears to be associated with important health outcomes.

Key words: Dietary fats, Coronary Heart Disease, Stroke, Mortality, Diabetes, Meta-analysis.

No. of words: 250

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality worldwide [1], and CVD accounts for over 17 million deaths annually with almost 1 million deaths in the US [1]. Similarly, the prevalence of type 2 diabetes (T2D) is rapidly increasing all over the world with a predicted 592 million cases by 2035 [2]. Diet is one of the most important modifiable risk factors for CVD and current guidelines recommend a low-fat diet and substantial limiting of saturated fatty acids (SFA) while increasing intake of unsaturated fatty acids – both monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) [3].

The effects of different types of dietary fat on health have long been of interest. This dietary strategy attracts considerable controversy and has been investigated in epidemiological and clinical studies. Results from relatively old meta-analyses did not support the association of SFAs with all-cause, CVD, or T2D mortality [4-6]. In the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort study the authors evaluated SFA intake and coronary heart disease (CHD) with 12-year follow-up [7]. Higher SFA intake was not associated with higher CHD risk [7]. Conflicting results were obtained in another study where higher dietary intakes of major SFAs were significantly associated with an increased risk of CHD [8]. In fact, there are several studies in the medical literature with contradictory findings regarding the potential role of dietary fats on hard health outcomes.

In 2017, after a review of the existing evidence, the American Heart Association (AHA) endorsed the recommendation to reduce intake of SFA and replace it with unsaturated fats, especially PUFA, in order to reduce the incidence of CVD [9]. This was based on several studies reporting PUFA intake to be associated with lower all-cause and CVD mortality [10, 11]. However, there are also studies which did not demonstrate a significant link between PUFA intake and risk of all-cause mortality [12, 13], including very recent randomized controlled trials (RCTs), which showed no effect of PUFA supplementation on CVD events [14, 15]. The recent 18-country observational Prospective Rural Urban Epidemiology (PURE) study suggested that total fat and types of fat were related to lower all-cause mortality. Total fat and types of fat were not associated with CVD, myocardial infarction (MI), or CVD mortality, whereas saturated fat had an inverse association with stroke [16]. However, the results in PURE may be confounded by poverty in some regions and the results do not apply to high-income countries, where diets are vastly different.

Intake of MUFAs has been reported to improve blood lipid profiles, inflammatory markers, and common CVD risk factors, but little evidence exists to associate consumption of MUFAs with lower CVD mortality [17-19]. In a recent study performed among 63,442 women from the Nurses' Health Study and 29,942 men from the Health Professionals Follow-Up Study, the authors reported no link between MUFA intake and CHD mortality [20]. In another study, the associations of specific dietary fats with all-cause and cause-specific mortality were examined, and hazard ratios (HRs) for all-cause mortality comparing extreme quintiles of specific dietary fats were 1.08, (95%CI, 1.03-1.14) for SFA, 0.81 (0.78-0.84) for PUFA and 0.89 (0.84-0.94) for MUFA [21].

Based on the available data, the associations between different fats with mortality still appear to be conflicting. Public concerns have increasingly been raised regarding the link between higher SFA intake and the prevalence of various chronic disorders [22, 23]. Therefore, it is still unclear which types of fat should be promoted to improve health outcomes. To address these concerns, we examined the association between fat consumption (total, MUFA, PUFA, SFA) and all-cause and cause-specific (CHD, stroke and T2D) mortality in a large, nationally representative US cohort. Furthermore, we performed a comprehensive systematic review and meta-analysis to examine these possible associations by using all existing prospective cohort studies.

METHODS

[A] NHANES study

Population:

This was a prospective cohort study using data from the US National Health and Nutrition Examination Survey (NHANES 1999-2010). The National Center for Health Statistics (NCHS) Research Ethics Review Board approved the underlying protocol, and written informed consent was obtained from all participants. Details on NHANES Laboratory/Medical Technologists Procedures and Anthropometry Procedures have been described elsewhere [24, 25].

Baseline data in NHANES were gathered when individuals participated in a household interview and a medical examination, during which they provided blood and urine samples. Demographic information, including sex, age, ethnic origin, household income, education, and smoking status was obtained during the household interview (26). A digital scale was used to measure weight to the nearest 100 g and a fixed stadiometer to measure height to the nearest mm. Body mass index (BMI) was calculated as weight in kg divided by the square of height in m. Waist

circumference (WC) was measured at the iliac crest to the nearest mm, using a steel tape [26]. A blood specimen was drawn from the participant's antecubital vein. Fasting blood glucose (FBG) was measured by a hexokinase method using a Roche/Hitachi 911 Analyzer and Roche Modular P Chemistry Analyzer (NJ, USA). Other laboratory-test details are available in the NHANES Laboratory/Medical Technologists Procedures Manual [27]. Details on C-reactive protein (CRP) measurement are available elsewhere [26]. Hypertension (HTN) was diagnosed in individuals with SBP ≥140 mmHg and/or DBP ≥90 mmHg, and in participants on antihypertensive medications [28]. T2D was defined as a self-reported history of diabetes or fasting blood glucose (FBG) ≥126 mg/dL [29].

Dietary intake was assessed *via* 24h recall obtained by a trained interviewer, with the use of a computer-assisted dietary interview system with standardized probes, i.e. the United States Department of Agriculture Automated Multiple-Pass Method (AMPM) [30, 31]. Briefly, the type and quantity of all foods and beverages consumed in a single 24h period before the dietary interview (from midnight to midnight) were collected using the AMPM. The AMPM is designed to enhance complete and accurate data collection while reducing respondent burden [31, 32]. In the current study we used the data on fatty acids intake such as total daily fat intake, total SFA intake (the sources of SFA in the diet have been described previously [33]), total MUFA intake and total PUFA intake, saturated fatty acids (SFA) 4:0 (butanoic), SFA 6:0 (hexanoic), SFA 8:0 (octanoic), SFA 10:0 (decanoic), SFA 12:0 (dodecanoic), SFA 14:0 (tetradecanoic), SFA 16:0 (hexadecanoic), SFA 18:0 (octadecanoic), MUFA 16:1 (hexadecenoic), MUFA 18:1 (octadecenoic), MUFA 20:1 (eicosenoic), MUFA 22:1 (docosenoic), PUFA 18:2 (octadecadienoic), PUFA 18:3 (octadecatrienoic), PUFA 18:4 (octadecatetraenoic), PUFA 20:4 (eicosatetraenoic), PUFA 20:5 (eicosapentaenoic), PUFA 22:5 (docosapentaenoic) and PUFA 22:6 (docosahexaenoic).

Mortality:

A full description of mortality linkage methods is available from the National Center for Health Statistics (NCHS). The anonymized data of NHANES 1999-2010 participants were linked to longitudinal Medicare and mortality data using the NHANES assigned sequence number. Mortality follow-up data are available from the date of survey participation until December 31, 2011 (median follow-up: 12 years). We examined all-cause mortality, as well as mortality due to

CHD (I00-I09, I11, I13, I20-I51), cerebrovascular disease (I60-I69), and diabetes (E10-E14). Cause of death was determined using ICD-10.

Statistical analysis:

Analyses were conducted according to the guidelines set by the Centers for Disease Control (CDC) and Prevention for analysis of the NHANES dataset, accounting for the masked variance and using their suggested weighting methodology [34]. Continuous and categorical demographic variables were compared across total fat consumption quartiles using analysis of variance (ANOVA) and Chi-square tests, respectively.

We constructed Kaplan-Meier survival curves according to quartiles of total fat intake compared differences for the composite endpoint (all-cause mortality) across groups using the *log-rank* test. Multivariable Cox proportional hazards were applied to determine the HRs and 95% confidence intervals (95% CIs) of mortality (all-cause, CHD, CVD, T2D and cerebrovascular) for total fat, MUFA, PUFA, SAF consumption; the first quartile (Q1) was always used as reference. To derive the HR and 95%CI we performed analyses using 2 different models, *Model 1:* adjusted for age, race, education, marital status, poverty to income ratio, total energy intake, physical activity and smoking; *Model 2:* additionally adjusted for alcohol consumption, dietary cholesterol, body mass index, hypertension, and non-HDL cholesterol. The Cox regression was applied for the second model (adjusted for age, race, education, marital status, poverty to income ratio, total energy intake, physical activity, smoking, alcohol consumption, dietary cholesterol, body mass index, hypertension, and non-HDL cholesterol), to have same covariates with same outcomes (all-cause, CHD, stroke and T2D death).

A two-sided $p<0.05$ was used to characterise significant results. All statistical analysis was conducted in R (version 3.4.2 R Core Team, 2017), Comprehensive Meta-Analysis V3 software (Biostat 2014, Englewood, NJ) and SPSS® complex sample module version 22.0 (IBM Corp, Armonk, NY).

[B] Systematic Review and Meta-Analysis

Literature search and study selection:

The meta-analysis was designed, conducted and reported according to Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines [35]. The primary exposures of interest

were fat type consumption and various sub-types, whereas the primary outcomes were all-cause and cause-specific mortality. Prospective cohort studies published up to 31 November 2018, without language restriction, were searched using PubMed, SCOPUS, Web of Science and Google Scholar databases. The query syntax of searching is shown in the Supplemental Methods (**Supplemental Table 1**). This was complemented by hand searches of the reference list of eligible articles, and email correspondence with authors for additional relevant data. After excluding duplicates and based on titles and abstracts, we excluded studies on animals, baseline age <20 years, or populations with prior CHD, T2D or any other chronic disease. In addition, supplementary hand searching of reference lists of previous reviews or meta-analyses was conducted.

Study Selection:

Study selection started with the removal of duplicates, followed by screening of titles and abstracts by 2 reviewers (MM and MB). To avoid bias, the reviewers were blinded to the names, qualifications or institutional affiliations of the study authors. The agreement between the reviewers was excellent (Kappa index: 0.92; $p<0.001$). Disagreements were resolved at a meeting between reviewers and third reviewer (DPM) prior to selected articles being retrieved (a flow chart is available in **Supplemental Figure 1**).

We included studies if they met all the following criteria: (1) evaluated fat intake, (2) were population-based cohort studies and reported mortality data, and, (3) relative risk (RR), HR or odds ratio (OR) estimates with 95%CIs adjusted for multivariable factors were available or could be calculated. Studies were excluded according to the following criteria: (1) reviews, letters, unpublished data or comments, (2) those published in languages other than English, (3) not population-based cohort studies, and, (4) RR or HR estimates with 95%CI were not available or could not be calculated (despite individual contact attempts with the given investigators). Narrative reviews, comments, opinion pieces, editorials, letters or any other publications lacking primary data and/or explicit method descriptions, were also excluded.

Data extraction and management:

Full text of studies meeting inclusion criteria were retrieved and screened to determine eligibility by two reviewers (MM, NK). The study quality assessment was performed according to

the Newcastle-Ottawa Scale (NOS, **Supplemental Table 2**) [36]. By evaluation of selection, comparability and outcome, the rating system scores studies from 0 (highest degree of bias) to 9 (lowest degree of bias). Additionally, we investigated the funding sources of the eligible studies. Following assessment of methodological quality, two reviewers (MM, MB) extracted data using a purpose-designed data extraction form. Information extracted per study included: author, year and references, country, study name, age, follow-up time (years), number of cases, and number of participants, exposure categories, outcome and main confounders. Extractions were compared and any differences of opinion were resolved by discussion and consultation with a third reviewer (DPM). Any further calculations on study data considered necessary, was conducted by the first reviewer (MM) and checked by the second reviewer (MB).

Data synthesis and statistical analyses:

For studies that reported results from different multivariable-adjusted models, the model with the most confounding factors was extracted for the meta-analysis. DerSimonian-Laird method or generic inverse variance methods were used for random effects meta-analyses to calculate pooled HRs, 95%CI and *p* value for heterogeneity. HRs comparing the highest score category with the lowest category were combined across studies to generate the summary associations. The extent of heterogeneity across studies was examined using the I^2 test [37-39]; an I^2 >50% together with a two-sided p<0.05 indicated significant heterogeneity [37-39].

Publication bias:

Potential publication bias was explored using Begg's rank correlation and Egger's weighted regression tests. Duval and Tweedie 'trim and fill' method was used to adjust the analysis for the effects of publication bias [40]. Meta-analyses were conducted using the Comprehensive Meta-Analysis (CMA) V3 software (Biostat, NJ) [41].

RESULTS

[A] NHANES study

Overall, 24,144 participants were included (mean age was 49.6 years and 48.5% were men). Their demographic characteristics according to total fat intake quartiles are shown in **Table 1**.

Participants in the highest quartile of total fat were significantly younger than those in the lowest quartile (40.0 *vs* 54.8, $p<0.001$, **Table 1**). For the lowest category of fat consumption, females were in the majority, while males were in the majority in the highest category ($p<0.001$, **Table 1**). We found the following crude reported mean and SEM (g/day) for intake of total fat (overall=78.7±0.3, males=90.2±0.5 *vs* females=66.2±0.4; $p<0.001$), PUFA (overall=16.8±0.8, males=19.2±0.4 *vs* females=14.1±0.5; $p<0.05$), MUFA (overall=28.6±0.1, male=33.1±0.2 *vs* female=23.1±0.2; $p<0.05$), and SFA (overall=25.6±0.1, males=29.5±0.1 *vs* females=21.4±0.1; $p<0.001$).

During the follow-up of up to 12 years of NHANES 1999-2010, 3,632 all-cause deaths were recorded, including 714 CHD-related deaths and 233 due to stroke. Kaplan-Meier survival plots showed that subjects in the highest quartiles of total fat intake had significantly lower all-cause mortality than those in the lowest quartiles (log-rank $p<0.0001$; **Figure 1 & Central Illustration**).

Results from multivariable Cox regression models for risk of death across fat quartiles are shown in **Table 2**. With regard to total fat, in *Model 1*, subjects with the highest consumption had a 16% lower risk of mortality (HR: 0.84, 95%CI: 0.81, 0.88); this association was diluted but was still significant in *Model 2* (HR: 0.90, 95%CI: 0.82, 0.99, **Table 2, Central Illustration**). In the *Model 1*, subjects in the highest quartile had 12% lower risk for CHD mortality (HR: 0.88, 95%CI: 0.86, 0.89, **Table 2**), while this association was no longer evident after further adjustments in *Model 2* ($p=0.421$ for trend; **Central Illustration**). We did not find significant association between intake of total fat and both stroke and T2D mortality in both models ($p>0.365$, **Table 2**).

With regard to SFA intake, increase in risk of all-cause mortality was observed for both the 1st and 2nd model (*Model 2*=Q2: 1.05, 95%CI: 1.02, 1.08; Q3: 1.14, 95%CI: 1.10, 1.19, Q4: 1.08, 95%CI: 1.04, 1.11, **Table 2, Central Illustration**). We observed that subjects in the highest quartile (Q4) of the SFA had significantly higher risk of CHD mortality even after adjustment for wide range of co-variables (HR: 1.11, 95%CI: 1.07, 1.17, **Table 2, Central Illustration**). In *Model 2*, subjects in the highest quartile had a 13 and 4% greater risk of stroke (HR: 1.13, 95%CI: 1.06, 1.22) and T2D (HR: 1.04, 95%CI: 1.01, 1.08) mortality, respectively, compared with the first quartile (Q1).

With regard to MUFA, we found that (*Model 1*) subjects in the highest quartile had a significantly lower risk of all-cause mortality compared with the first quartile (Q2: 1.02, 95%CI: 0.29, 3.96; Q3: 0.85, 95%CI: 0.80, 0.91, and Q4: 0.71, 95%CI: 0.69, 0.74, **Table 2, Central Illustration**). However, no significant association was observed for this outcome in the fully adjusted *Model 2*. Further, either in the partially or fully adjusted model we observed insignificant association between CHD, stroke and T2D mortalities with MUFA intake (**Table 2**).

With regard to PUFA intake, we observed a protective association with all-cause mortality in both minimally and fully adjusted model - in the *Model 2* subjects in the highest quartile had 19% lower risk of all-cause mortality (HR: 0.81, 95%CI: 0.78-0.84, **Table 2, Central Illustration**). Further, subjects in the highest quartile of PUFA intake in both in first and second model had a 43 and 25% lower risk of CHD mortality (*Model 1*= Q4: 0.57, 95%CI: 0.55, 0.60; *Model 2* = Q4: 0.75, 95%CI: 0.62-0.90, **Table 2, Central Illustration**). Similar inverse associations were observed in both models for both stroke and T2D mortality - in the fully adjusted model, subjects in the highest quartile of PUFA both had 15 and 14% lower risk of stroke and T2D mortality (0.85, 95%CI: 0.80-0.91; and 0.84, 95%CI: 0.79-0.92, respectively; **Table 2**).

[B] Meta-Analysis

Of 49 eligible full articles, 29 cohorts met the inclusion criteria (**Supplemental Figure 1**). An overview of key characteristics of the 29 prospective cohort studies is shown in **Supplemental Table 3**. A total of 1,148,117 participants, with 253,592 deaths, were included in the analysis. The duration of follow-up ranged from 3.7 to 32.0 years (mean= 13.3 years [156 months]). Results of NOS quality assessment are shown in the **Supplemental Table 2**, with seven studies scoring 8, and no study scoring less than 7.

***Total fat consumption and all-cause and cause-specific mortality*:**

We found an inverse and significant association between total fat consumption and all-cause mortality (HR: 0.89, 95%CI: 0.82-0.97, p=0.009, n=11 studies, I^2: 27%, **Figure 2, Central Illustration**). No significant association was observed between total fat with both CVD and CHD mortality (HR: 0.92, 95%CI: 0.79-1.08, p=0.340, n=8 studies, I^2: 46%, **Supplemental Figure 2**,

and HR: 1.03, 95%CI: 0.99-1.09, p=0.115, n=7 studies, I^2: 42%, respectively; **Central Illustration**).

We observed a non-significant association between SFA and all-cause mortality (HR: 1.04, 95%CI: 0.98-1.11, p=0.139, n=18 studies, I^2: 40%, **Supplemental Figure 3, Central Illustration**); no significant association was also showed between SFA intake and CVD mortality (HR: 0.96, 95%CI: 0.84-1.11, p=0.643, n=9 studies, I^2: 30%, **Supplemental Figure 4**). While we found a significant association with CHD mortality (HR: 1.10, 95%CI: 1.01-1.20, p<0.001, n=19 studies, I^2: 52%, **Figure 3, Central Illustration**). No significant association was observed between SFA and stroke mortality (HR: 1.03, 95%CI: 0.85-1.26, p=0.703, n=3 studies, I^2: 41%).

There was an inverse association between MUFA consumption and risk of all-cause mortality (HR: 0.94, 95%CI: 0.89-0.99, p=0.028, n=15 studies, I^2: 56%, **Figure 4, Central Illustration**), while there was an inverse however non-significant association between MUFA intake and CVD mortality (HR: 0.89, 95%CI: 0.77-1.03, p=0.120, n=13 studies, I^2: 32%, **Supplemental Figure 5**), and CHD mortality (HR: 0.99, 95%CI: 0.89-1.10, p=0.896, n=9 studies, I^2: 49%, **Supplemental Figure 6, Central Illustration**). Finally, we showed an inverse and significant association between MUFA intake and stroke mortality (HR: 0.80, 95%CI: 0.67-0.96, p=0.019, n=3 studies, I^2: 0%).

There was an inverse link between PUFA consumption and risk of all-cause mortality (HR: 0.88, 95%CI: 0.83-0.94, p<0.001, n=14 studies, I^2: 63%, **Figure 5, Central Illustration**), while no significant association between PUFA intake and CVD was observed (HR: 0.98, 95%CI: 0.85-1.12, p=0.773, n=8 studies, I^2: 47.4, **Supplemental Figure 7**) and CHD (HR: 0.96, 95%CI: 0.85-1.07, p=0.480, n=8 studies, I^2: 51.0, **Supplemental Figure 8, Central Illustration**) mortality was observed. There was an inverse significant effect of PUFA consumption on the risk of stroke mortality (HR: 0.84, 95%CI: 0.80-0.90, p<0.001, n=2 studies, I^2: 0.0).

Sensitivity analysis:

In leave-one-out sensitivity analyses, the pooled effect estimates remained similar for the association of total fat (HR: 0.89, 95%CI: 0.82-0.97, p=0.009), SFA (HR: 1.03, 95%CI: 0.99-1.08, p=0.113), MUFA (HR: 0.93, 95%CI: 0.87-0.99, p=0.043) and PUFA (HR: 0.86, 95%CI: 0.80-0.93, p<0.001) intake on all-cause mortality.

Publication bias:

Both Egger's linear regression (intercept=0.461, 95%CI: -2.45, 3.37, p=0.523) and Begg's rank correlation test (Kendall's Tau with continuity correction=0.285, z=0.901, p=0.415) were not indicative of publication bias. After adjustment of effect size for potential publication bias using the 'trim and fill' correction, no potentially missing studies were imputed in funnel plot. The 'fail-safe N' test showed that 126 studies would be needed to bring the weighted mean difference down to a non-significant ($p \geq 0.05$) value.

DISCUSSION

Using a large and representative samples of USA adults, with long follow-up periods we have found that reported total dietary fat intake is linked to lower all-cause mortality (10-13%). SFA intake was associated with all-cause, CHD, stroke and T2D mortality. In contrast, PUFA intake showed an inverse association; higher intake was associated with lower risk of all-cause and cause-specific mortality. MUFA intake was not significantly associated with any type of mortality. These results were robust even after adjustments for a wide range of clinical, nutritional and socio-economic factors. We further pooled data from all the published prospective studies with >1.1 million participants; this confirmed the results based on NHANES cohort studies and revealed a protective association of total fat, MUFA and PUFA on all-cause mortality, while no link was observed between total fat, MUFA and PUFA intake with CVD and CHD mortality (with significant association with stroke mortality). SFA intake was associated with higher risk of CHD mortality.

Currently, there are considerable discrepancies between studies regarding fat intake and its relationship with all-cause and cause-specific mortality [10,11,13,42-58]. Our results showed an inverse association of all-cause mortality with total fat, MUFA and PUFA consumption. The PURE study (18 countries, n=135 335, follow-up of 7.4 years) study reported that intake of total fat and each type of fat was associated with lower risk of all-cause mortality (total fat: HR 0·77 [95% CI 0·67-0·87]; saturated fat, HR 0·86 [0·76-0·99]; monounsaturated fat: HR 0·81 [0·71-0·92] and polyunsaturated fat: HR 0·80 [0·71-0·89]) [42]. Another study that investigated 83,349 women from the Nurses' Health Study (follow up of 32 years) and 42,884 men from the Health Professionals Follow-up Study (follow up of 26 years), reported that the HRs of all-cause mortality comparing

extreme quartiles of specific dietary fats was 1.08, (95% CI: 1.03-1.14) for saturated fat, 0.81 (95% CI: 0.78-0.84) for polyunsaturated fat and 0.89 (95% CI: 0.84-0.94) for monounsaturated fat [11]. In contrast, Wakai *et al.* [10] and Leosdottir *et al.* [13], reported no significant association between total fat consumption and risk of all-cause mortality. We reported small (6% lower risk) but potentially protective association of MUFA intake on all-cause mortality (significant in the meta-analysis with longer follow-up), which is in line with the PREvención con DIeta MEDiterránea study (n=7038, 6 years follow-up) that reported inverse associations with all-cause death for PUFA and MUFA intakes [43]. Further, the above-mentioned study by Wakai *et al.* (Japan Collaborative Cohort Study; 58,672 individuals; 19.3-year follow up) found an inverse association between total fat intake and all-cause mortality (HR: 0.91, 95%CI:0.93-0.99) [10]. Surprisingly, a recent meta-analysis of 49 RCTs (but with fewer participants and a much shorter follow-up in comparison to our meta-analysis) investigated the link between PUFA intake (PUFA supplementation, but not habitual PUFA intake) and mortality, and reported that increasing PUFA intake probably has little or no effect on all-cause mortality. In our study habitual PUFA intake was associated with lower all-cause mortality by 14-19%. Furthermore, the investigators reported that increasing PUFA has little or no effect on CVD mortality (we also observed only 2% risk reduction in the meta-analysis) [59].

We have shown that total fat, MUFA and PUFA consumption were not significantly associated with either CVD or CHD mortality. The Esrey *et al.* study (n=4546, follow-up 12 years,), reported no significant association between total fat and CHD (RR: 0.99, 95%CI: 0.95-1.03) mortality [45]. Similar results were observed by Xu *et al.* in their study with 2938 subjects and 7.2-year follow-up (RR: 0.77, 95%CI: 0.40-1.44) [51]. With regard to MUFA intake and CVD mortality, most of the studies are in line with our results. A single study, conducted by Guasch-Ferré *et al.* (n=7038, 6 years of follow-up) reported a surprisingly large protective effect of MUFA consumption on CVD mortality (RR: 0.50, 95%CI: 0.30-0.80) [43], while the result of other available studies on the link between MUFA intake and CVD mortality no significant association was observed [10,42,44,47,50,59]. We believe the effect of PUFA intake on CVD mortality requires further investigation, as the available studies have conflicting results, and most suggest no link [10,13,42,44]. However, the same study by Guasch-Ferré *et al.* study again reported very protective effect of PUFA consumption on CVD mortality (RR: 0.68, 95%CI: 0.48-0.96) [43].

We observed strong and significant association between SFA-intake and all-cause and cause-specific mortality in two investigated cohorts of NHANES study but after pooling all the studies

in meta-analysis, SFA intake was no longer related to all-cause mortality, but it was associated with 10% higher risk of CHD mortality. Several available studies reported no link between SFA intake and all-cause mortality [13,42,43,50,44,58]. The Malmo Diet and Cancer Study (n=28,098, 6-year follow up) demonstrated no significant link between SFA and all-cause mortality (RR: 0.89, 95%CI: 0.64-1.23) [13]. The same results were observed in relatively small (n=501) the Baltimore Longitudinal Study of Aging (BLSA) with 18-year follow-up (RR: 1.16, 95%CI: 0.86-1.56) [58]. The effect of SFA intake on CHD mortality has been already observed [45,51-53,61]. A study in a Greek population (n=28,572, 4.5 years follow-up), reported a highly significant link (RR: 1.76, 95%CI: 1.11-2.77) between SFA and CHD mortality [61].

Our study has some strengths and limitations. Our observational study was based on a well-known, long-term large population-based US cohort. The availability of detailed data on covariates allowed us to better control for confounding. In NHANES, we used a validated 24-h food recall method, and our data was collected by trained personnel in settings, which allow us to report health data accurately. Although 24-h recall methods may be prone to bias and confounding, alternatives such as food-frequency questionnaires are also subjective estimates. Dietary intake of nutrients is complex to measure, and no instrument is perfect. It has been suggested that combining questionnaire data with biomarker levels is an attractive alternative, but this adds complexity and expense to studies [62]. Furthermore, we evaluated over 1.1 million subjects in our meta-analysis, pooling all the published studies to reinforce our cohort results. However, varying definitions of the exposure also pose a significant limitation to the interpretation and reliability of the meta-analysis, which might be sensitive to the categorization of fat intake and the varying definitions. Heterogeneity of the results of the component studies was relatively low or modest, which indicated that each result was broadly consistent and most variation may be attributable to chance alone. The limitation of the cohort study is its observational design, which means we cannot exclude the possibility that our findings may be influenced by unmeasured or residual confounding factors or indeed reverse causality whereby ill health alters diet. Studies focusing on single nutrients can be complicated by the fact that a diet low in one energy source (e.g. fat) may contain higher amounts of alternative energy sources e.g. carbohydrate [63] but also that people (non)adherent to reduce one energy source, might be also (non)adherent to others (like proteins or carbohydrate). In the NHANES 1999-2010 results, an increase in BMI and in the percentage of obesity from Q1 to Q4 was observed, whereas this is not reflected by the energy intake. Although,

our analysis was corrected for several covariates (however not all data was available, including details on medication used), it would be better for the future studies to consider more variables or use a different design to minimize the impact of other factors, as demographic details, risk factors and the gender balance of the participants varied between quartiles of fat intake in this analysis. As our analysis was based on quantities of fat, rather than specific foods, it is hard to interpret the results in terms of specific advice for patients.

In conclusion, our results highlighted the divergent associations between total fat, MUFA and PUFA and all-cause mortality, while suggesting that not only quantity but also quality is critical for long-term outcomes. SFA intake was mainly associated with higher CHD mortality, and PUFA with significantly lower all-cause mortality and stroke events. No significant association was observed between total fat and MUFA with CVD and CHD mortality. Further well-design studies are necessary to explain all the inconsistencies between dietary fat intake and all-cause and cause-specific mortality and to provide clear recommendations on target levels of fats in the healthy diet. For now, however, based on these data, and the results of prior trials, it seems sensible to focus on the quality of dietary fats with a reduction in SFA intake and increase PUFA and MUFA intake to lower future CVD outcomes and mortality.

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FIGURES LEGENDS:

Central Illustration: An effect of total dietary fats, saturated, mono- and polyunsaturated fats on all-cause and CHD mortality based in NHANES and meta-analysis results**.**

Figure 1: Risk of all-cause death across the category of total fat intake in NHANES 1999-2010

Figure 2. Forest plot of total fat consumption and all-cause mortality.

Figure 3. Forest plot of high-saturated fatty acids consumption and risk of coronary heart disease mortality.

Figure 4. Forest plot of monounsaturated fatty acids consumption and risk of all-cause mortality.

Figure 5. Forest plot of polyunsaturated fatty acids consumption and risk of all-cause mortality.

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Table 1. Characteristics of the study participants based on total fat consumption (NHANES, 1999-2010).

		Total Fat Consumption (NHANES 1999-2010)				
		Q1 (n = 5996)	**Q2 (n = 6142)**	**Q3 (n = 6008)**	**Q4 (n = 5998)**	***P-value***
Macronutrients' amounts (g/day; calories/day; %energy*)		Carbohydrate: 161 (644), Protein: 45 (180), **Fat: 32 (288; 25%)**	Carbohydrate: 212 (848), Protein: 63 (252), **Fat: 55 (495; 31%)**	Carbohydrate: 254 (1016), Protein: 79 (316), **Fat: 80 (720; 35%)**	Carbohydrate: 328 (1312), Protein: 109 (436), **Fat: 120 (1080; 38%)**	
Age (Years)		51.2±0.2	48.2±0.2	45.6±0.1	40.8±0.2	<0.001
Gender	**Men (%)**	36.9	32.9	52.9	58.4	<0.001
	Women (%)	63.1	67.1	47.1	41.6	
Race/Ethnicity	**Mexican-American (%)**	23.5	19.4	16.5	14.4	<0.001
	Non-Hispanic White (%)	42.2	46.5	48.9	55.5	
	Non-Hispanic Black (%)	17.8	19.6	21.8	21.5	
Education level: <9th grade, (%)		15.6	14.3	12.5	8.5	<0.001
Smoking (%)		22.3	21.0	25.8	23.6	<0.001
Body Mass Index (kg/m^2) [1]		27.2±0.2	28.5±0.2	29.3±0.2	29.6±0.2	<0.001
Diabetes (%)		9.0	10.6	12.4	13.2	<0.001
Hypertension (%)		18.5	19.5	20.1	18.9	<0.001
Obesity (%)		31.8	35.1	39.4	41.2	<0.001

Metabolic syndrome (%)	29.1	31.2	32.3	30.5	<0.001
Dietary cholesterol (g/day) [2]	108.0 (66.0-173.0)	182.0 (124.0-285.0)	252.0 (176.0-392.0)	383.0 (263.5-594.0)	<0.001
Protein (g/day) [2]	45.15 (32.67-59.72)	63.22 (50.48- 80.03)	79.59 (64.63-98.95)	109.68 (89.06 -137.36)	<0.001
Carbohydrate (g/day) [2]	161.17 (117.57- 215.46)	212.57 (165.41-269.91)	254.77 (197.18-321.40)	328.01 (254.02 -421.27)	<0.001
*Groups across the quartiles were compared by either chi-square or analysis of variance. Values expressed as mean ± standard error of mean [1] or median and (25^{th}-75^{th}) [2]. NHANES: National Health and Nutrition Examination Surveys. *refers only to dietary fats*.					

Table 2. Multivariable-adjusted hazard ratios (95% confidence intervals) for mortality across the categories of different fats in NHANES 1999-2010 study.

		Total Fat			*p-value*	SFA			*p-value*
		Q2	**Q3**	**Q4**		**Q2**	**Q3**	**Q4**	
Total mortality	***Model 1***	1.10 (0.58-2.13)	1.02 (0.29,3.96)	0.84 (0.81,0.88)	0.235	1.40 (1.35,1.57)	1.68 (1.61,1.75)	1.73 (1.66,1.80)	<0.001
	Model 2	0.98 (0.50-1.90)	0.89 (0.84,0.95)	0.90 (0.82,0.99)	0.015	1.05 (1.02,1.08)	1.14 (1.10,1.19)	1.08 (1.04,1.11)	<0.001
CHD	***Model 1***	1.03 (0.97, 1.09)	0.99 (0.95, 1.02)	0.88 (0.86,0.89)	0.292	1.02 (0.84, 1.24)	0.93 (0.77, 1.14)	1.42 (1.27,1.96)	<0.001
	Model 2	1.13 (0.97, 1.31)	0.93 (0.79, 1.10)	1.07 (0.99,1.15)	0.421	1.09 (0.93, 1.29)	1.06 (1.03-1.09)	1.13 (1.06,1.21)	<0.001
Stroke	***Model 1***	0.97 (0.91, 1.02)	1.01 (0.95, 1.08)	1.04 (0.96,1.12)	0.362	1.18 (0.95, 1.46)	0.88 (0.66, 1.17)	1.23 (1.11,1.43)	0.012
	Model 2	1·03 (0·78, 1·35)	1.24 (0.62,2.48)	1.05 (0.99,1.11)	0.665	1.02 (0.84, 1.22)	1.05 (1.02-1.08)	1.11 (1.07, 1.15)	<0.001
T2D	***Model 1***	1·10 (0·84, 1·44)	1·06 (0·80, 1·39)	1·18 (0·90,1·56)	0.462	0.88 (0.73, 1.06)	0.97 (0.81, 1.17)	1.20 (1.11,1.36)	0.182
	Model 2	0.99 (0.97, 1.02)	0.98 (0.95, 1.01)	1·05 (0·77,1·43)	0.582	0.99 (0.77, 1.26)	1.05 (0.99, 1.12)	1.04 (1.01, 1.08)	0.241
		MUFA				**PUFA**			
		Q2	**Q3**	**Q4**		**Q2**	**Q3**	**Q4**	
Total mortality	***Model 1***	1.02 (0.29,3.96)	0.85(0.80, 0.91)	0.71(0.69, 0.74)	0.024	0.95 (0.49-1.96)	0.72 (0.62-0.83)	0.68 (0.65-0.70)	<0.001
	Model 2	0.98 (0.50,1.90)	0.93(0.79, 1.10)	0.91(0.76,1.09)	0.362	0.85 (0.79-0.91)	0.78 (0.72- 0.84)	0.81 (0.78-0.84)	<0.001
CHD	***Model 1***	0.95 (0.59, 1.52)	0.93(0.59, 1.48)	1.04(0.84,1.29)	0.526	1.12 (0.52-1.35)	0·70 (0·60-0·82)	0.57 (0.55, 0.60)	<0.001
	Model 2	0.91 (0.75, 1.11)	0.87(0.61,1.23)	0.95(0.77,1.17)	0.421	0.83 (0.77- 0.89)	0·87 (0·73-1·04)	0·75 (0·62-0·90)	0.016
Stroke	***Model 1***	1.04 (0.72,1.49)	0.82(0.51, 1.32)	0.86(0.53,1.40)	0.625	1.19 (0.93-1.53)	1.01 (0.91-1.11)	0·83 (0·72-0·96)	0.382
	Model 2	1.02 (0.64,1.64)	1.20(0.79, 1.83)	0.80(0.61,1.06)	0.692	0.96 (0.79-1.16)	0·81 (0·71-0·91)	0·85 (0·80-0.91)	<0.001
T2D	***Model 1***	0.91 (0.57, 1.45)	0.84(0.66, 1.07)	0.88(0.73, 1.06)	0.452	0.96 (0.79, 1.17)	0.85 (0.79, 0.91)	0.78 (0.72,0.84)	<0.001
	Model 2	0.94 (0.55, 1.59)	0.89(0.74, 1.06)	1.14(0.93, 1.39)	0.762	0.99 (0.77, 1.26)	0.88 (0.66, 1.17)	0.84 (0.79,0.92)	0.162

Model 1: Adjusted for age, race, education, marital status, poverty to income ratio, physical activity and smoking.

Model 2: Adjusted for age, race, education, marital status, poverty to income ratio, physical activity, smoking, alcohol consumption, dietary cholesterol, body mass index and hypertension, non-HDL cholesterol.

ABBREVIATIONS: MUFA: mono-unsaturated fatty acids, SFA: saturated fatty acids, PUFA: polyunsaturated fatty acids, CHD: coronary heart disease, T2D: diabetes, HDL: high-density lipoprotein, NHANES: National Health and Nutrition Examination Surveys.

P-values describe any difference across all 4 levels.

Supplemental Table 1. Full search terms and strategy for papers indexed in investigated databases.		
No	**Concept**	**Search terms**
1	**Fat**	"Dietary fat" [Text Word] OR "fatty acids" [Text Word] OR "monounsaturated fat" [Text Word] OR "MUFA" [Text Word] OR "polyunsaturated fat" [Text Word] OR "PUFA" [Text Word] OR "unsaturated fatty acids" [Text Word] OR "SFA" OR "olive oil" [Text Word] OR "oleic acid" [Text Word] OR "Mediterranean diet" [Text Word] OR ω-3 FA" [Mesh] OR "omega-3 FA" [Mesh] OR "omega-3 OR "fish oils" [Mesh]
2	**Mortality**	mortality[tiab] OR death[tiab] OR dead[tiab] OR all-cause[tiab] OR all cause[tiab] OR fatal[tiab] OR event[tiab] OR nonfatal[tiab] OR non-fatal[tiab] OR Mortality[MeSH:NoExp] OR mortality[MeSH subheading]
3	**Cardiovascular**	cardiovascular[tiab] OR vascular[tiab] OR CVD[tiab] OR Cardiovascular Diseases[Mesh:NoExp]
4	**Stroke**	cerebrovascular[tiab] OR stroke[tiab] OR TIA[tiab] OR transient ischemic*[tiab] OR CVA[tiab] OR cerebral infarction[tiab] OR Cerebrovascular accident [MeSH:NoExp] OR stroke [MeSH:NoExp]
5	**Diabetes**	diabetes mellitus[MeSH Terms] OR diabetes OR mellitus OR diabetes mellitus OR diabetes mellitus, type 2[MeSH Terms] OR type 2 diabetes mellitus OR type 2 diabetes OR diabetes mellitus[MeSH Terms] OR diabetes AND mellitus OR diabetes mellitus OR diabetes OR diabetes insipidus[MeSH Terms] OR diabetes AND insipidus OR diabetes insipidus
6	**Combination**	#2 OR #3 OR #4 OR #5
7	**Combination Exposure And Outcome**	#1 AND #6
8	**Limit**	Rats[Mesh:NoExp]) OR Mice[Mesh:NoExp]) OR rat[Title/Abstract]) OR rats[Title/Abstract]) OR mouse[Title/Abstract]) OR mice[Title/Abstract]) OR vivo[Title/Abstract]) OR vitro[Title/Abstract])
9	**Limit**	#7 NOT #8

For Supplemental Table 2:

NEWCASTLE – OTTAWA QUALITY ASSESSMENT SCALE COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average *healthy adults* in the community★

b) somewhat representative of the average *healthy adults* in the community ★

c) selected group of users *e.g. nurses, volunteers, vegetarian*

d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort

a) drawn from the same community as the exposed cohort★

b) drawn from a different source

c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure

a) secure record (*e.g. 7 day food diary)* ★

b) structured interview/≥ 2 *dietary recalls/diet history/ food frequency questionnaire validated for dairy components* ★

c) written self-report (*e.g. <2 dietary recalls/non-validated food frequency questionnaire or not reported whether food frequency questionnaire was validated*)

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes★

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for *age, sex, smoking, total energy intake, and body mass index*★

b) study controls for any additional factor (*e.g. physical activity, alcohol intake, family history of diabetes, dietary factors*) ★

Outcome

1) Assessment of outcome

a) independent blind assessment (e.g. clinical diagnosis/complete medical information available). ★

b) record linkage/*medical record or validated self-report* ★

c) non-validated self-report

d) no description

2) Was follow-up long enough for outcomes to occur

a) yes/ *follow up period for outcome of interest is 10 years or over* ★

b) no

3) Adequacy of follow-up of cohorts

a) complete follow-up - all subjects accounted for ★

b) subjects lost to follow-up unlikely to introduce bias - small number lost ≤*20%* follow-up, or description provided of those lost ★

c) follow-up rate <*80%* or no description of those lost

d) no statement

Supplemental Table 2. Quality assessment of cohort studies which included in meta-analysis.

Studies	Selection				Comparability	Outcome			Total score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	
Dilis 2012 (1)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Esrey 1996 (2)	C	A★	B★	A★	A★	B★	A★	B★	7
Nagata 2012 (3)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Pietinen 1997 (4)	C	A★	B★	A★	A★	B★	A★	B★	7
Solfrizzi 2005 (5)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Trichopoulou 2005 (6)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Trichopoulou 2003 (7)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Xu 2006 (8)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Ascherio 1996 (9)	C	A★	B★	A★	A★	B★	A★	B★	7
Boniface 2002	C	A★	B★	A★	A★ B★	B★	A★	B★	8

(10)									
Goldbourt 1993 (11)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Leosdottir 2005 (12)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Mann 1997 (13)	C	A★	B★	A★	A★	B★	A★	B★	7
McGee 1985 (14)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Sauvaget 2004 (15)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Tucker 2005 (16)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Misirli 2012 (17)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Trichopoulou 2006 (18)	C	A★	B★	A★	A★	B★	A★	B★	7
Chien 2013(19)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Wakai 2014 (20)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Shekelle 1981 (21)	C	A★	B★	A★	A★	B★	A★	B★	7
Kushi 1985 (22)	C	A★	B★	A★	A★	B★	A★	B★	7
Dehghan 2017 (23)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Guasch-Ferré 2015 (24)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Wang 2017 (25)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Zhuang 2019 (26)	C	A★	B★	A★	A★ B★	B★	B	B★	7
Zhuang 2019 (27)	C	A★	B★	A★	A★ B★	B★	B	B★	7

Supplemental Table 3. Characteristics of the prospective cohort studies included in the present meta-analysis.

Author, year and reference	Country, region/cohort	Age	Follow-Up Time (Years)	No. of cases	No. of subjects	Exposure	Definition of the exposure	Outcome	Main confounders
Dilis, 2012 (1)	European Prospective Into Cancer and Nutrition GRE	20-86	10	240	23,929	MUFA, SFA, PUFA	per 1 standard deviation increment	CHD mortality	Age, BMI, height, PA, years of schooling and energy intake entered, alcohol consumption, smoking status and arterial blood pressure
Esrey, 1996 (2)	Lipid Research Clinics Prevalence Study USA	30-79	12.4	92	4,546	Total, MUFA, PUFA, SFA	1% increase in fatty acids intake	CHD mortality	Age, sex, energy intake, serum lipids, systolic blood pressure, cigarette smoking, BMI, glucose intolerance
Nagata, 2012 (3)	Takayama study JAP	≥35	16	4616	28,356	Total, MUFA, PUFA, SFA	Q4 vs Q1	All-cause mortality CVD mortality	Age, non-alcohol energy, and protein expressed as percentage of non-alcohol energy and was additionally adjusted for fat subtypes expressed as percentage of non-alcohol energy as appropriate, height, BMI, PA, smoking status, alcohol intake, education, marital status, menopausal status, histories of diabetes and hypertension, and intakes of fruits, vegetables, and dietary fibre
Pietinen, 1997 (4)	Finland Finish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study	50-69	6.1	635	21,930	MUFA, SFA	Q5 vs Q1	CHD mortality	Age, smoking, BMI, blood pressure, energy intake, alcohol, education, PA
Solfrizzi, 2005 (5)	Italian Longitudinal Study on Aging ITA	65-84	8.5	91	278	MUFA	Not reported	All-cause mortality	Age, sex, waist-hip ratio, smoking status, Charlson co-morbidity index, and total energy intake
Trichopoulou, 2005 (6)	European Prospective Into Cancer and Nutrition Elderly EU	>60	7.4	-	74,607	MUFA, PUFA, SFA	Not reported	All-cause mortality	Age, sex, diabetes mellitus at baseline, waist to hip ratio, BMI, educational achievement, smoking status, PA at occupation, PA score at leisure, alcohol intake, and total energy intake

Trichopoulou, 2003 (7)	European Prospective Into Cancer and Nutrition GRE	20-86	3.7	275	22,043	MUFA, PUFA, SFA	per 1 standard deviation increment	All-cause mortality	Age, sex, waist-to-hip ratio, energy expenditure score, years of education, smoking status, BMI, and total energy intake
Xu, 2006 (8)	Strong Heart Study USA	47-79	7.2	138	2,938	Total, MUFA, PUFA, SFA	Not reported	CHD mortality	Age, sex, energy, study centre, diabetes status, BMI, HDL, LDL, triacylglycerol, Smoking, alcohol consumption, hypertension, percentage of energy from protein, and total energy intake
Ascherio, 1996 (9)	United States Health Professionals' Follow-up Study	40-75	6	229	43,757	-	Q5 vs. Q1	40-75 CHD Deaths	Age, energy, BMI, smoking habits, alcohol consumption, physical activity, history of hypertension or high blood cholesterol, family history of MI <60-years, profession, dietary fibre
Boniface, 2002 (10)	United Kingdom	40-75	16	155	2,676	Total, SFA	Q5 vs. Q1	CHD Deaths	Age, alcohol consumption, smoking habits, frequency of exercise, BMI, blood pressure, social class, deprivation index
Goldbourt, 1993 (11)	Israel	40+	23	3473, 1098	11,876	-	Q5 vs. Q1	Total deaths and CHD deaths	Age, presence of initial malignant disease,
Leosdottir 2005 (12)	Sweden Malmo Diet and Cancer Study	≈59	6.6	1250, 339	28,098	Total, MUFA, PUFA, SFA	Q4 vs. Q1	Total and CVD deaths	Age, alcohol, smoking, social class, marital status, physical activity, BMI, fibre intake, monounsaturated and polyunsaturated fats, total fat intake for ratio between unsaturated and saturated fats
Mann, 1997 (13)	United Kingdom	16-79	13.3	64, 392	10,802	SFA	Q3 vs. Q1	CHD deaths, all-cause mortality	Age, sex, smoking habit, social class
McGee, 1985 (14)	United States Honolulu Heart Program	45-60+	10	542; 61, 99	7,088	SFA	≥50 g vs. <10g SFA	Total deaths; stroke deaths; CHD deaths	Age, SBP, BMI, physical activity, cigarettes smoked
Sauvaget, 2004 (15)	Japan Adult Health Study (subcohort of the Life Span Study)	35-89	14	90	3,731	SFA, MUFA, PUFA	Q3 vs Q1	Stroke deaths	Radiation dose, city of exposure, smoking and drinking status, BMI, history of hypertension and diabetes, fruit and vegetable intake, markers of nutritional status, lymphocyte count,

									blood cholesterol level, total energy intake, weight
Tucker, 2005 (16)	United States Baltimore Longitudinal Study of Aging	34-80	18	71	501	SFA	Not reported	CHD deaths	Age at first visit, total energy intake, BMI, smoking, alcohol use, dietary supplements, physical activity
Misirli, 2012 (17)	Greece European Prospective Investigation into Cancer and Nutrition (EPIC Study), Greek-EPIC Cohort	25-67	10.6	196	23,601	MUFA, SFA	12 g/day increments	Stroke deaths	Sex, age, smoking status, BMI, education, physical activity level, energy intake, hypertension, diabetes mellitus, Mediterranean diet score
Trichopoulou, 2006 (18)	Greece European Prospective Investigation into Cancer and Nutrition (EPIC Study), Greek-EPIC Cohort	NR	4.5	80	1013	MUFA, SFA, PUFA	Not reported	CVD deaths	Gender, age, educational level, smoking, waist-to-height, hip circumference, physical activity, metabolic activity task score, total energy intake, treatment with insulin, treatment for hypertension at enrolment, treatment for hypercholesterolaemia at enrolment, flour, flakes, starches, pasta, rice, other grain, bread, crisp bread, rusks, breakfast cereals, biscuit, dough, pastry
Chien, 2013 (19)	Japan (Chin-Shan)	≈60	≈10	568	3,602	SFA	56.3% vs. 45% of total fat	Total deaths	Age, gender, BMI, smoking, drinking, marital status, education level, job and sports activity, hypertension, diabetes, LDL-C and HDL-C
Wakai, 2014 (20)	Japan Collaborative Cohort Study JAP	≈56	19.3	11,656 ; 1,665	58,672	Total, MUFA, PUFA, SFA	7.3 vs. 3.0% E for SFA	Total and CVD death	Age, area, education, smoking, alcohol consumption, BMI, sleep duration, walking, consumption of vegetables and fruit, and total energy intake
Shekelle, 1981 (21)	U.S.A. (Western Electric Study)	40-55	19	215	1,900	SFA	1-unit increase	CHD deaths	Age, SBP, smoking, serum cholesterol, alcohol, BMI, ancestry
Kushi, 1985 (22)	U.S.A.-Ireland (Ireland-Boston Heart Study)	40-60	23	110	1,001	SFA	Top 3rd vs. Bottom 3rd	CHD deaths	Age, cohort, SBP, serum cholesterol, LVH, smoking, alcohol
Dehghan, 2017 (23)	The Prospective Urban Rural Epidemiology	35-70	7.4	5796	135,335	Total, MUFA, PUFA, SFA	Q5 vs. Q1	Total deaths and CVD deaths	Age and sex, education, smoking, physical activity, waist to hip ratio, history of diabetes, urban or rural location, and total energy intake.

Guasch-Ferré, 2015 (24)	PREvención con DIeta MEDiterránea	67	6	414, 336	7,038	Total, MUFA, PUFA, SFA	Q5 vs. Q1	Total deaths and CVD deaths	Age, sex, total energy intake, alcohol intake, fiber, protein intake, BMI, smoking status, educational level, leisure-time physical activity, baseline diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of antihypertensive medication
Wang, 2017 (25)	Nurses' Health Study and the Health Professionals Follow-up Study	-	32 and 26	20314, 33304	83,349	Total, MUFA, PUFA, SFA	Q4 vs. Q1	Total deaths	Age, Caucasian, marital status body-mass index, physical activity, smoking status, alcohol consumption, multivitamin use, vitamin E supplementation use, current aspirin use, family history of myocardial infarction, family history of diabetes, family history of cancer, history of hypertension, history of hypercholesterolemia, intakes of total energy, dietary cholesterol and percentage of energy intake from dietary protein, and menopausal status and hormone use in women.
Zhuang, 2019 (26)	China Health and Nutrition Survey	-	14	1,007	14,117	PUFA	Q4 vs. Q1	Total deaths	Age, gender, BMI, education, marital status, residence, physical activity, smoking, alcohol drinking status, history of hypertension, history of diabetes, intake of total energy, vegetables, fruits, red meat and saturated fat.
Zhuang, 2019 (27)	NIH-AARP Diet and Health Study	50-71	16	129,328	521,120	MUFA, PUFA, SFA	Q4 vs. Q1	CVD, Total, T2D,	Multivariable models were adjusted for age, gender, BMI, race, education, marital status, household income, smoking, alcohol, physical activity, multi-vitamin use, aspirin use, history of hypertension, history of hypercholesterolemia, perceived health condition, history of heart disease, stroke, diabetes, and cancer at baseline, hormones use for women, intake of total energy, percentages of energy intake from protein, and remaining fatty acids where appropriate.

Mazidi, 2019*	United States NHANES; 1999-2010	48.5	12	24,144	6,581	Total, MUFA, PUFA, SFA	Q4 vs. Q1	Total, CHD, stroke, T2D	Age, race, education, marital status, poverty to income ratio, physical activity and smoking, alcohol consumption, dietary cholesterol, body mass index, hypertension, and non-HDL cholesterol

** Mazidi NHANES cohort study from the recent paper.*

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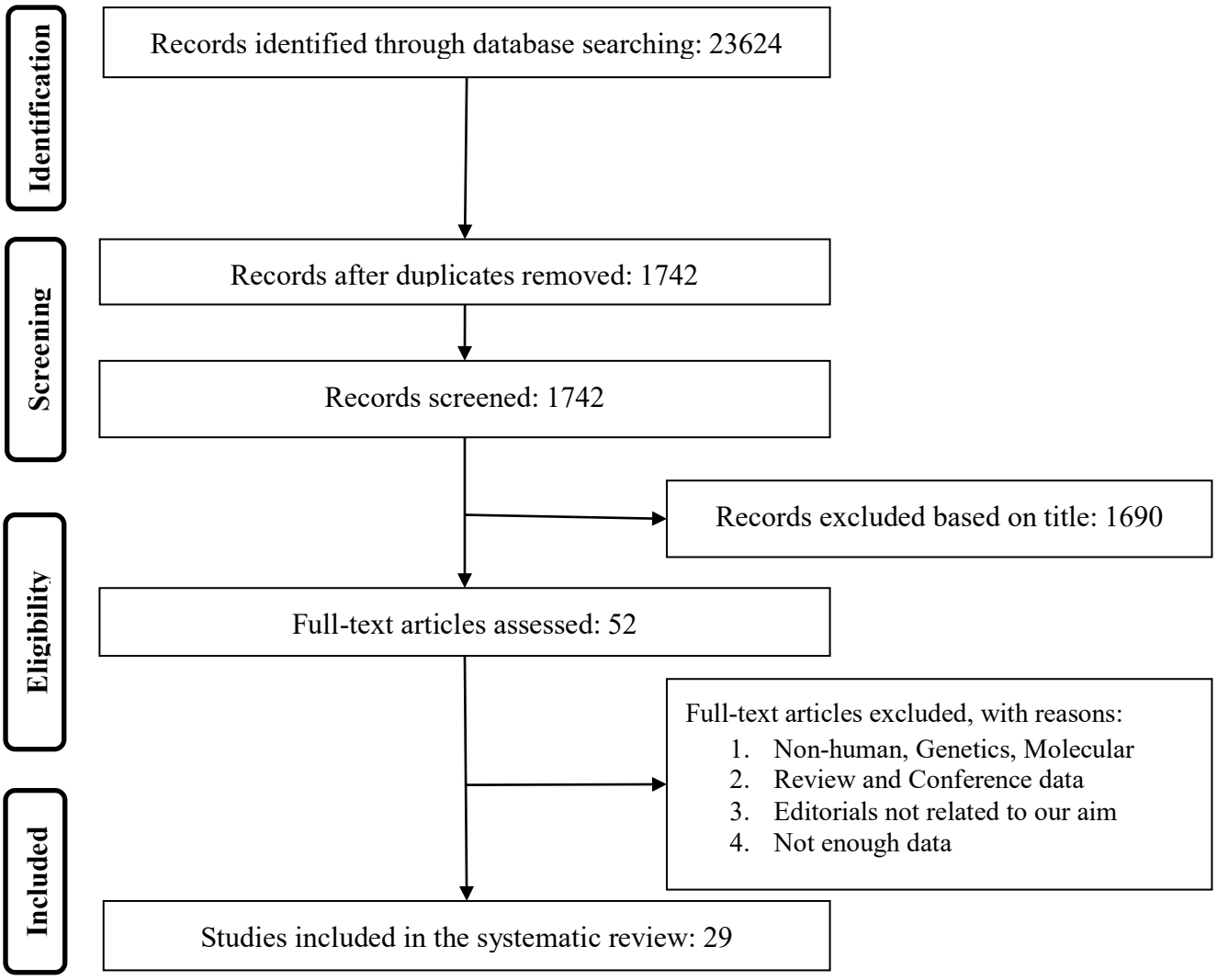
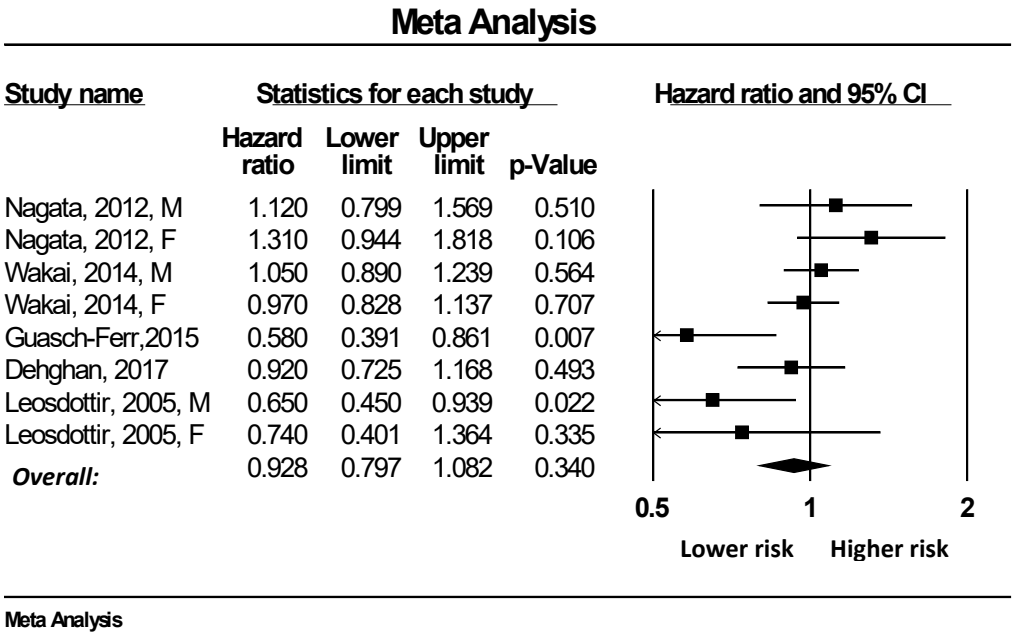
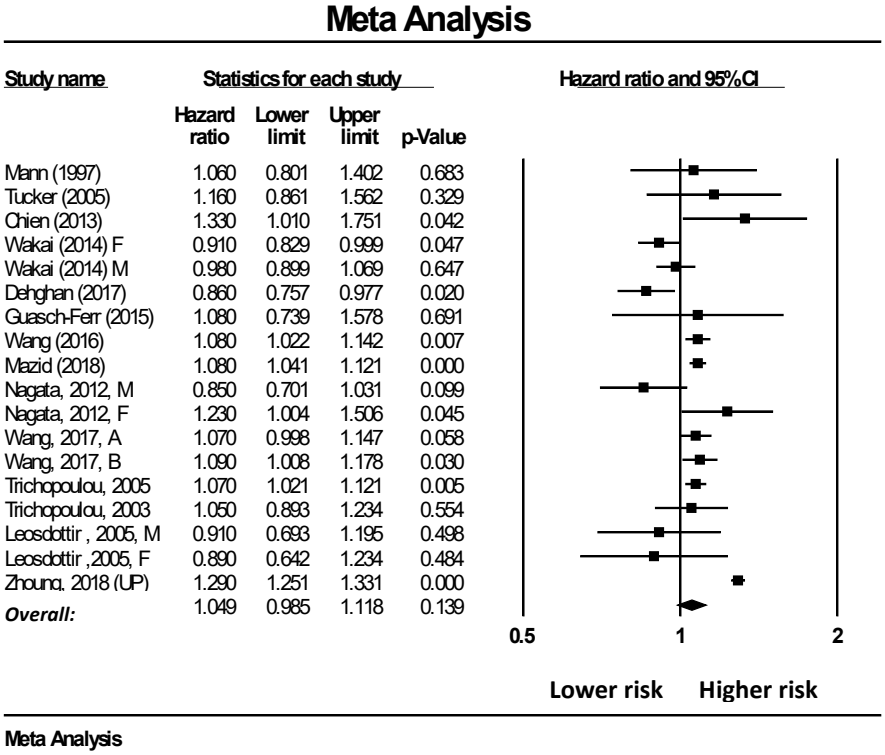
Supplemental Figure 1. Flow chart diagram of studies selection.



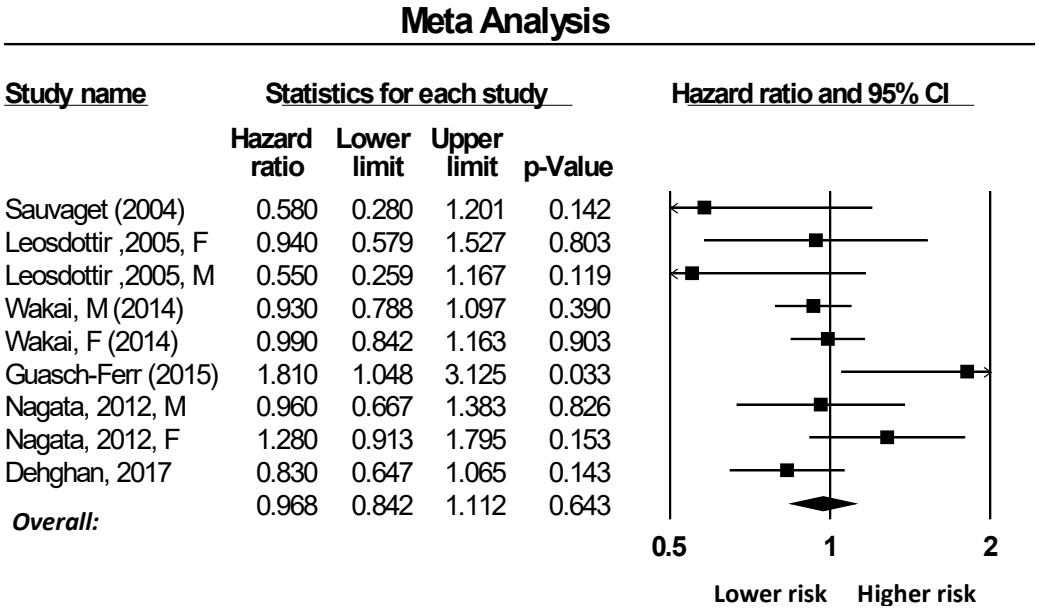
Supplemental Figure 2. Forest plot of total fat consumption and cardiovascular disease mortality.



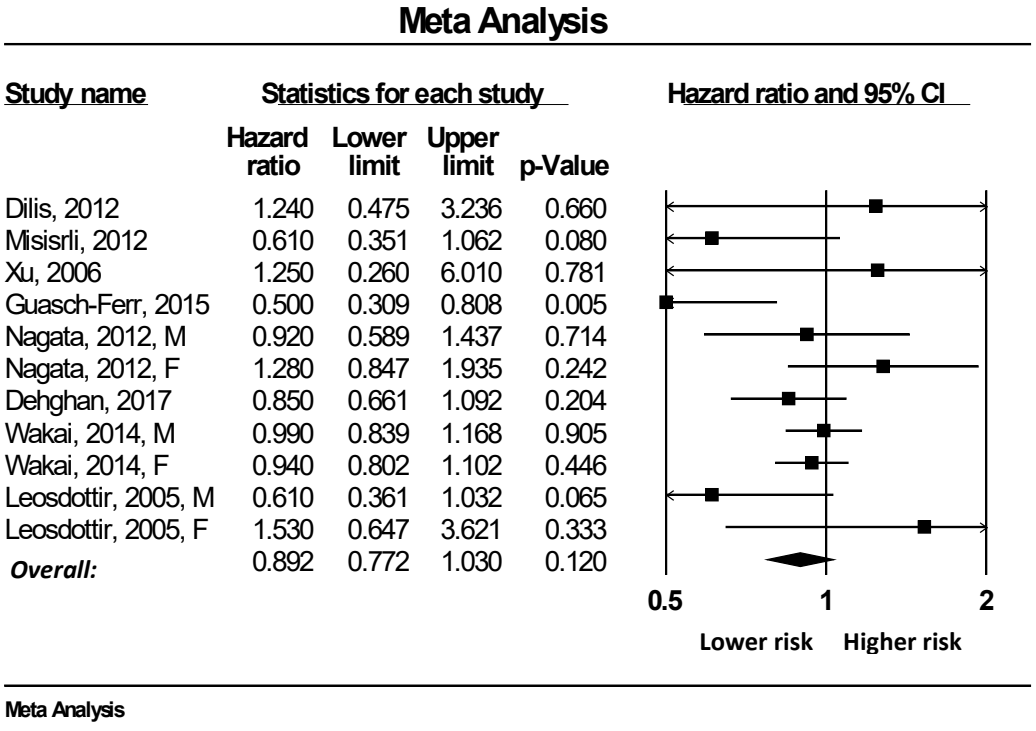
Supplemental Figure 3. Forest plot of saturated fatty acids (SFA) consumption and all-cause mortality.



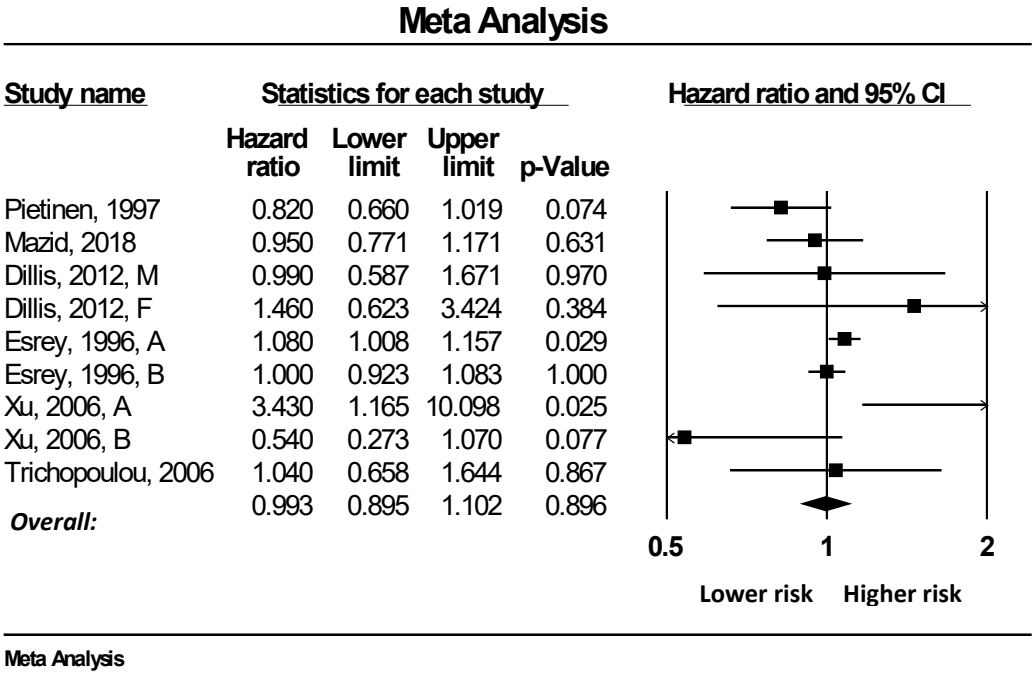
Supplemental Figure 4. Forest plot of saturated fatty acids (SFA) consumption and cardiovascular disease mortality.



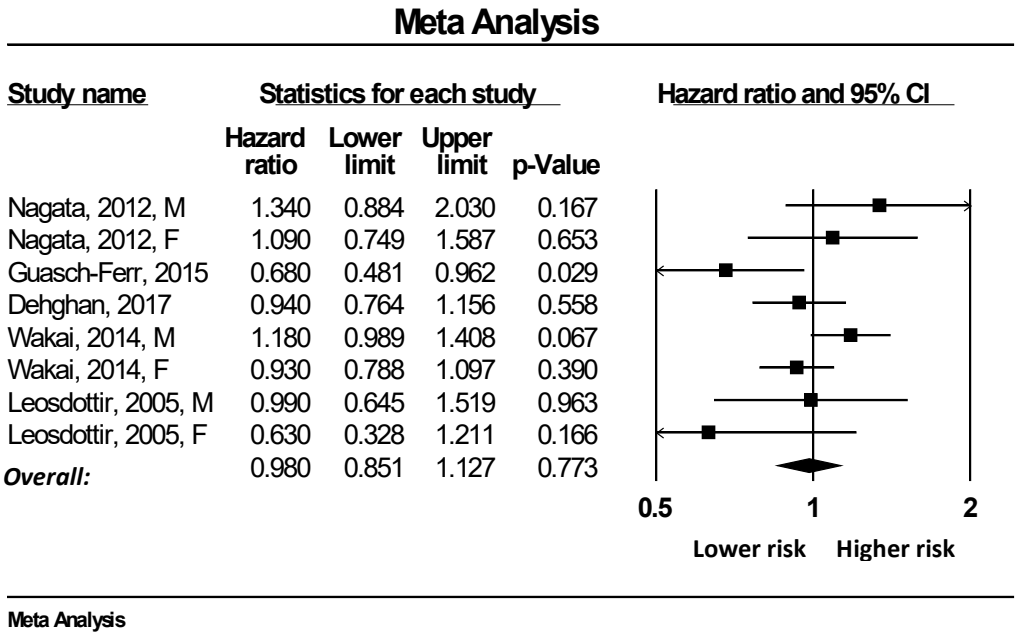
Supplemental Figure 5. Forest plot of monounsaturated fatty acids (MUFA) consumption and cardiovascular disease mortality.



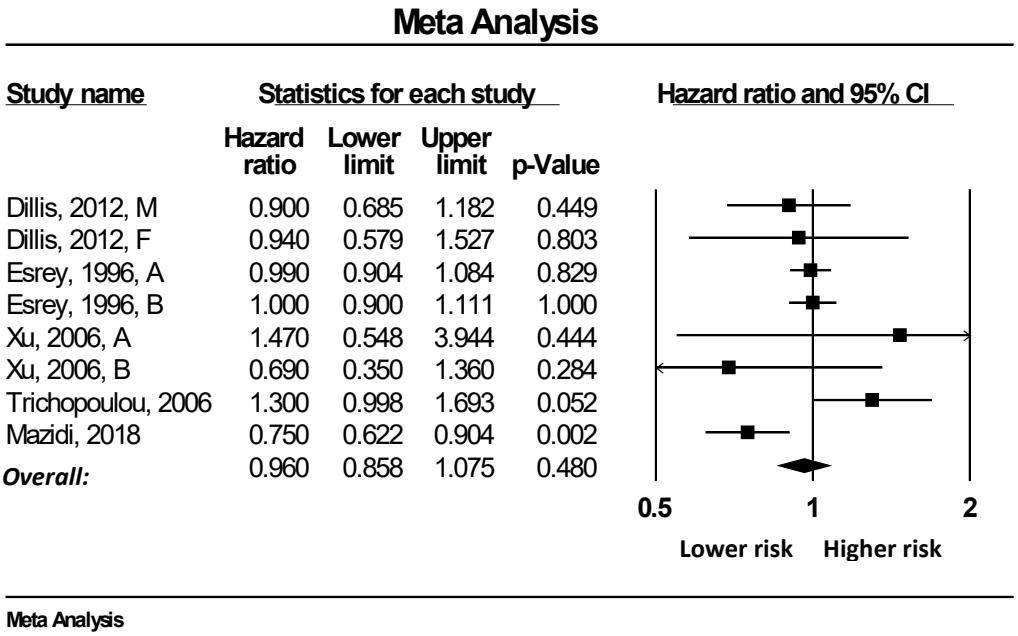
Supplemental Figure 6. Forest plot of monounsaturated fatty acids (MUFA) consumption and coronary heart disease mortality.

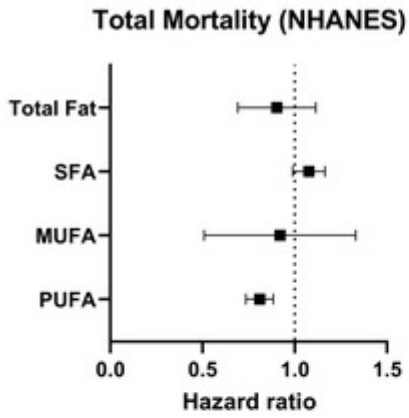


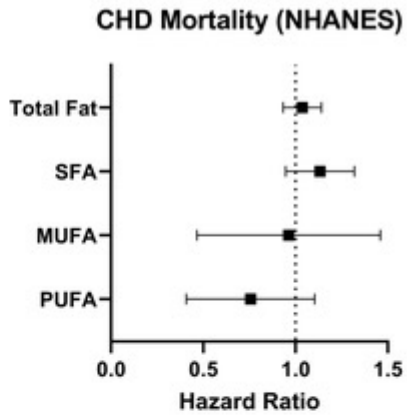
Supplemental Figure 7. Forest plot of polyunsaturated fatty acids (PUFA) consumption and cardiovascular disease mortality.

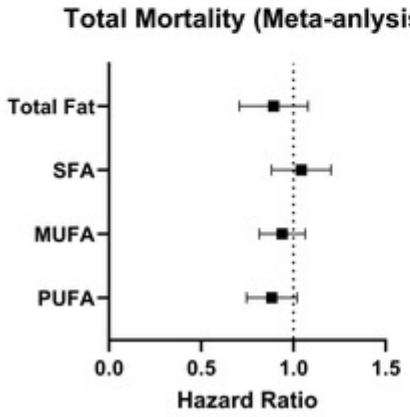


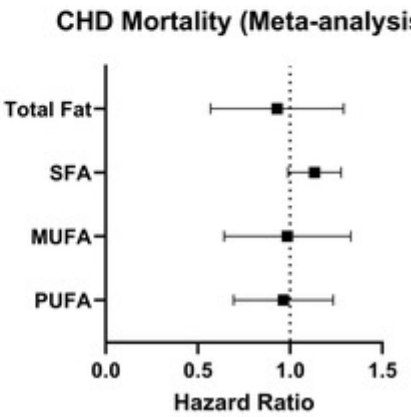
Supplemental Figure 8. Forest plot of polyunsaturated fatty acids (PUFA) consumption and coronary heart disease mortality.




Total Mortality (NHANES)
Total Fat
SFA
MUFA
PUFA
0.0
0.5
1.0
1.5
Hazard ratio


CHD Mortality (NHANES)
Total Fat
SFA
MUFA
PUFA
0.0
0.5
1.0
1.5
Hazard Ratio


Total Mortality (Meta-anlysis)
Total Fat
SFA
MUFA
PUFA
0.0
0.5
1.0
1.5
Hazard Ratio


CHD Mortality (Meta-analysis)
Total Fat
SFA
MUFA
PUFA
0.0
0.5
1.0
1.5
Hazard Ratio