Dietary saturated fat intake and atherosclerotic vascular disease mortality in elderly women: a prospective cohort study¹–³

Lauren C Blekkenhorst, Richard L Prince, Jonathan M Hodgson, Wai H Lim, Kun Zhu, Amanda Devine, Peter L Thompson, and Joshua R Lewis

ABSTRACT

Background: The reduction of saturated fatty acid (SFA) intake has been the basis of long-standing dietary recommendations. However, recent epidemiologic studies have reported conflicting evidence in the relation between SFA consumption and risk of atherosclerotic vascular disease (ASVD) mortality.

Objective: We investigated the association of SFA intake with serum lipid profiles and ASVD mortality in a population-based 10-y cohort study.

Design: At baseline (1998) 1469 women living in Perth, Western Australia, with a mean ± SD age of 75.2 ± 2.7 y had SFA intake measured by using a validated food-frequency questionnaire. Outcome data were serum lipids at baseline and ASVD deaths over 10 y (13,649 person-years of follow-up), retrieved from the Western Australian Data Linkage System. Other risk factors for ASVD were assessed and adjusted for in multivariable analyses.

Results: ASVD deaths occurred in 9.1% (134) of participants. The highest quartile of SFA intake (>31.28 g/d) had an ~16% cumulative mortality risk compared with ~5% in the lowest quartile (<17.39 g/d) (HR: 3.07; 95% CI: 1.54, 6.11; P = 0.001). Baseline SFA intake was associated with baseline serum total and LDL cholesterol in multivariable-adjusted models (β: 0.199, SE: 0.056, P < 0.001 and β: 0.190, SE: 0.051, P < 0.001, respectively). However, baseline serum total and LDL cholesterol were not associated with ASVD mortality.

Conclusions: High SFA intake was associated with the risk of ASVD mortality in this population of elderly women. Although there was a strong positive association between SFA intake and LDL cholesterol, LDL cholesterol was not associated with ASVD mortality in this cohort. Nevertheless, these data support dietary advice to reduce SFA intake.

Keywords: cardiovascular diseases, dietary fats, epidemiologic studies, fatty acids, vascular diseases

INTRODUCTION

Atherosclerotic vascular disease (ASVD)⁴ is a leading cause of morbidity and mortality in both developed and developing countries, contributing to >17.3 million deaths worldwide (1). ASVD is characterized by the presence of atherosclerotic lesions, a progressive inflammatory condition affecting the inner layer of the medium- and large-sized arteries, resulting in widespread vascular endothelial damage (2). Atherosclerosis is a complex disease considered to be affected by multiple factors, including the imbalance between total cholesterol, LDL cholesterol, and HDL cholesterol. When the inner layer of blood vessels is exposed to increased concentrations of LDL cholesterol, the endothelium allows entry of lymphocytes and monocytes, which migrate deeper into the vessel wall, triggering reactions that lead to inflammation and plaque formation (1–3). In contrast, HDL has a protective effect against atherosclerosis by suppressing or reversing LDL accumulation within atherosclerotic plaques, reducing oxidation and cytotoxicity (4). Excess accumulation of LDL has been shown to be associated with a 3.8-fold greater risk of cardiac events in high-risk individuals (5), and multiple primary and secondary randomized controlled trials using LDL-lowering agents have consistently demonstrated reductions in the risk of ASVD events (6–9).

Recommendations concerning dietary SFAs and heart disease were established in the late 1950s (10). The replacement of dietary SFAs with PUFAs and/or MUFAs have been the basis of professional advice for decades (11). Limiting SFAs is recommended to reduce the risk of ASVD mainly due to the effect on blood cholesterol (12). This view resulted from early experimental studies showing SFAs increase serum cholesterol (13, 14) and epidemiologic studies demonstrating associations between serum cholesterol and risk of ASVD mortality (15). However,

¹From the School of Medicine and Pharmacology, University of Western Australia, Queen Elizabeth Medical Centre Unit, Perth, Australia (LCB, RLP, WHL, KZ, and JRL); the Departments of Endocrinology and Diabetes (RLP, KZ, and JRL), Renal Medicine (WHL), and Cardiology (PLT), Sir Charles Gairdner Hospital, Perth, Australia; the School of Medicine and Pharmacology, University Western Australia, Royal Perth Hospital, Perth, Australia (LCB and JMH); and the School of Exercise and Health Science, Edith Cowan University, Perth, Australia (AD).
²Supported by Healthway (the Health Promotion Foundation of Western Australia) for the CAIFOS/CARES study and by project grants 254627, 303169, and 572604 from the National Health and Medical Research Council of Australia.
³Address correspondence to LC Blekkenhorst, School of Medicine and Pharmacology, Royal Perth Hospital Unit (M570), The University of Western Australia, 35 Stirling Highway, Crawley WA 6009 Australia. E-mail: lauren.blekkenhorst@research.uwa.edu.au.
⁴Abbreviations used: ASVD, atherosclerotic vascular disease; FFQ, food-frequency questionnaire; ICD-9-CM, International Classification of Diseases, Ninth Revision, Clinical Modification; ICD-10-AM, International Classification of Diseases, 10th Revision, Australian Modification; IHD, ischemic heart disease.

Received November 2, 2014. Accepted for publication March 30, 2015. First published online May 6, 2015. doi: 10.3945/ajcn.114.102392.
many recent epidemiologic studies have failed to demonstrate a definite association of SFA intake with serum cholesterol and risk of ASVD (16, 17).

As a result of these more recent concerns, a 10-y prospective study sought to investigate the association of SFA intake with serum cholesterol concentrations at baseline and the risk of 10-y ASVD mortality in a cohort of elderly women to explore the hypothesis that SFA intake would be associated with serum cholesterol concentrations and subsequently a higher risk of ASVD mortality.

METHODS

Participants

Participants of this study (n = 1500) were recruited at random by using the Australian electoral roll in 1998 to a 5-y randomized, double-blinded, placebo-controlled calcium intervention trial of 1.2 g elemental calcium in the form of 2 tablets of calcium carbonate taken every day with meals in the morning and afternoon or an identical placebo. Participants were ambulant and were included on the basis of absence of disease considered likely to impair a 5-y survival by the participant. This resulted in 175 of 1469 (11.9%) of participants with prevalent ASVD being included in the study. The study took place in Perth, Western Australia, and was approved by the Human Rights Committee of the University of Western Australia. Participants were then followed for an additional 5-y observational extension study.

Dietary saturated fat intake

Dietary intake was assessed at baseline by using a self-administered semiquantitative food-frequency questionnaire (FFQ). This validated FFQ measures the usual frequency of food intake over a period of 12 mo and comprises a list of 74 items with 10 frequency response options ranging from “never” to “3 or more times per day” (18, 19). The questionnaire calculates portion size by using 3 photographs of scaled portions for 4 different food types. Nutrient intake calculations were analyzed by the Cancer Council Victoria by using the NUTTAB95 food composition database (20) and were supplemented by other data where necessary. The process of collection was identical, whereby a research assistant supervised the completion of the questionnaire in small groups. Food models, cups, spoons, and charts were provided for accuracy. A total of 1485 participants completed a FFQ, and after excluding implausible scores (n = 16) for total energy intake (<2092 kJ (500 kcal/d) or >14,644 kJ (3500 kcal/d)), 1469 of 1500 (97.7%) participants were available to be included in the analysis.

Baseline ASVD risk assessment

A comprehensive medical history and list of medications were obtained from all participants, and the accuracy was verified by participants’ general practitioners where possible. These data were coded by using the International Classification of Primary Care–Plus method (21). This coding method allows aggregation of different terms for similar pathologic entities as defined by the International Classification of Diseases, 10th Revision coding system. These data were then used to determine the presence of preexisting diabetes (T89001–90009). Cardiovascular medications included statins, low-dose aspirin, and antihypertensive medication. Smoking status was coded as nonsmoker or ex-smoker/current smoker if they had consumed more than 1 cigarette/d for more than 3 mo at any time in their life. Body weight (kg) was measured by using electronic scales (August Sauter GmbH) to the nearest 0.1 kg with participants wearing light clothes and no shoes. Height (m) was measured by using a wall-mounted stadiometer (Holttain Limited) to the nearest 0.1 cm without socks or shoes. From height and weight measurements, BMI was calculated as weight (kg)/height (m)2. Amount of physical activity was assessed at baseline by using a questionnaire (22, 23). Activity amount was calculated in kJ/d with a validated method using body weight, number of hours and type of physical activity, and energy costs of such activities (24, 25).

Prevalent ASVD at baseline was determined retrospectively from hospital discharge data (1980–1998) defined by using di- agnosis codes from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) (26). These codes included ischemic heart disease (ICD-9-CM codes 410–414); cerebrovascular disease, excluding hemorrhage (ICD-9-CM codes 433–438); heart failure (ICD-9-CM code 428); and peripheral arterial disease (ICD-9-CM codes 440–444).

Biochemical assessments

Serum lipid profiles were obtained at baseline (1998). Total cholesterol, HDL cholesterol, and triglyceride concentrations were measured in 1050 of 1469 (71.5%) participants by using a Hitachi 917 auto analyzer (Roche Diagnostics GmbH). LDL cholesterol was calculated on 1042 of 1469 (70.9%) participants by using the Friedewald method (27). Baseline creatinine was measured in 1328 of 1469 (90.4%) participants by using an isotope dilution mass spectrometry traceable Jaffe kinetic assay on a Hitachi 917 analyzer (Roche Diagnostics GmbH). Estimated glomerular filtration rate using creatinine was calculated with the Chronic Kidney Disease EPIdemiology equation (28) and was added to the multivariable-adjusted models because this has been shown to predict ASVD in this cohort (29).

ASVD mortality

The primary outcome was an ASVD death. ASVD-coded deaths were retrieved from the Western Australian Data Linkage System for each study participant between 1998 and 2008, inclusive. Complete follow-up death records were available for all participants. ASVD deaths were defined by using the primary diagnosis codes from the ICD-9-CM (26) and the International Classification of Diseases, 10th Revision, Australian Modification (ICD-10-AM) (30). Primary diagnosis codes included ischemic heart disease (ICD-9-CM codes 410–414 and ICD-10-AM codes I20–I25); cerebrovascular disease, excluding hemorrhage (ICD-9-CM codes 433–438 and ICD-10-AM codes I63–I69, G45.9); heart failure (ICD-9-CM code 428 and ICD-10-AM code I50); and peripheral arterial disease (ICD-9-CM codes 440–444 and ICD-10-AM codes I70–I74).

Statistical analyses

The analysis plan included assessment of preexisting baseline risk factors and medication use. Descriptive statistics of continuous variables were expressed as mean ± SD and categorical
variables as number and proportion (%). Nonnormally distributed variables, including physical activity, were expressed as median and interquartile range. Serum lipid concentrations were compared with SFA intake (per SD) by using unadjusted, age- and energy-adjusted, and multivariable-adjusted (age, BMI, physical activity, prevalent diabetes, statin medication, and energy intake) linear regression models. Time to participants’ death was calculated in days from baseline and then divided into months to obtain time to death up to ±10 y after each participant’s baseline visit. Cox regression was selected for the analysis with the primary outcome being the HR of time to ASVD death, adjusting for potential ASVD risk factors (age, BMI, physical activity, renal function, smoking history, prevalent diabetes, prevalent ASVD, low-dose aspirin medication, antihypertensive medication, statin medication, and energy intake). Covariates were entered into the model as continuous variables with the exception of smoking history, prevalent diabetes, prevalent ASVD, low-dose aspirin medication, antihypertensive medication, and statin medication, which were entered as categorical (yes/no) variables. Forward stepwise Cox regression was used to yield the best predictive model with potential ASVD and dietary confounders. The confounding variables included were age (per SD), BMI (per SD), physical activity (per SD), renal function (per SD), smoking history (yes/no), prevalent diabetes (yes/no), prevalent ASVD (yes/no), low-dose aspirin medication (yes/no), antihypertensive medication (yes/no), statin medication (yes/no), energy intake (per SD), dietary cholesterol (per SD), SFAs (per SD), MUFAs (per SD), PUFAs (per SD), and fiber intake (per SD). To investigate a “dose” relation between dietary SFAs and ASVD death, quartiles of SFA intake were calculated and tested in the multivariable-adjusted model, including age, BMI, physical activity, renal function, smoking history, prevalent diabetes, prevalent ASVD, low-dose aspirin medication, antihypertensive medication, statin medication, and energy intake. To assess the extent of reverse causality bias, we excluded all ASVD deaths that occurred within the first 24 mo and then reanalyzed the data. Clinical significance was set at a 2-tailed $P < 0.05$. All data were analyzed by using IBM SPSS Statistics Version 21 (SPSS, Inc.).

### RESULTS

#### Characteristics of study participants

Table 1 outlines the baseline demographic and cardiovascular disease risk factor characteristics of the 1469 participants demonstrating the expected high prevalence of ASVD risk factors in this population. A total of 636 (43.3%) participants were taking low-dose aspirin and 268 (18.2%) were taking statins. Mean total serum cholesterol was 5.9 mmol/L, 3.7 mmol/L, and 1.4 mmol/L, respectively.

#### Serum lipid concentrations

The baseline cross-sectional associations between SFA intake and serum lipid concentrations are shown in Table 2. For every 1 SD (11.26 g/d) higher intake of SFAs, total cholesterol and LDL cholesterol concentrations were 5.9 mmol/L, 3.7 mmol/L, and 1.4 mmol/L, respectively.
cholesterol were 0.29 mmol/L and 0.28 mmol/L higher, respectively (age- and energy-adjusted models). After adjusting for age, BMI, physical activity, prevalent diabetes, statin medication, and energy intake, results were slightly lower ($\beta$: 0.199, SE: 0.056, $P < 0.001$ and $\beta$: 0.190, SE: 0.051, $P < 0.001$, respectively). SFA intake was not associated with serum HDL cholesterol or triglycerides in unadjusted and adjusted models.

**ASVD mortality**

During 13,649 person-years of follow-up, 134 of 1469 (9.1%) participants died of ASVD. For every 1 SD (11.26 g/d) higher intake of SFAs, there was a 77% higher risk of 10-y ASVD death (multivariable-adjusted HR: 1.77; 95% CI: 1.31, 2.37; $P < 0.001$).

To evaluate the most predictive model with potential ASVD and dietary confounders, we used a forward stepwise Cox regression analysis (Table 3). The analysis identified increases in age, higher intake of SFAs and prevalent ASVD, and low-dose aspirin and antihypertensive therapy as predictors of increased ASVD death risk. The analysis also identified higher intake of MUFAs as a predictor of reduced risk by $\sim 50\%$ per SD increase (8.7 g/d). Using a backward stepwise Cox regression model did not affect these results.

To further explore a dose relation between SFAs and ASVD mortality, we conducted a quartile analysis, as shown in Figure 1. Survival was worst in the highest quartile of SFA intake (>31.28 g/d) compared with the lowest (<17.39 g/d) (multivariable-adjusted HR: 3.07; 95% CI: 1.54, 6.11; $P = 0.001$). To assess the extent of reverse causality bias, we excluded all ASVD deaths that occurred within the first 24 mo. The highest quartile of SFA intake (>31.28 g/d) compared with the lowest quartile (<17.39 g/d) remained statistically significant (multivariable-adjusted HR: 2.96; 95% CI: 1.44, 6.08; $P = 0.003$).

**Sensitivity analysis**

The primary proposed pathway linking SFA intake with ASVD is via total and LDL cholesterol concentrations. Therefore, we investigated the relation of total and LDL cholesterol with ASVD mortality. Total cholesterol (per SD) was not related to ASVD mortality in the multivariable-adjusted Cox regression model with age, BMI, physical activity, renal function, smoking history, prevalent diabetes, prevalent ASVD, low-dose aspirin medication, antihypertensive medication, statin medication, and energy intake. ASVD, atherosclerotic vascular disease.

**TABLE 3**

| Most parsimonious model for prediction of atherosclerotic vascular disease mortality in elderly women$^1$ |
|-----------------|-----------------|--------------|
| All participants | HR (95% CI) | $P$ value |
| Age (per SD) | 1.35 (1.13, 1.61) | 0.001 |
| Prevalent ASVD | 1.95 (1.25, 3.04) | 0.003 |
| Low-dose aspirin medication | 1.76 (1.18, 2.63) | 0.006 |
| Antihypertensive medication | 1.55 (1.08, 2.22) | 0.016 |
| SFAs (per SD) | 2.12 (1.57, 2.85) | <0.001 |
| MUFAs (per SD) | 0.50 (0.36, 0.69) | <0.001 |

$^1$Results are presented as HRs (95% CIs) using forward stepwise Cox regression analysis. Baseline variables included were age (per SD), BMI (per SD), physical activity (per SD), renal function (per SD), smoking history, prevalent diabetes, prevalent ASVD, low-dose aspirin medication, antihypertensive medication, statin medication, energy intake (per SD), dietary cholesterol (per SD), SFAs (per SD), MUFAs (per SD), PUFAs (per SD), and fiber intake (per SD). ASVD, atherosclerotic vascular disease.

**FIGURE 1** Survival outcomes for quartiles of SFA intake. Cox regression analysis for quartiles of SFAs (g/d) and 10-y ASVD deaths ($n = 134$) for quartile 1 (black dashed line: <17.39 g/d referent category), quartile 2 (solid light gray line: 17.39–23.40 g/d; HR: 1.28; 95% CI: 0.74, 2.21; $P = 0.370$), quartile 3 (solid dark gray line: 23.41–31.28 g/d; HR: 1.35; 95% CI: 0.74, 2.45; $P = 0.327$), and quartile 4 (solid black line: >31.28 g/d; HR: 3.07; 95% CI: 1.54, 6.11; $P = 0.001$). Multivariable-adjusted model includes age, BMI, physical activity, renal function, smoking history, prevalent diabetes, prevalent ASVD, low-dose aspirin medication, antihypertensive medication, statin medication, and energy intake. ASVD, atherosclerotic vascular disease.

**DISCUSSION**

In this study population, for every $\sim 11$ g/d higher intake of SFAs, there was a 77% greater risk of ASVD mortality after adjusting for other cardiovascular disease risk factors. Participants consuming the highest SFA intakes (>31.28 g/d) were at the highest risk, with a $\sim 3$-fold increase in risk of ASVD death over the subsequent 10 y.

The positive association between SFA intake and risk of ASVD mortality in this cohort of elderly women supports epidemiologic studies in younger female cohorts <$75$ y (31–35) but not others (17, 36–38). A recent systematic review and meta-analysis summarizing prospective observational and randomized controlled trials of the relation between dietary fatty acid intakes and ischemic heart disease (IHD) concluded that current evidence does not support dietary recommendations to decrease SFA intake and increase PUFA intake (39). These conflicting results may be due to limitations inherent in epidemiologic studies, including diet and lifestyle change throughout the study duration, inaccuracy of results produced from nutritional assessment tools, cohorts with differing cardiovascular risks, incomplete adjustments for confounding variables, and inaccurate assessment of outcome data such as the inclusion of cerebral hemorrhage, which is not related to the disease process of interest.

To address some of these concerns, we compared nutritional intakes at 5 and 7 y by using the same FFQ (40). SFA intake (g/d) over 7 y was relatively stable, varying by $\sim 4.2\%$ and only
increasing slightly as a proportion of total energy intake (~0.7%). Second, while accepting that FFQs have general limitations in the ability to measure diet accurately, in this study, the completion of FFQs was supervised in small groups, and the use of food models, cups, spoons, and charts was provided for accuracy of portion size estimates. Third, comprehensive cardiovascular and dietary confounding variables were assessed at baseline and were included in the analyses for ASVD mortality. Last, there was complete ascertainment of ASVD-related mortality data for all participants by using the comprehensive morbidity data systems available in Western Australia (41). This included the exclusion of non-atherosclerotic causes of death such as cerebral hemorrhage.

It has been hypothesized that there is a relation between fatty acid balance and IHD outcomes (42). In this study, there was indeed evidence of a ~50% lower risk of ASVD mortality with higher intakes of MUFAs in addition to the adverse association of SFAs. Studies investigating the replacement of dietary SFA intake with unsaturated fatty acids have been widely reported (42, 43). In particular, the Nurses’ Health Study demonstrated a ~30% reduction in the risk of IHD over 14 y of follow-up when replacing 5% of energy from SFAs with 5% of energy from MUFAs (31). In addition to MUFAs being a predictor for lower ASVD mortality risk in this cohort of elderly women, low-dose aspirin and antihypertensive therapy were predictors for higher ASVD mortality risk. This may be a result of these medications being used in high-risk individuals in whom the medications did not completely reverse the risk.

In this cohort of elderly women, an SFA intake of ~11 g/d was positively associated with total and LDL cholesterol concentrations, 0.29 mmol/L and 0.28 mmol/L, respectively. These findings support intervention studies that demonstrate reducing SFA intake lowers total and LDL cholesterol concentrations, due in large part to the decrease in LDL cholesterol (44–46). A decrease in total and LDL cholesterol is thought to lower the risk of ASVD mortality, but the importance of total and LDL cholesterol as a cardiovascular disease risk factor in older people is uncertain (47).

The relation of SFA intake with ASVD mortality was further investigated by examining the relation between baseline total and LDL cholesterol concentrations and ASVD mortality. Similar to some studies (33, 49) but not others (48, 49), neither total nor LDL cholesterol concentrations were related to ASVD mortality. A review of the relation between serum cholesterol and all-cause mortality in older people (~80 y) using both observational studies and randomized controlled trials also concluded that the relation between total cholesterol and CVD mortality varied considerably (50). These data suggest that total and LDL cholesterol concentrations may not be wholly involved in ASVD mortality. Other possibilities, which include the oxidation of LDL cholesterol and particle size of LDL, have been associated with inflammation of the inner layer of arteries (51). A systematic review of SFA intake and inflammatory markers suggests a potential positive association between SFA intake and high-sensitivity C-reactive protein (52), a predictor of incident IHD (53).

The observational nature of this study is a limitation in identifying a causal relation, and although comprehensive cardiovascular and dietary confounders were available for adjustment, residual confounding may still occur. The findings of this study are also limited to elderly women; therefore, caution should be exercised in generalizations to other populations.

In conclusion, this study demonstrated a positive association between baseline SFA intake and baseline total and LDL cholesterol concentrations, as well as a positive association between baseline SFA intake and 10-y ASVD mortality, which was most evident in intakes of SFAs >31 g/d and an association independent of the relation between SFA intake and cholesterol concentrations. These data support the hypothesis that in elderly women, high SFA intake is associated with an increased risk of 10-y ASVD mortality and supports current advice to lower SFA intake. Further investigation is needed to elucidate the mechanism whereby SFAs increase the risk of ASVD mortality in this elderly population.

The authors’ responsibilities were as follows—LCB, RLP, and JRL: study concept and design, analysis and interpretation of data, drafting of the manuscript, statistical analysis, and study supervision; LCB, RLP, WHL, KZ, AD, and JRL: acquisition of data; LCB, RLP, WHL, JMH, WHL, KZ, AD, PLT, and JRL: critical revision of the manuscript for important intellectual content; RLP, WHL, KZ, AD, and JRL: funding acquisition; and all authors: full responsibility for the integrity of the data in the study. The salary of JMH is supported by a National Health and Medical Research Council Senior Research Fellowship. The salary of JRL is supported by a Raine Medical Research Foundation Priming Grant. Neither of these funding agencies had any role in the conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. The authors reported no conflicts of interest.


