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# Serum Fatty Acid and Risk of Coronary Artery Disease
## — Circulatory Risk in Communities Study (CIRCS) —

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***Background:*** Few prospective studies have explored the association between fatty acids (FA) and risk of CAD. Understanding of the role of each individual serum FA as a coronary risk or protective factor is still limited. The aim was to investigate which serum FA are associated with the incidence of CAD in Japanese subjects.

***Methods and Results:*** A prospective nested case-control study of 40–85-year-old Japanese subjects was undertaken using frozen serum samples collected from 12,840 participants who participated in cardiovascular risk surveys from 1984 to 1998 for 1 community and 1989–1997 for 2 other communities. Three control subjects per case were matched by sex, age, community, year of serum storage and fasting status. By 2005 we had identified 152 incident cases of CAD. Mean n-3-polyunsaturated and saturated FA did not differ between cases and controls, while mean n-6-polyunsaturated FA was higher in controls compared with cases. The multivariable OR of CAD for the highest vs. lowest quartiles of miristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), and linoleic acid (18:2) were 2.8 (95% CI: 1.5–5.2), 2.7 (95% CI: 1.4–5.5), 3.2 (95% CI: 1.7–6.1) and 0.4 (95% CI: 0.2–0.7), respectively.

***Conclusions:*** High serum miristic acid, palmitic acid and palmitoleic acid have an adverse effect, and high serum linoleic acid had a protective effect, on the risk of CAD.

**Key Words:** Coronary artery disease; Follow-up study; Risk; Serum fatty acid

Accumulated evidence suggests that dietary intake of saturated fatty acids may increase the risk of cardiovascular disease,[1,2] whereas polyunsaturated fatty acids are cardioprotective.[3,4] Conversely, the association between monounsaturated fatty acid intake and risk of cardiovascular disease has been inconclusive. Recent review of evidence from systematic reviews and meta-analyses indicated that only several meta-analyses found a benefit in a diet rich in monounsaturated fatty acids on cardiovascular risk, but most meta-analyses failed to find any significant association.[5] Most of the previous studies focused on the effect of classification groups or specific fatty acids,[2,5–15] while relatively few studies have examined the effects of each individual biomarker of fatty acid intake on the risk of coronary artery disease (CAD).[16,17] Most of the aforementioned studies assessed fatty acid intake using a survey questionnaire,[2,5–12,14,15] while 3 studies measured serum or plasma fatty acids using gas-liquid chromatography.[13,16,17] Although fatty acids in blood and adipose tissue have been used as a gold standard to validate dietary polyunsaturated fatty acids, biomarkers of mono-unsaturated and saturated fatty acids are not reflected by fatty acid intake.[18,19]

Each individual fatty acid composition in the same classification group or within classification groups can cause different effects with regard to risk of CAD. A diet high in stearic acid lowered low-density lipoprotein cholesterol (LDL-C) when compared with other saturated fatty acids (e.g., palmitic acid), but tended to increase LDL-C when compared with unsaturated fatty acids.[9] Furthermore, a substitution of 5% energy intake from linoleic acid for dietary saturated fat intake was associated with a decreased

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risk of CAD by 9% (relative risk [RR], 0.91; 95% CI: 0.86–0.96) and decreased mortality from CAD by 13% (RR, 0.87; 95% CI: 0.82–0.94) in a meta-analysis of 8 cohort studies.[12] Studies conducted in Japan showed an inverse association of n-3 polyunsaturated fatty acids with CAD events[14] and cardiovascular mortality.[15] No prospective study, however, has been carried out to explore the effect of individual biomarkers of fatty acid intake on risk of CAD in Japanese subjects. We conducted a prospective nested-control study in 3 Japanese communities using stored serum samples to examine the role of each individual serum fatty acid on the risk of CAD in Japanese men and women.

## Methods

The present study was an ancillary study to the Circulatory Risk in Communities Study (CIRCS).[20] The CIRCS is a dynamic cohort of Japanese men and women aged ≥30 years in 5 communities across Japan, conducted by a research team of the Osaka Center for Cancer and Cardiovascular Disease Prevention, Osaka University and the University of Tsukuba. The surveyed populations in the present study consisted of 12,840 participants (5,382 men and 7,458 women) 40–85 years of age who participated in cardiovascular risk surveys between 1984 and 1998 in Kyowa (a mid-eastern rural community), and between 1989 and 1997 in Ikawa (a northeast rural community) and Noichi (a southwest rural community). The participants of Kyowa, Ikawa and Noichi aged 40–85 years of age numbered 6,518, 2,570 and 3,752, respectively. The census populations of Kyowa, Ikawa and Noichi for those aged 40–85 years were 8,557, 29,815, and 7,169, respectively. The participation rate in cardiovascular risk surveys for men and women 40–85 years of age was 76% in Kyowa, 86% in Ikawa, and 54% in Noichi, and 69% for the total population. A 1.0–2.0-mL serum sample obtained from each participant was stored at −80°C for 1–21 years (median, 11.0 years). Participants with a history of stroke or CAD (n=478) were excluded from analysis. The participants were followed to determine the incidence of CAD occurring by the end of 2005. Informed consent was obtained from community leaders and, verbally, from individual participants according to the guidelines of the Council for International Organizations of Medical Science.[21] The Ethics Committees of the Osaka Center for Cancer and Cardiovascular Disease Prevention and University of Tsukuba approved this study.

All potential cases of CAD were extracted from the national insurance claims, ambulance records, death certificates (as the underlying cause of death; ICD 9 classification, 410–414, 428, 429), reports by local physicians, and reports by public health nurses and health volunteers. To confirm the diagnosis of CAD, we called, visited or invited the susceptible subjects to participate in annual cardiovascular risk surveys in order to obtain clinical histories. For non-fatal cases, study physicians obtained medical histories and reviewed medical records from local clinics and hospitals. For almost all fatal cases, information was obtained from their families, and medical records were reviewed.

The criteria for CAD were confirmed in the medical records according to the modified CAD criteria proposed by the World Health Organization Expert Committee.[22] Definite myocardial infarction (MI) was indicated by typical chest pain, lasting ≥30 min with the appearance of abnormal and persistent Q or QS waves on electrocardiogram (ECG), or changes in cardiac enzyme activity or both. Probable MI was indicated by typical chest pain in which ECG or enzyme activity were not available. Angina pectoris was defined as repeated episodes of chest pain during effort, especially when walking, usually disappearing rapidly after the cessation of effort or with the use of sublingual nitroglycerin. Sudden cardiac death was defined as death ≤1 h after onset, a witnessed cardiac arrest, or abrupt collapse not preceded by >1 h of symptoms. CAD included definite or probable MI, angina pectoris, and sudden cardiac death.

For each new case of CAD, 3 control subjects were selected randomly from the participants with no incidence of CAD, matched for sex, age (±2 years), community, year of serum storage, and fasting status at serum collection (<8 and ≥8 h).

Non-fasting venous blood was collected in a 7–10-mL plain tube and allowed to stand for <30 min for serum separation. The serum samples were aliquoted immediately and placed on dry ice at survey sites and then stored at −80°C until analysis.

Lipids were extracted from the stored serum with chloroform and methanol and were saponified with potassium hydroxide and ethanol. Fatty acids were transesterified with the Boron Trifluoride-methanol, and the methyl esters were analyzed in a gas chromatograph with a 3-m glass column with 3-mm internal diameter. An injection temperature of 250°C, a column temperature of 220°C, and a column flow of 40 m/min of nitrogen were used. Peaks were determined using a flame ionization detector and were quantified with an electronic integrator. Composition of individual serum fatty acids was expressed as a percentage of the total area of 13 major fatty acid peaks from 14:0 to 22:6.

An interview was conducted to ascertain history of cigarette smoking (never, ex, current), ethanol intake (never, ex, and current: <46 g/day ethanol, and ≥46 g/day ethanol), and medication use for hypertension and diabetes. Height in stocking feet and weight in light clothing were measured. Body mass index (BMI) was calculated as weight (kg)/height ($m^2$).

Systolic and diastolic blood pressure (SBP and DBP) were measured using a standard mercury sphygmomanometer by trained observers. Blood pressure was measured on the right arm of seated participants after a 5-min rest. Hypertension was defined as SBP ≥160 mmHg and/or DBP ≥95 mmHg and/or use of antihypertensive medication; normotension was defined as SBP <140 mmHg and DBP <90 mmHg and not taking antihypertensive medication. All others were classified as having borderline hypertension.

Serum total cholesterol was measured using an enzymatic method and serum triglycerides were measured on fluorometry at the Osaka Medical Center for Health Science and Promotion, an international member of the US National Cholesterol Reference Method Laboratory Network. This laboratory has been standardized by the Lipid Standardization Program, conducted by the Centers for Disease Control (Atlanta, GA, USA), and has successfully met the criteria for both reproducibility and accuracy of cholesterol and triglycerides measurements.[23,24] Serum glucose was measured using the hexokinase method. Impaired glucose tolerance was defined as fasting glucose 6.1–6.9 mmol/L and/or non-fasting glucose 7.8–11.0 mmol/L,

**Table 1. Unadjusted Baseline Characteristics vs. Presence of CAD**

| | Total | | Men | | Women | |
|---|---|---|---|---|---|---|
| | CAD cases | Controls | CAD cases | Controls | CAD cases | Controls |
| n | 152 | 456 | 93 | 279 | 59 | 177 |
| Age (years) | 66.3 | 66.2 | 64.2 | 64.1 | 69.5 | 69.6 |
| SBP (mmHg) | 139 | 137 | 140* | 136 | 136 | 137 |
| DBP (mmHg) | 79 | 79 | 81 | 81 | 74 | 76 |
| Hypertension | 46 | 41 | 44 | 35 | 51 | 49 |
| BMI (kg/m$^2$) | 23.3 | 23.6 | 23.1 | 23.4 | 23.6 | 23.9 |
| Ethanol intake (g/day) | 11.7 | 13.7 | 20.6 | 22.0 | 0.19 | 0.64 |
| Current smokers | 35 | 31 | 54 | 48 | 5 | 5 |
| Serum cholesterol (mmol/L) | 5.34* | 5.17 | 5.20* | 4.96 | 5.56 | 5.49 |
| Triglycerides (mmol/L) | 1.60* | 1.44 | 1.58 | 1.44 | 1.62 | 1.43 |
| Impaired glucose tolerance | 15 | 14 | 18 | 18 | 9 | 8 |
| Diabetes mellitus | 15*** | 7 | 13 | 8 | 19*** | 4 |
| Saturated FA (mean %) | 34.47 | 33.85 | 34.84 | 34.43 | 33.89 | 32.95 |
| Miristic (14:0) | 1.26* | 1.16 | 1.26 | 1.19 | 1.27* | 1.13 |
| Palmitic (16:0) | 25.33* | 24.77 | 26.32 | 25.65 | 24.74 | 23.96 |
| Stearic (18:0) | 7.87 | 7.92 | 7.88 | 7.96 | 7.87 | 7.86 |
| Monounsaturated FA (mean %) | 24.44* | 23.74 | 24.72* | 23.60 | 24.01 | 23.95 |
| Palmitoleic (16:1) | 3.61* | 3.31 | 3.52* | 3.20 | 3.75 | 3.48 |
| Oleic (18:1) | 20.8 | 20.4 | 21.20* | 20.40 | 20.26 | 20.48 |
| n-6-polyunsaturated FA (mean %) | 30.90* | 32.14 | 30.11 | 31.25 | 32.15 | 33.54 |
| Linoleic (18:2) | 25.39* | 26.65 | 24.78 | 25.97 | 26.36 | 27.73 |
| $\gamma$-linolenic (18:3n6) | 0.28 | 0.27 | 0.25 | 0.24 | 0.34 | 0.32 |
| Dihomo-r-linolenic (20:3) | 0.96 | 0.92 | 0.88 | 0.84 | 1.08 | 1.04 |
| Arachidonic (20:4) | 4.26 | 4.30 | 4.19 | 4.20 | 4.37 | 4.45 |
| n3-polyunsaturated FA (mean %) | 8.78 | 8.86 | 8.82 | 9.17 | 8.72 | 8.36 |
| $\alpha$-linolenic (18:3,n3) | 0.93 | 1.00 | 0.87 | 0.94 | 1.04 | 1.07 |
| Eicosapentaenoic (20:5) | 3.04 | 2.94 | 3.09 | 3.11 | 2.97 | 2.68 |
| Docosapentanenoic (22:5) | 0.62 | 0.59 | 0.66 | 0.64 | 0.57 | 0.52 |
| Docosahexaenoic (22:6) | 4.18 | 4.33 | 4.21 | 4.49 | 4.15 | 4.09 |

*P<0.05, ***P<0.001 (vs. controls). Data given as mean or % or as defined. BMI, body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure; FA, fatty acids; SBP, systolic blood pressure.

without medication use for diabetes. Diabetes was defined as fasting glucose ≥7.0 mmol/L and/or non-fasting glucose ≥1.1 mmol/L and/or use of medication for diabetes.

### Statistical Analysis

Student's t-test was used to compare the mean baseline cardiovascular risk factors and serum fatty acid composition between incident CAD cases and control subjects. The chi-squared test was used to compare proportions between CAD cases and control subjects. The conditional odds ratios (OR) and 95% CI for CAD were estimated according to 1-SD increment and quartiles of serum fatty acid composition with conditional logistic regression models. Adjustment was made for BMI, alcohol intake (never, former, current: <46 g/day ethanol, and ≥46 g/day ethanol) and cigarette smoking status (never, ex-smoker, and current). We did not adjust for hypertension, diabetes mellitus, serum triglycerides or total serum cholesterol because we considered them potential mediators of any association between fatty acids and CAD. Lipid-lowering medication was not adjusted for in the analysis. Overall, the prevalence of lipid-lowering medication usage was only 3.1% (statins were not widely used in the baseline period of the 1980–1990s) in the present study, and there was no significant difference (P>0.05) between those who developed CAD (2.6%) and those who were free of CAD (3.3%). Therefore, lipid-lowering medication was unlikely to have affected the association that we observed.

Linear regression was used to test for linear trends across the serum fatty acid composition categories, using a median serum fatty acid composition for each serum fatty acid composition category. All statistical probability values were 2-tailed, and P<0.05 was regarded as statistically significant. SAS version 9.1.3 (Statistical Analysis System, Cary, NC, USA) was used for analysis.

## Results

During the follow-up period, we identified 152 incident CAD events. **Table 1** lists the risk characteristics of CAD and serum fatty acid composition compared with controls. Mean age was 64.2 years in men and 69.5 years in women, and 64.1 years in CAD cases and 69.6 years in controls. Serum cholesterol and the prevalence of diabetes mellitus were higher in CAD cases than in controls. SBP and serum cholesterol were higher in CAD cases than in controls for men, but no difference was found for women. The prevalence of diabetes mellitus was higher in CAD cases than in

**Table 2. Multivariate Conditional OR of CAD According to 1-SD FA Increase**

| Serum FA | Total | Women | Men | P for sex-interaction |
|---|---|---|---|---|
| | OR (95% CI) | OR (95% CI) | OR (95% CI) | |
| **Saturated FA** | 1.43 (1.11–1.83)* | 1.47 (1.02–2.11) | 1.39 (0.98–1.97) | 0.695 |
| Miristic (14:0) | 1.34 (1.10–1.64)** | 1.43 (1.05–1.95)* | 1.26 (0.96–1.64) | 0.323 |
| Palmitic (16:0) | 1.49 (1.16–1.91)** | 1.47 (1.03–2.09)* | 1.47 (1.03–2.10)* | 0.708 |
| Stearic (18:0) | 0.92 (0.70–1.22) | 1.04 (0.67–1.61) | 0.88 (0.61–1.28) | 0.668 |
| **Monounsaturated FA** | 1.30 (1.05–1.62)* | 1.02 (0.71–1.47) | 1.50 (1.13–1.98)** | 0.131 |
| Palmitoleic (16:1) | 1.46 (1.16–1.85)** | 1.44 (0.97–2.15) | 1.43 (1.07–1.92)* | 0.780 |
| Oleic (18:1) | 1.18 (0.96–1.46) | 0.91 (0.64–1.28) | 1.39 (1.07–1.82)* | 0.073 |
| **n-6-Polyunsaturated FA** | 0.66 (0.52–0.84)*** | 0.69 (0.48–0.98)* | 0.65 (0.47–0.91)* | 0.864 |
| Linoleic (18:2) | 0.67 (0.53–0.85)*** | 0.70 (0.50–0.98)* | 0.65 (0.47–0.91)* | 0.953 |
| γ-linolenic (18:3n6) | 1.09 (0.88–1.34) | 1.08 (0.78–1.51) | 1.11 (0.84–1.45) | 0.755 |
| Dihomo-r-linolenic (20:3) | 1.19 (0.96–1.49) | 1.18 (0.83–1.68) | 1.22 (0.91–1.63) | 0.612 |
| Arachidonic (20:4) | 0.93 (0.75–1.14) | 0.94 (0.68–1.31) | 0.93 (0.71–1.23) | 0.832 |
| **n3-Polyunsaturated FA** | 0.99 (0.80–1.22) | 1.20 (0.87–1.66) | 0.87 (0.66–1.15) | 0.159 |
| α-linolenic (18:3,n3) | 0.84 (0.67–1.06) | 0.91 (0.65–1.29) | 0.79 (0.57–1.08) | 0.505 |
| Eicosapentaenoic (20:5) | 1.10 (0.90–1.36) | 1.26 (0.93–1.72) | 1.01 (0.76–1.35) | 0.353 |
| Docosapentanenoic (22:5) | 1.23 (0.98–1.54) | 1.41 (0.94–2.09) | 1.12 (0.85–1.48) | 0.264 |
| Docosahexaenoic (22:6) | 0.87 (0.70–1.09) | 1.07 (0.74–1.54) | 0.77 (0.59–1.03) | 0.171 |

*P<0.05, **P<0.01, ***P<0.001 (vs. controls). Adjusted for BMI, current alcohol intake and cigarette smoking status as well as matching for sex, age, community, year of serum stored, and fasting status. Abbreviations as in Table 1.

controls for women, but not for men. Mean DBP, BMI, ethanol intake and triglycerides and the prevalence of hypertension, current smokers and impaired glucose tolerance did not differ between CAD cases and controls for total subjects, men or women. Overall, mean miristic acid, palmitic acid and palmitoleic acid were higher in CAD cases than in controls, while mean linoleic acid was lower in CAD cases than in controls. Other serum fatty acid compositions did not differ between CAD cases and controls for total subjects.

The multivariate OR and 95% CI for CAD associated with a 1-SD increase in each fatty acid are listed in **Table 2**. Saturated fatty acids, particularly for miristic acid (14:0) and palmitic acid (16:0), and monounsaturated fatty acids, particularly for palmitoleic acid (16:1), were associated with increased risk of CAD. An inverse association was observed between n6 polyunsaturated fatty acids and risk of CAD. Increased linoleic acid (18:2) was associated with reduced risk of CAD. The association between all fatty acids and incidence of CAD did not vary significantly between men and women.

**Table 3** lists multivariate OR of CAD according to quartiles for each fatty acid. Higher serum saturated fatty acids, particularly miristic acid (14:0) and palmitic acid (16:0), and monounsaturated acids, particularly palmitoleic acid (16:0), were associated with increased risk of CAD. The multivariable odds ratios of CAD for the highest versus lowest quartiles were 2.87 (1.41–5.82) for saturated fatty acids, 2.80 (1.51–5.18) for miristic acid, 2.72 (1.35–5.48) for palmitic acid, 2.25 (1.21–4.18) for monounsaturated acids and 3.18 (1.66–6.09) for palmitoleic acid. Higher serum n6-polyunsaturated fatty acids were associated with reduced risk of CAD; the multivariate OR for the highest vs. the lowest quartiles was 0.36 (95% CI: 0.18–0.70) and for the third vs. the lowest quartiles was 0.38 (95% CI: 0.21–0.69). Linoleic acid was associated with approximately 65% reduced risk of CAD in the highest and the third vs. the lowest quartiles, and a 47% reduced risk in the second vs. the lowest quartiles. No significant association was observed for serum n3-polyunsaturated fatty acids and increased risk of CAD. Similar results for serum fatty acids, except for monounsaturated, were obtained after we further adjusted for serum triglycerides. Monounsaturated fatty acids were positively associated with risk of CAD after adjustment for serum triglycerides (data not shown). We further excluded fatal outcome (n=19) in the sensitivity analysis, and similar results were observed (data not shown).

## Discussion

In the present study, n-6-polyunsaturated fatty acids, and especially serum linoleic acid were inversely associated with the risk of CAD but not n-3-polyunsaturated fatty acids. High saturated fatty acid and palmitoleic acid were associated with higher risk of CAD in both men and women.

Polyunsaturated fatty acids have been suggested to be cardioproctective.[3,4] In the present study, we found that only linoleic acid, the primary source of polyunsaturated fatty acids, but no other n-6-polyunsaturated fatty acids, was inversely associated with risk of CAD. This suggests that even within the polyunsaturated fatty acid subgroups such as n3-polyunsaturated fatty acids and n6-polyunsaturated fatty acids, specific individual fatty acids can have different effects with regard to risk of CAD. Although there was less evidence for the association of linoleic acid with the risk of CAD in Japanese subjects, the beneficial effect of serum linoleic acid on stroke has been demonstrated in our previous Japanese population study.[25] In a series of long-term feeding studies, higher intake of linoleic acid decreases ventricular fibrillation, suggesting that linoleic acid may have anti-arrhythmic effects.[26] A meta-analysis of 16 clinical trial studies observed that linoleic acid lowers LDL-C and increases high-density lipoprotein

**Table 3. Multivariable OR of CAD According to FA Quartile**

| Serum FA | CAD | | | | P for trend |
|---|---|---|---|---|---|
| | Quartiles of fatty acid | | | | |
| | 1 (low) | 2 | 3 | 4 (high) | |
| **Saturated FA** | | | | | |
| CAD cases, n | 30 | 36 | 36 | 50 | |
| Control, n | 113 | 114 | 117 | 112 | |
| Multivariable OR (95% CI) | 1.00 | 1.42 (0.79–2.56) | 1.58 (0.83–3.00) | 2.87 (1.41–5.82)** | 0.005 |
| **Miristic (14:0)** | | | | | |
| CAD cases, n | 25 | 39 | 35 | 53 | |
| Control, n | 111 | 119 | 112 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 1.65 (0.92–2.96) | 1.70 (0.93–3.11) | 2.80 (1.51–5.18)** | 0.002 |
| **Palmitic (16:0)** | | | | | |
| CAD cases, n | 30 | 38 | 34 | 50 | |
| Control, n | 114 | 113 | 115 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 1.54 (0.87–2.73) | 1.49 (0.79–2.80) | 2.72 (1.35–5.48)** | 0.009 |
| **Stearic (18:0)** | | | | | |
| CAD cases, n | 38 | 47 | 35 | 32 | |
| Control, n | 112 | 114 | 116 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 1.16 (0.65–2.05) | 0.76 (0.40–1.45) | 0.67 (0.32–1.43) | 0.185 |
| **Monounsaturated FA** | | | | | |
| CAD cases n | 29 | 39 | 33 | 51 | |
| Control, n | 114 | 114 | 114 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 1.50 (0.84–2.69) | 1.32 (0.24–2.44) | 2.25 (1.21–4.18)* | 0.021 |
| **Palmitoleic (16:1)** | | | | | |
| CAD cases, n | 27 | 32 | 35 | 58 | |
| Control, n | 113 | 115 | 114 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 1.31 (0.71–2.42) | 1.55 (0.84–2.86) | 3.18 (1.66–6.09)*** | 0.001 |
| **Oleic (18:1)** | | | | | |
| CAD cases, n | 28 | 41 | 41 | 42 | |
| Control, n | 113 | 114 | 116 | 113 | |
| Multivariable OR (95% CI) | 1.00 | 1.54 (0.87–2.72) | 1.54 (0.86–2.76) | 1.66 (0.89–3.11) | 0.123 |
| **n-6-Polyunsaturated FA** | | | | | |
| CAD cases, n | 54 | 39 | 27 | 32 | |
| Control, n | 114 | 113 | 115 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 0.61 (0.37–1.03) | 0.38 (0.21–0.69)** | 0.36 (0.18–0.70)** | 0.001 |
| **Linoleic (18:2)** | | | | | |
| CAD cases, n | 59 | 36 | 26 | 31 | |
| Control, n | 113 | 114 | 115 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 0.53 (0.32–0.90)* | 0.35 (0.20–0.63)*** | 0.35 (0.18–0.65)*** | 0.001 |
| **γ-linolenic (18:3n6)** | | | | | |
| CAD cases, n | 35 | 36 | 42 | 39 | |
| Control, n | 114 | 116 | 111 | 115 | |
| Multivariable OR (95% CI) | 1.00 | 1.08 (0.61–1.92) | 1.40 (0.76–2.57) | 1.26 (0.68–2.32) | 0.353 |
| **Dihomo-γ-linolenic (20:3)** | | | | | |
| CAD cases, n | 31 | 35 | 45 | 41 | |
| Control, n | 112 | 113 | 118 | 113 | |
| Multivariable OR (95% CI) | 1.00 | 1.20 (0.66–2.19) | 1.45 (0.82–2.55) | 1.48 (0.79–2.77) | 0.173 |
| **Arachidonic (20:4)** | | | | | |
| CAD cases, n | 44 | 39 | 30 | 39 | |
| Control, n | 114 | 113 | 115 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 0.89 (0.52–1.50) | 0.60 (0.34–1.06) | 0.77 (0.43–1.37) | 0.207 |
| **n3-Polyunsaturated FA** | | | | | |
| CAD cases, n | 47 | 30 | 37 | 38 | |
| Control, n | 113 | 114 | 116 | 113 | |
| Multivariable OR (95% CI) | 1.00 | 0.62 (0.36–1.08) | 0.80 (0.47–1.08) | 0.81 (0.46–1.44) | 0.685 |

(**Table 3** continued the next page.)

| Serum FA | CAD | | | | P for trend |
|---|---|---|---|---|---|
| | Quartiles of fatty acid | | | | |
| | 1 (low) | 2 | 3 | 4 (high) | |
| **α-linolenic (18:3,n3)** | | | | | |
| CAD cases, n | 43 | 37 | 38 | 34 | |
| Control, n | 113 | 116 | 113 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 0.83 (0.48–1.42) | 0.90 (0.52–1.57) | 0.76 (0.41–1.42) | 0.469 |
| **Eicosapentaenoic (20:5)** | | | | | |
| CAD cases, n | 41 | 27 | 42 | 42 | |
| Control, n | 111 | 117 | 114 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 0.63 (0.36–1.09) | 1.03 (0.62–1.71) | 1.05 (0.61–1.81) | 0.475 |
| **Docosapentanenoic (22:5)** | | | | | |
| CAD cases, n | 37 | 41 | 33 | 41 | |
| Control, n | 112 | 117 | 115 | 112 | |
| Multivariable OR (95% CI) | 1.00 | 1.04 (0.58–1.85) | 0.91 (0.48–1.73) | 1.17 (0.59–2.31) | 0.758 |
| **Docosahexaenoic (22:6)** | | | | | |
| CAD cases, n | 42 | 45 | 33 | 32 | |
| Control, n | 113 | 113 | 116 | 114 | |
| Multivariable OR (95% CI) | 1.00 | 1.07 (0.65–1.78) | 0.78 (0.45–1.36) | 0.71 (0.39–1.27) | 0.155 |

*P<0.05, **P<0.01, ***P<0.001 (vs. controls). Adjusted for BMI, current alcohol intake, cigarette smoking status as well as matching for sex, age, community, year of serum stored, and fasting status. Abbreviations as in Table 1.

cholesterol,[27] consequently reducing the risk of CAD. In the present study, however, the inverse association between linoleic acid and risk of CAD was not attributed to the decreasing effect of total cholesterol. First, serum linoleic acid was weakly and positively associated with serum total cholesterol (Pearson correlation=0.08, P=0.04; data not shown). Second, the significant inverse association between linoleic acid and risk of CAD remained even after adjustment for serum total cholesterol and lipid-lowering medication; the multivariable HR for the highest vs. the lowest quartiles was 0.33 (95% CI: 0.17–0.62; data not shown). Another explanation of the inverse association is the anti-inflammation effect of polyunsaturated fatty acids, which do not involve cyclooxygenase or lipoxygenase.[28] Furthermore, linoleic acid intake improves insulin sensitivity and reduces the risk of type 2 diabetes, which may also contribute to reduce the risk of CAD.[29,30]

Serum linoleic acid and n-3-polyunsaturated fatty acids better reflect dietary intake than serum monounsaturated fatty acids and saturated fatty acids.[18,19] In a previous Japanese study of middle-aged men, the Pearson correlation between dietary intake of fatty acids (expressed as % of total fatty acids) assessed on 24-h dietary intake recall and serum fatty acid composition was 0.34 (P<0.001) for linoleic acid, 0.26 (P<0.001) for n-3 polyunsaturated fatty acids, −0.10 (P=0.03) for monounsaturated fatty acids, and −0.005 (P=0.90) for saturated fatty acids.[19] The difference in serum fatty acid composition and dietary intake is probability due to absorption, metabolism and other factors that have an impact on metabolic efficiency.[31]

A recent meta-analysis of 10 cohort studies found that the RR of a fixed-effect model comparing the highest and lowest dietary linoleic acid category was 0.85 (95% CI: 0.78–0.92) for risk of total CAD events.[12] Furthermore, a substitution of 5% energy from dietary linoleic acid intake for dietary saturated fat intake was associated with a decreased risk of 9% for CAD events in a meta-analysis of 8 cohort studies.[12] These results, however, contradict the Sydney Diet Heart Study, a randomized controlled trial for secondary prevention of CAD, in which the dietary saturated fat (animal fats, common margarines, and shortening) was replaced with dietary linoleic acid (safflower oil and safflower oil polyunsaturated margarine) in the intervention group.[11] The Sydney Diet Heart Study found that the selective increase in dietary linoleic acid in patients with established CAD increased the mortality rate from cardiovascular disease and CAD.[11] The inconsistent findings may be attributed to differences in study design and population characteristics.

A recent review of 19 cohort studies reported that docosapentaenoic acid, docosahexaenoic acid and α-linolenic acid were associated with lower risk of fatal CAD.[32] In the present study, however, no association was observed between serum n-3 polyunsaturated and risk of CAD, and the result remained even when we excluded fatal cases in the sensitivity analysis. One possible reason for lack of the expected association was the relatively high level of n-3-polyunsaturated fatty acids reported in the Japanese general population compared with Western general populations.[33] Energy provided by dietary intake of n-3-polyunsaturated fatty acids for Japanese men and women (1.3–1.4% kcal) is higher than in Western populations (0.7–0.8% kcal).[34] This implies that populations with high serum n-3-polyunsaturated are protected against CAD. Furthermore, a systematic review indicated that modest consumption of fish (1–2 servings per week), especially fish higher in n-3 polyunsaturated fatty acids, reduces the risk of CAD.[35] Higher intake, however, did not further lower the risk of CAD, suggesting that the association is non-linear and that there is a threshold effect of n-3 polyunsaturated fatty acids on the risk of CAD.[35] The present study has described the evidence for the threshold effect in a population with a relatively high intake of n-3 polyunsaturated fatty acids.

## Limitations and Strengths

As noted, the present cohort has its limitations. First, the dehydration of polyunsaturated fatty acids during

preservation at −80°C may be a potential problem. A subsample analysis in our previous study, however, showed that there were no material changes in serum n-3 or n-6 polyunsaturated fatty acid composition measured at 2 time points in 8 years.[25] Second, non-fasting serum used in the present study and the time of blood collection may affect fatty acid Subsample analysis in our previous study, however, suggested no significant difference in serum acid composition between fasting and non-fasting samples.[25] Third, we did not have information on the absolute values of fatty acids, thus we were unable to examine the variability in the effect size of fatty acids on risk of CAD using an absolute approach. In a relative approach (weight percentage), the percentage of individual fatty acids was calculated based on the sum of all fatty acids and was not independent of other fatty acids.[36] The association between percentage and absolute concentration of serum fatty acids, however, was highly correlated (Pearson correlation coefficient >0.60).[37] In that study the author suggested that the choice to use a relative or absolute approach may not matter in studying the association of serum fatty acids with disease outcome.[37]

The strengths of this cohort study include the prospective nested case-control design, in which serum samples were stored and only incident CAD cases and selected controls were analyzed, to avoid possible effects of disease and of changes in diet after disease diagnosis. We used incident CAD as the outcome measure because it reflects more directly the association with risk factors than does mortality from CAD.

## Conclusions

In conclusion, our longitudinal study showed the adverse effect of high serum miristic acid, palmitic acid and palmitoleic acid, and the beneficial effect of serum linoleic acid on risk of CHD in a Japanese general population.

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### Disclosures

The authors declare no conflicts of interest.

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