Association of serum n-6 and n-3 polyunsaturated fatty acids with lipids in 3 populations of middle-aged men1–3


ABSTRACT

Background: The association of serum polyunsaturated fatty acids (PUFAs) with lipids in different populations is not known.

Objective: Our aim was to examine the association of serum n-6 (omega-6) or n-3 (omega-3) PUFAs with triglycerides or HDL-cholesterol concentrations in 261 white, 285 Japanese, and 212 Japanese American men aged 40–49 y.

Design: We used a population-based cross-sectional study. Of the original sample (n = 926), those taking lipid-lowering medications or who had diabetes (n = 168) were excluded. Serum fatty acids were analyzed by capillary gas-liquid chromatography. Multiple regression models as a function of tertile groups of each PUFA were used.

Results: Serum n-6 PUFAs were significantly inversely associated with triglycerides across populations after adjustment for age, body mass index, pack-years of smoking, and ethanol consumption [β = -0.39 (P < 0.001), -0.38 (P < 0.001), and -0.33 (P < 0.001) in whites, Japanese, and Japanese Americans, respectively]. Marine n-3 PUFAs were significantly inversely associated with triglycerides across populations [β = -0.15 (P < 0.001), -0.22 (P < 0.001), and -0.13 (P < 0.001) in whites, Japanese, and Japanese Americans, respectively]. n-6 PUFAs were significantly positively associated with HDL cholesterol in whites (β = 4.49, P < 0.001) and Japanese (β = 3.73, P < 0.01). Marine n-3 PUFAs were significantly positively associated with HDL cholesterol in Japanese (β = 2.15, P < 0.05), and eicosapentaenoic acid was significantly positively associated with HDL cholesterol in whites (β = 2.68, P < 0.01).

Conclusion: Serum n-6 and n-3 PUFAs are inversely associated with triglycerides across populations. Am J Clin Nutr 2009;90:49–55.

INTRODUCTION

Dietary intake of polyunsaturated fatty acids (PUFAs) may play an important role in influencing coronary heart disease (CHD) risk. n-6 (omega-6) PUFAs, such as linoleic acid (LA) found in vegetable oils, may reduce CHD risk as a result of its favorable effects on serum total cholesterol, LDL cholesterol, insulin sensitivity, or hemostatic factors (1, 2). Recent large prospective studies, eg, the Health Professional Follow-up Study and the Nurses’ Health Study, have found that dietary intake of LA is inversely associated with the risk of fatal CHD (3). n-3 (omega-3) PUFAs derived from fish may also reduce CHD risk. Large epidemiologic studies have shown an inverse association of dietary intake of marine n-3 PUFAs with the risk of CHD (4–7).

Evidence suggests that serum concentrations of PUFAs also are protective against CHD risk. Case-control and prospective studies showed an inverse association of circulating LA with nonfatal CHD events (8) and cardiovascular mortality (8, 9). Additionally, case-control and prospective studies have reported that circulating n-3 PUFAs were inversely associated with fatal and nonfatal CHD events, (8, 10–12).

Studies have shown that dietary intake of n-3 PUFAs has beneficial effects on lipid profiles. Recent extensive studies on clinical trials have showed that n-3 PUFAs decreased triglycerides by substantial amounts (13, 14). However, few studies have examined the association of serum n-6 PUFAs with triglyceride concentrations. To the best of our knowledge, the association of serum n-3 or n-6 PUFAs with triglycerides or HDL cholesterol in different populations has not been previously studied.

The purpose of the study was to examine the association of serum n-3 or n-6 PUFAs with triglycerides and HDL cholesterol, in the electron-beam tomography, risk factor assessment in Japanese and US men in the post-World War II birth cohort (ERA-JUMP Study) (15–17), a population-based cross-sectional

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study of white, Japanese, and Japanese American men aged 40–49 y.

SUBJECTS AND METHODS

Subjects and basic measurements

From 2002 to 2006, 926 men aged 40–49 y were randomly selected: 313 Japanese from Kusatsu, Shiga, Japan; 310 whites from Allegheny County, Pennsylvania (15–17); and 303 Japanese Americans from offspring of fathers who participated in the Honolulu Heart Program (18), Honolulu, Hawaii. These offspring were the third or fourth generation of Japanese Americans without ethnic admixture (16). All participants were without clinical cardiovascular disease, type 1 diabetes, or other severe diseases (15). Fasting samples were collected from all participants (15–17). Additionally, the current study excluded 160 subjects who were taking lipid-lowering medications or had type 2 diabetes. Type 2 diabetes was defined as “a fasting glucose of ≥126 mg/dL, or taking diabetes medication.” We also excluded 8 subjects with missing data. Our final sample comprised 261 whites, 285 Japanese, and 212 Japanese Americans. Informed consent was obtained from all participants. The study was approved by the institutional review boards of Shiga University of Medical Science (Otsu, Japan), the University of Pittsburgh (Pittsburgh, PA), and the Kuakini Medical Center (Honolulu, HI).

All participants underwent a physical examination, lifestyle questionnaire, and laboratory assessment as described previously (15–17). Serum samples were stored at −80°C and shipped on dry ice to the University of Pittsburgh, and concentrations of LDL cholesterol, HDL cholesterol, triglycerides, glucose, and insulin were measured as previously described (15). Serum fatty acids were analyzed by capillary-gas-liquid chromatography (PerkinElmer Clarus 500; PerkinElmer, Waltham, MA) (17). The coefficients of variation between runs for the major n-6 PUFAs—LA (18:2n–6) and arachidonic acid (AA) (20:4n–6)—were 1.6% and 2.8%, respectively. The coefficients of variation for the marine n-3 PUFAs—eicosapentaenoic acid (EPA; 20:5n–3), docosapentaenoic acid (22:5n–3), and docosahexaenoic acid (DHA; 22:6n–3)—were 2.5%, 2.5%, and 7.0%, respectively. The CVs for other major fatty acids—palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n–9), α-linolenic acid (ALA; 18:3n–3), and total fatty acid amount—were 1.2%, 4.0%, 2.3%, 7.9%, and 5.7%, respectively. Fatty acids were expressed as a percentage of total serum fatty acids.

Statistical analyses

To compare risk factors for CHD and fatty acids between populations, we performed an analysis of variance test and multiple comparison tests by using a Bonferroni test. To examine associations of serum n–6 PUFAs and serum marine-derived n–3 PUFAs with various lipids (HDL cholesterol and triglycerides), we made tertile groups of n–6 and n–3 PUFAs for each population and compared age- and multivariable-adjusted tertile-specific concentrations of lipids. To examine the linear trend of tertile-specific concentrations of lipids, general linear-model analyses were used. In model I, age, body mass index, pack-years of smoking, and ethanol consumption were adjusted.

In model II, n–6 PUFAs were further adjusted for all marine n–3 series (marine n–3 PUFAs, EPA, and DHA); marine n–3 PUFAs were further adjusted for all n–6 series (total n–6 PUFAs, LA, and AA). Statistical significance was considered to be \( P \leq 0.05 \). All statistical analyses were performed with SAS for Windows (SAS system release 9.1; SAS Institute, Cary, NC).

RESULTS

The results shown in Table 1 and Table 2 have been previously reported without the exclusion of subjects who were taking lipid-lowering medications or who had type 2 diabetes (17). Risk factors for CHD (blood pressure, hypertension, triglycerides, glucose, and cigarette smoking) for whites were similar to or more favorable compared with those for the Japanese (Table 1). Whites, however, were significantly more obese than the Japanese (\( P < 0.01 \)). Japanese Americans were similarly obese compared with whites but had a significantly higher prevalence of hypertension (\( P < 0.01 \)).

Whites had significantly higher concentrations of n–6 PUFAs than the Japanese (Table 2; \( P < 0.01 \)) but similar concentrations of n–6 PUFAs compared with Japanese Americans. Whites had >2-fold lower concentrations of marine n–3 PUFAs than did the Japanese (\( P < 0.01 \)). The Japanese had a higher total fatty acid amount compared with Japanese Americans (\( P < 0.01 \)).

Serum n–6 and n–3 PUFAs were significantly inversely associated with triglycerides across all 3 populations. The associations remained significant even after adjusting for age, body mass index, pack-years of smoking, ethanol consumption, and n–3 or n–6 PUFAs (Table 3; \( P < 0.001 \) for all populations). Serum LA and AA, the 2 main components of n–6 PUFAs, also were significantly inversely associated with triglycerides across populations (\( P < 0.001 \) for LA and \( P < 0.01 \) for AA). Serum EPA and DHA were also significantly inversely associated with triglycerides across populations (\( P < 0.01 \), \( P < 0.001 \), \( P < 0.05 \) for EPA, and \( P < 0.001 \), \( P < 0.001 \), \( P < 0.05 \) for DHA among whites, Japanese, and Japanese Americans, respectively).

n–6 PUFAs were significantly positively associated with HDL cholesterol in whites (Table 4; \( P < 0.001 \) for total n–6 and LA, \( P < 0.01 \) for AA) and in Japanese (\( P < 0.01 \) for total n–6 and LA). EPA was significantly positively associated with HDL cholesterol in whites (\( P < 0.01 \)). Marine n–3 PUFAs were significantly positively associated with HDL cholesterol in Japanese (\( P < 0.05 \)). n–6 or n–3 PUFAs did not have significant associations with HDL cholesterol in Japanese Americans.

Both n–6 and n–3 PUFAs were positively associated with LDL cholesterol in whites (\( \beta = 8.90, P < 0.01; \beta = 11.52, P < 0.001 \), respectively) but were not associated with LDL cholesterol in whites or Japanese Americans.

DISCUSSION

This study shows significant inverse associations of both serum total n–6 PUFAs and marine n–3 PUFAs with triglycerides across all 3 populations even after adjustment for potential confounders.

There has been concern that some antihypertensive medications, such as diuretics and β-blockers, have potentially detrimental effects on lipid profiles (19, 20). The data that were
analyzed excluded those taking antihypertensive medications, and the results were very similar.

To the best of our knowledge, very few previous studies have investigated the associations between serum concentrations of n–6 PUFAs and lipids. Ferrucci et al in 2006 (21) reported a significant inverse association between serum total n–6 PUFAs with triglycerides among 1123 men and women; however, this analysis was not the main focus, and detailed results were not reported.

Serum concentrations of n–3 PUFAs had significant positive associations with HDL cholesterol in whites, but the associations were not significant in the Japanese and Japanese Americans. However, in a dietary study from 1996 to 1998, Okuda et al (22) reported a significant positive relation between n–3 PUFA intake and serum HDL cholesterol in a sample size of 635 Japanese and Japanese American men. This difference may be due to a larger sample size in the study by Okuda et al (22).

| TABLE 1 | Characteristics of the study participants in 2002–2006 |
| --- | --- | --- |
| Whites (n = 261) | Japanese (n = 285) | Japanese Americans (n = 212) |
| Age (y) | 44.9 ± 2.8 | 45.1 ± 2.8 | 46.0 ± 2.9 |
| BMI (kg/m²) | 27.6 ± 4.1 | 23.4 ± 2.9 | 27.1 ± 4.0 |
| Systolic blood pressure (mm Hg) | 122.7 ± 11.5 | 124.3 ± 15.7 | 126.5 ± 12.0 |
| Diastolic blood pressure (mm Hg) | 73.1 ± 8.9 | 76.0 ± 11.8 | 76.9 ± 8.7 |
| Hypertension (%) | 13.5 | 23.9 | 23.8 |
| LDL cholesterol (mg/dL) | 137.8 ± 33.1 | 131.5 ± 35.8 | 128.1 ± 33.2 |
| Triglycerides (mg/dL) | 149.8 ± 102.8 | 151.5 ± 74.7 | 168.1 ± 114.1 |
| HDL cholesterol (mg/dL) | 48.4 ± 12.9 | 54.2 ± 13.8 | 51.1 ± 12.4 |
| Glucose (mg/dL) | 99.2 ± 8.1 | 103.6 ± 8.3 | 105.9 ± 7.9 |
| Insulin (µIU/mL) | 14.5 ± 7.1 | 9.9 ± 4.3 | 13.3 ± 6.2 |
| Current cigarette smoker (%) | 7.6 | 50.9 | 12.1 |
| Pack-years of smoking | 3.6 ± 8.3 | 19.6 ± 16.7 | 3.9 ± 8.0 |
| Alcohol drinker (%) | 45.8 | 66.7 | 39.3 |
| Ethanol consumption (g/d) | 10.2 ± 12.2 | 25.9 ± 27.5 | 16.2 ± 26.0 |
| Hypertension medications (%) | 5.7 | 4.2 | 10.3 |

1 Hypertension was defined as systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg or taking hypertension medication. An alcohol drinker was defined as one who drank alcohol ≥2 d/wk. Ethanol consumption was defined as grams of ethanol intake per day. Statistical significance was based on ANOVA followed by Bonferroni test if the overall ANOVA was significant.
2 Mean ± SD (all such values).
3 Significantly different from Japanese Americans, P < 0.01.
4 Significantly different from whites, P < 0.01.
5 Significantly different from Japanese, P < 0.01.

| TABLE 2 | Serum proportions of fatty acids in the study participants in 2002–2006 |
| --- | --- | --- |
| Whites (n = 261) | Japanese (n = 285) | Japanese Americans (n = 212) |
| Total fatty acids (mg/dL) | 238.1 ± 51.2 | 243.6 ± 50.4 | 229.9 ± 63.8 |
| Fatty acids proportion (%) | | | |
| Total n–6 fatty acids | 41.9 ± 4.2 | 35.0 ± 4.3 | 42.2 ± 4.0 |
| Linoleic acid | 30.1 ± 4.1 | 26.4 ± 4.1 | 30.7 ± 4.2 |
| Arachidonic acid | 8.9 ± 1.9 | 6.6 ± 1.3 | 8.9 ± 2.2 |
| Marine-derived n–3 fatty acids | 4.2 ± 1.8 | 9.6 ± 3.0 | 5.4 ± 2.4 |
| Eicosapentaenoic acid | 0.8 ± 0.6 | 2.6 ± 1.5 | 1.1 ± 1.1 |
| Docosapentaenoic acid | 0.7 ± 0.2 | 0.9 ± 0.2 | 0.7 ± 0.2 |
| Docosahexaenoic acid | 2.4 ± 1.2 | 6.0 ± 1.7 | 3.3 ± 1.4 |
| Saturated fatty acids | 31.0 ± 2.5 | 31.7 ± 2.1 | 30.7 ± 2.1 |
| Monounsaturated fatty acids | 20.1 ± 3.2 | 21.2 ± 3.1 | 18.9 ± 3.2 |
| trans Fatty acids | 1.0 ± 0.5 | 0.6 ± 0.2 | 0.9 ± 0.4 |

1 All values are means ± SDs. Marine-derived n–3 fatty acids were calculated as the sum of eicosapentaenoic acid (20:5n–3), docosapentaenoic acid (22:5n–3), and docosahexaenoic acid (22:6n–3); total n–6 fatty acids as the sum of linoleic acid (18:2n–6), γ-linoleic acid (18:3n–6), dihomoy–γ-linolenic acid (20:3n–6), and arachidonic acid (20:4n–6); saturated fatty acids as the sum of myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0); monounsaturated fatty acids as the sum of palmitoleic acid (16:1n–7), oleic acid (18:1n–9), and cis-vaccenic acid (18:1n–7); and trans fatty acids as the sum of palmitelaidic acid (16:1) and linolealaidic acid (18:2t). Statistical significance was based on ANOVA followed by the Bonferroni test if the overall ANOVA was significant.
2 Significantly different from Japanese Americans, P < 0.01.
3 Significantly different from Japanese, P < 0.01.
It is well known that dietary and supplemental n–3 PUFAs reduce triglyceride concentrations in humans (23). Our study found significant inverse associations between serum total n–3 fatty acids with triglycerides across all populations. In accordance with our study, Block et al in 2008 (24) reported that concentrations of EPA and DHA in blood cell membranes were significantly inversely associated with triglyceride concentrations. However, in a study of 1460 men and women in Quebec, Canada, Dewailly et al (25) surprisingly reported significant positive associations between serum EPA and DHA with triglycerides. The authors state that the amount of fish intake of Quebecers was not high enough to see a beneficial effect of EPA and DHA on triglycerides.

ALA was significantly inversely associated with triglycerides in whites and Japanese and marginally positively associated with triglycerides in Japanese Americans (Table 3). Dietary studies of ALA on triglycerides have shown inconsistent results (13, 14). n–6 PUFA intakes have been reported to lower LDL cholesterol (26–28) and total cholesterol (29, 30). However, our data showed no significant associations in whites between n–6 PUFAs and LDL cholesterol. In Japanese, n–6 PUFAs were significantly positively associated with LDL cholesterol. n–3 PUFA intakes have been reported to raise LDL cholesterol by a small amount (13, 14). In accordance with these findings, our data show that n–3 PUFAs are significantly positively associated with LDL cholesterol in Japanese but not in whites or Japanese Americans. We could not find an explanation for this discrepancy.

Some have proposed that higher LA intakes can adversely affect the risk of CHD (31). This is partly based on the assumption that dietary LA increases blood and tissue concentrations of AA, which could favor the production of proinflammatory molecules and increase the risk of cardiovascular disease (32). However, studies have shown that changes in dietary LA do not appreciably alter AA concentrations (33–35). In accordance with these findings, our data show that n–3 fatty acids were calculated as the sum of eicosapentaenoic acid (EPA; 20:5n–3), docosapentaenoic acid (22:5n–3), and docosahexaenoic acid (DHA; 22:6n–3); total n–6 fatty acids were calculated as the sum of linoleic acid (LA; 18:2n–6), γ-linolenic acid (18:3n–6), dihomo-γ-linolenic acid (20:3n–6), and arachidonic acid (AA; 20:4n–6). Total n–6 refers to total n–6 fatty acids, and marine n–3 to marine-derived n–3 fatty acids. Marine-derived n–3 fatty acids were calculated as the sum of eicosapentaenoic acid (EPA; 20:5n–3), docosapentaenoic acid (22:5n–3), and docosahexaenoic acid (DHA; 22:6n–3); total n–6 fatty acids were calculated as the sum of linoleic acid (LA; 18:2n–6), γ-linolenic acid (18:3n–6), dihomo-γ-linolenic acid (20:3n–6), and arachidonic acid (AA; 20:4n–6). Total n–6 refers to total n–6 fatty acids, and marine n–3 to marine-derived n–3 fatty acids. ALA, a-linolenic acid.
and n-6 PUFAs in blood cell membranes could distinguish acute coronary syndrome patients from controls (24, 36, 37). Additionally, it was observed that n-6 and n-3 PUFAs were inversely associated with lipids in these studies.

Studies have reported that n-3 PUFAs decrease triglycerides and very-low-density lipoprotein (VLDL) in hypertriglyceridemic subjects, with an associated increase in HDL cholesterol. However, they appear to increase or have no effect on LDL cholesterol. n-3 PUFAs regulate activity of nuclear receptors, which results in repartitioning of fatty acids away from triglyceride storage and toward oxidation (38). This effect is mediated by a reduction in sterol regulatory element-binding protein-1c, the main transcription factor controlling lipogenesis. With a reduction in triglyceride synthesis and an increase in fatty acid oxidation in the hepatocyte, there is decreased substrate available for VLDL synthesis and secretion. n-3 PUFAs may also undergo extensive peroxidation, which appears to stimulate the degradation of apolipoprotein B, also resulting in the reduction of VLDL secretion (38, 39).

The Dietary Reference Intake Report and the 2005 Dietary Guidelines for Americans (40) support an acceptable macronutrient distribution range (the range of intakes for a particular energy source that is associated with reduced risk of chronic disease while providing adequate intakes of essential nutrients) of 5–10% dietary energy from n-6 PUFAs (41). Numerous metabolic studies have shown strong cholesterol-lowering effects for vegetable oils rich in LA when substituted for dietary saturated fat (42–44). In 1966 Brown et al (45, 46) showed that a diet with reduced saturated fat and increased PUFAs has a beneficial effect on serum cholesterol. In 1967 Keys et al reported that dietary PUFAs lower serum cholesterol in proportion to their concentration in the diet (29). However, there are no recommendations for serum concentrations of n-6 PUFAs or n-6 PUFAs intake in lowering serum cholesterol or triglycerides. Clinical implications for n-3 PUFAs in lowering serum triglycerides have been previously documented. Weber et al (23) reported that the smallest amount of n-3 PUFAs needed to statistically significantly lower serum triglycerides appears to be 1 g/d as provided by a fish diet.
A strength of this study is that we were able to investigate the associations of serum n–6 and n–3 PUFAs with lipids across population groups. To the best of our knowledge, this study is the first population-based study to identify the association of n–6 PUFAs with triglycerides and HDL cholesterol across population groups. The significant inverse associations between n–6 PUFAs and triglycerides across all 3 populations, even after adjustment for various potential confounders, were particularly surprising.

However, the study has several limitations. Unrecognized confounding factors may remain even after adjustment and exclusion of participants with diabetes and lipid-lowering medications. We did not collect precise dietary data in this study. However, the observed differences in serum n–3 and n–6 PUFAs in the 3 populations are reasonable on the basis of other studies that have evaluated precise dietary intake of these fatty acids and other dietary factors in these population groups (22, 47). The current study excluded women, and participants were only men who were aged 40–49 y, which limits the generalizability of the results.

Due to the cross-sectional study design, we cannot determine causal relations. Lower triglyceride and higher HDL cholesterol concentrations may be caused by n–3 fatty acids and other dietary factors in these population groups. The significant inverse associations between serum n–3 fatty acids and triglyceride concentrations could raise PUFA concentrations when expressed as a percentage of total fatty acids. However, we have analyzed the association of triglycerides with PUFAs, using the concentration of PUFAs (mg/dL), and have found similar results as those reported in Table 3.

In conclusion, we found that serum total n–6 and marine n–3 PUFAs were significantly and inversely related to serum triglycerides in a population-based sample of white, Japanese, and Japanese American men aged 40–49 y. Considering the risk of CHD due to elevated serum triglycerides, these findings may have prospects for reducing mortality.

The authors’ responsibilities were as follows—AS, JDC, HU, RWE, and LHK: developed the hypothesis of this study; AS, JDC, TK, AE-S, TO, YN, KS-T, BLR, DE, BJW, SK, and HU: involved in data collection; KRM, AS, JDC, HU, RWE, RDA, and LHK: led the data analyses; KRM and AS: prepared the first draft; and JDC, TK, AE-S, RDA, TO, RWE, YN, KS-T, BLR, AK, DE, BJW, SK, LHK, and HU: provided expert consultation on data interpretation. All authors were involved in review and revision of the manuscript and gave final approval of the version to be published. None of the authors had a conflict of interest.

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