Association of serum n−6 and n−3 polyunsaturated fatty acids with lipids in 3 populations of middle-aged men[1–3]

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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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[2] Supported by grant nos. R01-HL068200 and R01-HL071561 from the National Institutes of Health and grant nos. B 16790335 and A 13307016 from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

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Received July 28, 2008. Accepted for publication April 24, 2009.

First published online May 27, 2009; doi: 10.3945/ajcn.2008.26761.

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 $\geq$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 $P < 0.05$. 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 Japanese ($\beta = 8.90$, $P < 0.01$; $\beta = 11.52$, $P < 0.001$, 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

TABLE 1
Characteristics of the study participants in 2002–2006[1]

	Whites (n = 261)	Japanese (n = 285)	Japanese Americans (n = 212)
Age (y)	44.9 ± 2.8[2,3]	45.1 ± 2.8[4]	46.0 ± 2.9
BMI (kg/m^2)	27.6 ± 4.1[5]	23.4 ± 2.9[4]	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[3]	76.0 ± 11.8	76.9 ± 8.7
Hypertension (%)	13[3,5]	23.9	23.8
LDL cholesterol (mg/dL)	137.8 ± 33.1[3]	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[5]	54.2 ± 13.8[4]	51.1 ± 12.4
Glucose (mg/dL)	99.2 ± 8.1[3,5]	103.6 ± 8.3[4]	105.9 ± 7.9
Insulin (μIU/mL)	14.5 ± 7.1[5]	9.9 ± 4.3[4]	13.3 ± 6.2
Current cigarette smoker (%)	7.6[5]	50.9[4]	12.1
Pack-years of smoking	3.6 ± 8.33[5]	19.6 ± 16.7[4]	3.9 ± 8.0
Alcohol drinker (%)	45.8[5]	66.7[4]	39.3
Ethanol consumption (g/d)	10.2 ± 12.2[5]	25.9 ± 27.5[4]	16.2 ± 26.0
Hypertension medications (%)	5.7	4.2[4]	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, $P < 0.01$.

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 2
Serum proportions of fatty acids in the study participants in 2002–2006[1]

	Whites (n = 261)	Japanese (n = 285)	Japanese Americans (n = 212)
Total fatty acids (mg/dL)	238.1 ± 51.2	243.6 ± 50.4[2]	229.9 ± 63.8
Fatty acids proportion (%)			
Total n−6 fatty acids	41.9 ± 4.2[3]	35.0 ± 4.3[2]	42.2 ± 4.0
Linoleic acid	30.1 ± 4.1[3]	26.4 ± 4.1[2]	30.7 ± 4.2
Arachidonic acid	8.9 ± 1.9[3]	6.6 ± 1.3[2]	8.9 ± 2.2
Marine-derived n−3 fatty acids	4.2 ± 1.8[2,3]	9.6 ± 3.0[2]	5.4 ± 2.4
Eicosapentaenoic acid	0.8 ± 0.6[2,3]	2.6 ± 1.5[2]	1.1 ± 1.1
Docosapentaenoic acid	0.7 ± 0.2[3]	0.9 ± 0.2[2]	0.7 ± 0.2
Docosahexaenoic acid	2.4 ± 1.2[2,3]	6.0 ± 1.7[2]	3.3 ± 1.4
Saturated fatty acids	31.0 ± 2.5[3]	31.7 ± 2.1[2]	30.7 ± 2.1
Monounsaturated fatty acids	20.1 ± 3.2[2,3]	21.2 ± 3.1[2]	18.9 ± 3.2
trans Fatty acids	1.0 ± 0.5[2,3]	0.6 ± 0.2[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), dihomo-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 (16:1t) and linolelaidic acid (18:2tt). 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, $P < 0.01$.

TABLE 3
Associations between polyunsaturated fatty acids and triglycerides in US whites (n = 261), Japanese (n = 285), and Japanese Americans (n = 212) in 2002–2006[1]

	Whites				Japanese				Japanese Americans			
	Lower	Middle	Upper	β	Lower	Middle	Upper	β	Lower	Middle	Upper	β
Total n−6												
Crude	198.3 (1.0)	124.2 (1.0)	88.0 (1.0)	−0.41[2]	180.7 (1.0)	138.0 (1.0)	102.0 (1.0)	−0.29[2]	191.5 (1.1)	142.2 (1.1)	103.2 (1.1)	−0.31[2]
Model I	192.9 (1.0)	123.2 (1.0)	91.19 (1.0)	−0.38[2]	180.0 (1.0)	138.9 (1.0)	101.2 (1.0)	−0.29[2]	190.4 (1.1)	139.9 (1.1)	105.5 (1.1)	−0.30[2]
Model II	194.8 (1.0)	123.8 (1.0)	89.8 (1.0)	−0.39[2]	197.9 (1.0)	137.3 (1.0)	93.1 (1.0)	−0.38[2]	197.4 (1.1)	140.2 (1.1)	101.5 (1.1)	−0.33[2]
LA												
Crude	175.0 (1.1)	133.2 (1.1)	92.9 (1.1)	−0.32[2]	171.1 (1.0)	134.6 (1.0)	110.5 (1.0)	−0.22[2]	165.8 (1.1)	148.4 (1.1)	114.1 (1.1)	−0.19[2]
Model I	169.2 (1.1)	133.0 (1.1)	96.4 (1.1)	−0.28[2]	170.2 (1.0)	135.6 (1.0)	109.8 (1.0)	−0.22[2]	167.5 (1.1)	146.3 (1.1)	114.5 (1.1)	−0.19[2]
Model II	172.9 (1.1)	132.0 (1.1)	94.9 (1.1)	−0.03[2]	183.1 (1.0)	135.8 (1.0)	101.8 (1.0)	−0.29[2]	173.6 (1.1)	145.8 (1.1)	111.1 (1.1)	−0.22[2]
AA												
Crude	157.9 (1.1)	135.9 (1.1)	101.0 (1.1)	−0.22[2]	174.9 (1.0)	132.6 (1.0)	109.8 (1.0)	−0.23[2]	179.1 (1.1)	125.8 (1.1)	125.1 (1.1)	−0.18[2]
Model I	156.5 (1.1)	132.4 (1.1)	104.5 (1.1)	−0.20[2]	173.5 (1.0)	132.0 (1.0)	111.4 (1.0)	−0.22[2]	170.7 (1.1)	125.7 (1.1)	131.1 (1.1)	−0.13[3]
Model II	155.1 (1.1)	133.4 (1.1)	104.7 (1.1)	−0.20[2]	173.1 (1.0)	132.3 (1.0)	111.5 (1.0)	−0.22[2]	170.0 (1.1)	126.2 (1.1)	131.2 (1.1)	−0.13[3]
Marine n−3												
Crude	157.6 (1.1)	128.1 (1.1)	108.1 (1.1)	−0.19[2]	141.5 (1.1)	132.3 (1.1)	136.0 (1.1)	−0.02	163.9 (1.1)	130.2 (1.1)	1319 (1.1)	−0.11[4]
Model I	149.9 (1.1)	129.0 (1.1)	113.1 (1.1)	−0.14[2]	143.6 (1.1)	133.6 (1.1)	132.4 (1.1)	−0.04	157.1 (1.1)	128.9 (1.1)	138.9 (1.1)	−0.06
Model II	150.1 (1.0)	129.4 (1.0)	112.2 (1.0)	−0.15[2]	170.2 (1.0)	136.0 (1.0)	109.9 (1.0)	−0.22[2]	163.0 (1.1)	138.2 (1.1)	125.0 (1.1)	−0.13[3,2]
EPA												
Crude	141.2 (1.1)	134.2 (1.1)	114.7 (1.1)	−0.10[3]	138.9 (1.1)	135.9 (1.1)	134.7 (1.1)	−0.02	145.3 (1.1)	154.2 (1.1)	125.2 (1.1)	−0.07
Model I	135.0 (1.1)	134.8 (1.1)	119.2 (1.1)	−0.06	141.0 (1.1)	139.8 (1.1)	128.9 (1.1)	−0.05	143.0 (1.1)	150.7 (1.1)	130.3 (1.1)	−0.04
Model II	139.6 (1.0)	134.7 (1.0)	115.5 (1.0)	−0.1[3]	154.3 (1.0)	140.3 (1.0)	117.3 (1.0)	−0.14[2]	148.3 (1.1)	152.5 (1.1)	124.1 (1.1)	−0.09[4]
DHA												
Crude	159.3 (1.1)	129.5 (1.1)	105.0 (1.1)	−0.21[2]	138.7 (1.1)	133.4 (1.1)	137.6 (1.1)	−0.00	164.2 (1.1)	135.9 (1.1)	126.5 (1.1)	−0.13[3]
Model I	151.4 (1.1)	130.7 (1.1)	109.5 (1.1)	−0.16[2]	140.6 (1.1)	133.4 (1.1)	135.5 (1.1)	−0.02	157.1 (1.1)	133.1 (1.1)	134.8 (1.1)	−0.08
Model II	151.1 (1.0)	130.7 (1.0)	109.7 (1.0)	−0.16[2]	166.2 (1.0)	134.0 (1.0)	114.5 (1.0)	−0.19[2]	161.1 (1.1)	139.4 (1.1)	125.7 (1.1)	−0.12[3]
ALA												
Crude	136.0 (1.1)	125.6 (1.1)	126.5 (1.1)	−0.04	158.2 (1.1)	137.6 (1.0)	116.5 (1.1)	−0.15[2]	144.0 (1.1)	121.8 (1.1)	160.0 (1.1)	0.05
Model I	135.4 (1.1)	128.8 (1.1)	124.1 (1.1)	−0.04	157.0 (1.0)	137.8 (1.0)	117.3 (1.0)	−0.15[2]	143.5 (1.1)	119.9 (1.1)	163.0 (1.1)	0.06
Model II	132.8 (1.0)	126.2 (1.0)	129.2 (1.0)	−0.01	154.0 (1.0)	135.2 (1.0)	122.1 (1.0)	−0.12[2]	137.3 (1.1)	121.0 (1.1)	168.7 (1.1)	0.10[4]

[1] All values are means (SEs) unless stated otherwise. "Lower," "Middle," and "Upper" refer to tertile groups. For models, the outcome variable is triglycerides; the primary predictor variable is each fatty acid composition. "Crude" includes an adjustment for age; "model I" includes an adjustment for age, BMI, pack-years of smoking, and ethanol consumption; and "model II" includes a further adjustment for total n−3 or total n−6 polyunsaturated 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-linoleic 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, α-linolenic acid.

[2] $P < 0.001$ on the basis of a regression analysis of triglycerides as a function of tertile group of each fatty acid as a continuous variable.

[3] $P < 0.01$ on the basis of a regression analysis of triglycerides as a function of tertile group of each fatty acid as a continuous variable.

[4] $P < 0.05$ on the basis of a regression analysis of triglycerides as a function of tertile group of each fatty acid as a continuous variable.

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 no significant positive association between concentrations of LA and AA (data not shown). Our data also show significant inverse associations between serum AA and triglycerides across populations and do not suggest an increased risk of CHD. Studies have reported that n−3

TABLE 4
Associations between polyunsaturated fatty acids and HDL cholesterol in US whites (n = 261), Japanese (n = 285), and Japanese Americans (n = 212) in 2002–2006[1]

	Whites				Japanese				Japanese Americans			
	Lower	Middle	Upper	β	Lower	Middle	Upper	β	Lower	Middle	Upper	β
Total n−6												
Crude	44.3 (1.3)	46.8 (1.3)	54.1 (1.3)	4.92[2]	52.8 (1.4)	53.6 (1.4)	56.2 (1.4)	1.67	50.9 (1.5)	49.5 (1.5)	52.8 (1.5)	0.94
Model I	44.5 (1.3)	47.1 (1.2)	53.7 (1.3)	4.58[2]	52.1 (1.2)	53.0 (1.2)	57.7 1.25)	2.75[3]	50.8 (1.3)	49.8 (1.3)	52.6 (1.3)	0.90
Model II	44.6 (1.3)	47.2 (1.2)	53.6 (1.3)	4.49[2]	51.1 (1.3)	53.1 (1.2)	58.6 (1.3)	3.73[3]	50.3 (1.3)	49.7 (1.2)	53.2 (1.3)	1.45
LA												
Crude	46.5 (1.3)	45.3 (1.4)	53.4 (1.4)	3.44[2]	54.3 (1.4)	52.9 (1.4)	55.4 (1.4)	0.54	52.4 (1.5)	49.8 (1.5)	51.1 (1.5)	−0.66
Model I	46.9 (1.3)	45.4 (1.2)	53.0 (1.3)	3.05[3]	53.0 (1.2)	52.9 (1.2)	56.9 (1.2)	2.00[4]	51.5 (1.3)	50.3 (1.3)	51.4 (1.3)	−0.05
Model II	46.7 (1.3)	45.5 (1.2)	53.1 (1.3)	3.19[2]	52.2 (1.2)	52.9 (1.2)	57.7 (1.3)	2.81[3]	50.9 (1.3)	50.4 (1.3)	51.9 (1.3)	0.49
AA												
Crude	45.3 (1.4)	48.3 (1.4)	51.8 (1.4)	3.24[2]	52.0 (1.4)	52.2 (1.4)	58.4 (1.4)	3.23[3]	48.0 (1.5)	50.9 (1.5)	54.2 (1.4)	3.12[3]
Model I	45.6 (1.3)	49.0 (1.3)	50.6 (1.3)	2.49[3]	53.2 (1.2)	53.2 (1.2)	56.3 (1.2)	1.55	49.7 (1.3)	51.6 (1.3)	51.9 (1.3)	1.08
Model II	45.6 (1.3)	49.1 (1.3)	50.6 (1.3)	2.52[3]	53.3 (1.2)	53.1 (1.2)	56.3 (1.2)	1.53	50.0 (1.3)	51.4 (1.3)	51.8 (1.3)	0.93
Marine n−3												
Crude	46.1 (1.4)	48.8 (1.4)	50.3 (1.4)	2.08[4]	52.8 (1.4)	54.9 (1.4)	54.9 (1.4)	1.08	48.1 (1.5)	51.7 (1.5)	53.3 (1.5)	2.58[4]
Model I	47.2 (1.3)	48.7 (1.3)	49.3 (1.3)	1.03	53.4 (1.2)	54.7 (1.2)	54.7 (1.3)	0.66	49.3 (1.3)	52.1 (1.2)	51.9 (1.3)	1.30
Model II	47.2 (1.3)	48.7 (1.2)	49.4 (1.2)	1.10	51.9 (1.3)	54.6 (1.2)	56.2 (1.3)	2.15[4]	49.2 (1.3)	51.9 (1.3)	52.1 (1.3)	1.51
EPA												
Crude	46.0 (1.4)	47.5 (1.4)	51.8 (1.4)	2.91[3]	52.1 (1.4)	56.9 (1.4)	53.7 (1.4)	0.80	49.6 (1.4)	49.6 (1.5)	54.1 (1.5)	2.23[4]
Model I	46.6 (1.3)	47.5 (1.3)	51.2 (1.3)	2.30[4]	52.8 (1.2)	56.0 (1.2)	54.0 (1.2)	0.64	50.0 (1.2)	50.2 (1.3)	53.1 (1.3)	1.52
Model II	46.2 (1.2)	47.5 (1.2)	51.5 (1.2)	2.68[3]	52.0 (1.2)	56.0 (1.2)	54.8 (1.2)	1.39	49.9 (1.2)	50.2 (1.3)	53.2 (1.3)	1.63
DHA												
Crude	47.1 (1.4)	47.8 (1.4)	50.4 (1.4)	1.63	53.9 (1.4)	54.2 (1.4)	54.5 (1.4)	0.31	48.2 (1.5)	50.6 (1.4)	54.3 (1.5)	3.06[3]
Model I	48.1 (1.3)	47.9 (1.3)	49.4 (1.3)	0.65	54.4 (1.2)	54.1 (1.2)	54.2 (1.2)	−0.90	49.3 (1.3)	51.4 (1.2)	52.5 (1.3)	1.59
Model II	48.1 (1.3)	47.9 (1.2)	49.3 (1.3)	0.63	53.1 (1.3)	54.1 (1.2)	55.5 (1.3)	1.20	49.3 (1.3)	51.2 (1.2)	52.7 (1.3)	1.72
ALA												
Crude	48.3 (1.4)	49.0 (1.4)	48.0 (1.4)	−0.16	53.7 (1.4)	54.4 (1.4)	54.6 (1.4)	0.45	52.0 (1.5)	52.5 (1.5)	48.8 (1.5)	−1.59
Model I	48.7 (1.3)	48.3 (1.3)	48.3 (1.3)	−0.20	54.2 (1.2)	54.2 (1.2)	54.4 (1.2)	0.08	51.8 (1.2)	52.0 (1.3)	49.5 (1.3)	−1.15
Model II	48.9 (1.2)	48.5 (1.3)	47.9 (1.3)	−0.53	54.4 (1.2)	54.3 (1.2)	54.0 (1.2)	−0.17	51.9 (1.2)	51.9 (1.3)	49.4 (1.3)	−1.25

[1] Values are means (SEs) unless stated otherwise. "Lower," "Middle," and "Upper" refer to tertile groups. For models, the outcome variable is HDL cholesterol, and the primary predictor variable is the composition of each fatty acid. "Crude" includes an adjustment for age; "model I" includes an adjustment for age, BMI, pack-years of smoking, and ethanol consumption; and "model II" includes a further adjustment for total n−3 or total n−6 polyunsaturated 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); and total n−6 fatty acids were calculated as the sum of linoleic acid (LA; 18:2n−6), γ-linoleic 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, α-linolenic acid.

[2] $P < 0.001$ on the basis of a regression analysis of HDL cholesterol as a function of tertile group of each fatty acid as a continuous variable.

[3] $P < 0.01$ on the basis of a regression analysis of HDL cholesterol as a function of tertile group of each fatty acid as a continuous variable.

[4] $P < 0.05$ on the basis of a regression analysis of HDL cholesterol as a function of tertile group of each fatty acid as a continuous variable.

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 degredation 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 PUFA 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 PUFA needed to statistically significantly lower serum triglycerides appears to be $\approx$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−6 and n−3 PUFAs; however, the reverse could also be true. Triglycerides as a lipid fraction contain similar amounts of n−3 PUFAs, but fewer n−6 PUFAs compared with other lipid fractions such as cholesteryl esters and phospholipids (48, 49). VLDL, a triglyceride carrier, is relatively poor in cholesteryl esters and phospholipids. Lower 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 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.

REFERENCES

1. Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 2001;20:5–19.
2. Sanders TA, Oakley FR, Miller GJ, Mitropoulos KA, Crook D, Oliver MF. Influence of n−6 versus n−3 polyunsaturated fatty acids in diets low in saturated fatty acids on plasma lipoproteins and hemostatic factors. Arterioscler Thromb Vasc Biol 1997;17:3449–60.
3. Ascherio A. Epidemiologic studies on dietary fats and coronary heart disease. Am J Med 2002;113(Suppl 9B):9S–12S.
4. Dolecek TA. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. Proc Soc Exp Biol Med 1992;200:177–82.
5. Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ 1996;313:84–90.
6. Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 2007;369:1090–8.
7. Iso H, Kobayashi M, Ishihara J, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation 2006;113:195–202.
8. Harris WS, Poston WC, Haddock CK. Tissue n−3 and n−6 fatty acids and risk for coronary heart disease events. Atherosclerosis 2007;193:1–10.
9. Laaksonen DE, Nyyssonen K, Niskanen L, Rissanen TH, Salonen JT. Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. Arch Intern Med 2005;165:193–9.
10. Erkkila AT, Matthan NR, Herrington DM, Lichtenstein AH. Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD. J Lipid Res 2006;47:2814–9.
11. Simon JA, Hodgkins ML, Browner WS, Neuhaus JM, Bernert JT Jr, Hulley SB. Serum fatty acids and the risk of coronary heart disease. Am J Epidemiol 1995;142:469–76.
12. Harris WS, Reid KJ, Sands SA, Spertus JA. Blood omega-3 and trans fatty acids in middle-aged acute coronary syndrome patients. Am J Cardiol 2007;99:154–8.
13. Balk E, Chung M, Lichtenstein A, et al. Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease. Evid Rep Technol Assess 2004;Mar(93):1–6.
14. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis 2006;189:19–30.
15. Sekikawa A, Ueshima H, Kadowaki T, et al. Less subclinical atherosclerosis in Japanese men in Japan than in white men in the United States in the post-World War II birth cohort. Am J Epidemiol 2007;165:617–24.
16. Abbott RD, Ueshima H, Rodriguez BL, et al. Coronary artery calcification in Japanese men in Japan and Hawaii. Am J Epidemiol 2007;166:1280–7.
17. Sekikawa A, Curb JD, Ueshima H, et al. Marine-derived n−3 fatty acids and atherosclerosis in Japanese, Japanese-American, and white men: a cross-sectional study. J Am Coll Cardiol 2008;52:417–24.
18. Kagan A, Harris BR, Winkelstein W Jr, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: demographic, physical, dietary and biochemical characteristics. J Chronic Dis 1974;27:345–64.
19. Grimm RH Jr, Leon AS, Hunninghake DB, Lenz K, Hannan P, Blackburn H. Effects of thiazide diuretics on plasma lipids and lipoproteins in mildly hypertensive patients: a double-blind controlled trial. Ann Intern Med 1981;94:7–11.
20. Weidmann P, Uehlinger DE, Gerber A. Antihypertensive treatment and serum lipoproteins. J Hypertens 1985;3:297–306.
21. Ferrucci L, Cherubini A, Bandinelli S, et al. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 2006;91:439–46.
22. Okuda N, Ueshima H, Okayama A, et al. Relation of long chain n−3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese-American men in Hawaii: the INTERLIPID study. Atherosclerosis 2005;178:371–9.
23. Weber P, Raederstorff D. Triglyceride-lowering effect of omega-3 LC-polyunsaturated fatty acids: a review. Nutr Metab Cardiovasc Dis 2000;10:28–37.
24. Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. Atherosclerosis 2008;197:821–8.
25. Dewailly EE, Blanchet C, Gingras S, et al. Relations between n−3 fatty acid status and cardiovascular disease risk factors among Quebecers. Am J Clin Nutr 2001;74:603–11.
26. Howard BV, Hannah JS, Heiser CC, et al. Polyunsaturated fatty acids result in greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose-response comparison in a multiracial study group. Am J Clin Nutr 1995;62:392–402.

27. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 1992;12: 911–9.
28. Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N Engl J Med 1989; 321:436–41.
29. Fetcher ES, Foster N, Anderson JT, Grande F, Keys A. Quantitative estimation of diets to control serum cholesterol. Am J Clin Nutr 1967; 20:475–92.
30. Keys A, Parlin RW. Serum cholesterol response to changes in dietary lipids. Am J Clin Nutr 1966;19:175–81.
31. Dubnov G, Berry EM. Omega-6 fatty acids and coronary artery disease: the pros and cons. Curr Atheroscler Rep 2004;6:441–6.
32. Calder PC. Polyunsaturated fatty acids and inflammation. Biochem Soc Trans 2005;33:423–7.
33. Hussein N, Ah-Sing E, Wilkinson P, Leach C, Griffin BA, Millward DJ. Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. J Lipid Res 2005;46:269–80.
34. Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n−3) eicosapentaenoic acid in plasma phospholipids in healthy men. J Nutr 2007; 137:945–52.
35. Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. Am J Clin Nutr 2006;84:44–53.
36. Block RC, Harris WS, Pottala JV. Determinants of blood cell omega-3 fatty acid content. The Open Biomarkers J 2008;1:1–6.
37. Block RC, Harris WS, Reid KJ, Spertus JA. Omega-6 and trans fatty acids in blood cell membranes: a risk factor for acute coronary syndromes? Am Heart J 2008;156:1117–23.
38. Davidson MH. Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. Am J Cardiol 2006;98:27i–33i.
39. Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. Atherosclerosis 2008;197:12–24.
40. US Department of Health and Human Services, US Department of Agriculture. Dietary guidelines for Americans, 2005. 6th ed. Washington, DC: US Government Printing Office, 2005.
41. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 2009;119: 902–7.
42. Grundy SM, Bilheimer D, Blackburn H, et al. Rationale of the diet-heart statement of the American Heart Association. Report of Nutrition Committee. Circulation 1982;65:839A–54A.
43. Green JG, Brown HB, Meredith AP, Page IH. Use of fat-modified foods for serum cholesterol reduction. JAMA 1963;183:5–12.
44. Harris WS. Linoleic acid and coronary heart disease. Prostaglandins Leukot Essent Fatty Acids 2008;79:169–71.
45. Brown HB, Farrand M, Page IH. Design of practical fat-controlled diets. Foods, fat composition, and serum cholesterol content. JAMA 1966;196: 205–13.
46. Brown HB, Farrand ME. Pitfalls in constructing a fat-controlled diet. J Am Diet Assoc 1966;49:303–8.
47. Stamler J, Elliott P, Dennis B, et al. INTERMAP: background, aims, design, methods, and descriptive statistics (nondietary). J Hum Hypertens 2003;17:591–608.
48. Yeh LL, Bunker CH, Evans RW, Kuller LH. Serum fatty acid distributions in postmenopausal women. Nutr Res 1994;14:675–91.
49. Leaf DA, Connor WE, Barstad L, Sexton G. Incorporation of dietary n−3 fatty acids into the fatty acids of human adipose tissue and plasma lipid classes. Am J Clin Nutr 1995;62:68–73.