Published in final edited form as:
Br J Nutr. 2012 February ; 107(4): 567–572. doi:10.1017/S0007114511003199.

Significant inverse associations of serum n-6 fatty acids with plasma plasminogen activator inhibitor-1

Sunghee Lee[1], **J. David Curb**[2,4,5], **Takashi Kadowaki**[2], **Rhobert W. Evans**[1], **Katsuyuki Miura**[2], **Tomoko Takamiya**[1], **Chol Shin**[6], **Aiman El-Saed**[1], **Jina Choo**[7], **Akira Fujiyoshi**[2], **Teruo Otake**[1], **Sayaka Kadowaki**[2], **Todd Seto**[4], **Kamal Masaki**[4], **Daniel Edmundowicz**[3], **Hirotsugu Ueshima**[2], **Lewis H. Kuller**[1], and **Akira Sekikawa**[1,2]

[1]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[2]Department of Health Science, Shiga University of Medical Science, Otsu, Shiga, Japan

[3]Cardiovascular Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

[4]John A Burns School of Medicine, University of Hawaii, Honolulu, Hawaii

[5]Pacific Health Research Institute, Honolulu, Hawaii

[6]Department of Internal Medicine, Ansan Hospital, Korea University, Ansan, South Korea

[7]College of Nursing, Korea University, Seoul, South Korea

Abstract

Objective—Epidemiological studies suggested that n-6 fatty acids, especially linoleic acid (LA), have beneficial effects on coronary heart disease (CHD), whereas some *in vitro* studies suggested that n-6 fatty acids, specifically arachidonic acid (AA), may have harmful effects. We examined the association of serum n-6 fatty acids with plasminogen activator inhibitor-1 (PAI-1).

Methods and Results—A population-based cross-sectional study recruited 926 randomly selected men aged 40–49 without cardiovascular disease during 2002 to 2006 (310 Caucasian, 313 Japanese, and 303 Japanese-American men). Plasma PAI-1 was analyzed in free form, both active and latent. Serum fatty acids were measured with gas-capillary-liquid-chromatography. To examine the association between total n-6 fatty acids (including LA and AA, respectively) and PAI-1, multivariate regression models were used. After adjusting for confounders, total n-6 fatty acids, LA, and AA were inversely and significantly associated with PAI-1 levels. These associations were consistent across three populations.

Conclusions—Among 915 middle-aged men, serum n-6 fatty acids had significant inverse associations with PAI-1.

CORRESPONDENCE: Akira Sekikawa, MD, PhD, Graduate School of Public Health, University of Pittsburgh, 130 North Bellefield Avenue Suite 546, Pittsburgh, PA 15213, USA, Phone: 1-412-624-3225, Fax: 1-412-383-1956, akira@pitt.edu.

Disclosures

Conflict of interests: none.

Sunghee Lee wrote the first manuscript.
J. David Curb, Takashi Kadowaki, Rbobert W. Evans, Tomoko Takamiya, Aiman El-Saed, Sayaka Kadowaki, Todd Seto, Daniel Edmundowicz, Hirotsugu Ueshima, Lewis Kuller, and Akira Sekikawa collected the data.
Sunghee Lee, Teruo Otake, and Akira Sekikawa analyzed the data.
Sunghee Lee, Rhobert W. Evans, Katsuyuki Miura, Chol Shin, Jina Choo, Akira Fujiyoshi, Teruo Otake, Hirotsugu Ueshima, Lewis H. Kuller, and Akira Sekikawa interpreted the data.
J. David Curb, Rhobert W. Evans, Katsuyuki Miura, Jina Choo, Todd Seto, Kamal Masaki, Daniel Edmundowicz, Lewis H. Kuller and Akira Sekikawa gave critical comments.
Sunghee Lee with Akira Sekikawa revised the paper.

Keywords

plasminogen activator inhibitor-1; linoleic acid; fatty acids

Introduction

Plasminogen activator inhibitor-1 (PAI-1), a primary inhibitor of plasminogen activators, has an anti-fibrinolytic function.[1] High levels of PAI-1 are associated with an increased risk for developing coronary heart disease (CHD) or stroke.[2–4] This increased risk of CHD may be due to promoting platelet adhesion and acute thrombus formation.[4]

Epidemiological studies suggest that linoleic acid (LA), a major component of n-6 fatty acid, has beneficial effects on both CHD and its risk factors, whereas some *in vitro* studies suggest that another n-6 fatty acid, arachidonic acid (AA), may have adverse effects. The Nurses' Health Study showed that a high dietary intake of LA has a strong inverse association with CHD.[5] Additionally, a recent meta-analysis found that dietary intake of LA had a strong inverse association with non-fatal cardiovascular events.[6] The cardioprotective benefits[7] of n-6 fatty acids may be due to decreasing blood pressure,[8] reducing thrombosis,[9] and improving insulin sensitivity.[10] In contrast, several eicosanoids derived from AA are pro-inflammatory and pro-thrombotic, promoting vasoconstriction and enhance platelet aggregation. Thus, AA has been postulated to adversely affect CHD. However, recent studies identified AA-derived eicosanoids to have several beneficial attributes including vasodilation, platelet aggregation inhibition, and anti-inflammatory effects.[11] Interestingly, a recent meta-analysis showed that AA was not associated with fatal or non-fatal cardiovascular events.[6] These contradictory findings suggest that dietary or serum levels of AA have little association with CHD risk, possibly because AA levels are tightly regulated in the human body.[12–14] Although PAI-1 is known to be involved in developing atherothrombosis,[15, 16] very few studies reported their associations with n-6 fatty acids.

The purpose of this study was to test whether higher levels of serum n-6 fatty acids are associated with lower levels of PAI-1 in men aged 40–49. Additionally, we investigated whether higher levels of specific n-6 fatty acids, i.e., LA and AA, are associated with lower levels of PAI-1. To test these hypotheses, we examined data from a population-based cross-sectional study of 926 Caucasian, Japanese, and Japanese-American men aged 40–49 in the Electron-Beam Tomography, Risk Factor Assessment among Japanese and U.S. Men in the Post-World War II Birth Cohort (ERA-JUMP) study.[17]

Methods

Study population

Participants were a randomly selected population-based sample of 926 men aged 40–49 between 2002 and 2006 from three centers: 310 Caucasian men from Allegheny County, Pennsylvania, 313 Japanese men from Kusatsu, Shiga, Japan, and 303 Japanese-American men from Honolulu, Hawaii. Those with clinical cardiovascular or other severe diseases were excluded. Detailed descriptions of the study population were previously published.[17–19] Our final sample was 915 men (304 Caucasian, 313 Japanese, and 298 Japanese-American men) due to 11 missing data. Informed consent from each participant was obtained. The protocol for the study was approved by the Institutional Review Boards of the University of Pittsburgh (Pennsylvania), Shiga University of Medical Science (Otsu, Japan), and the Kuakini Medical Center (Honolulu, Hawaii).

Venipuncture was performed in all participants in the early morning after a 12-hour fast, as previously described.[17] Fasting serum and plasma samples were stored at −80°C, and shipped on dry ice to the Heinz Laboratory at the University of Pittsburgh to examine levels of low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), total cholesterol, triglycerides, insulin, and glucose, as published elsewhere.[17] A calorimetric-competitive-enzyme-linked immunosorbent assay was utilized to assess CRP (C-reactive protein). Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or anti-hypertensive medication usage. Diabetes mellitus was defined as fasting glucose level ≥7mmol/L (126mg/dL) or anti-diabetic medication usage. 'Alcohol drinker' was defined as a man who drinks alcohol two days or more per week. 'Current smoking' was defined as a man who smoked during the prior month.

Measurement of PAI-1

As previously reported,[20, 21] plasma PAI-1 was measured at the clinical biochemistry research laboratory of the University of Vermont. Briefly, plasma PAI-1 levels were analyzed in citrated plasma[20] by a two-site ELISA, which was sensitive to free PAI-1 form but not to complexes between t-PA and PAI-1[21], which was developed by Dr. Collen and colleagues.[22] Inter-assay coefficient of variations (CVs) for PAI-1 was 7.7%.

Measurements of serum fatty acids

To measure serum fatty acids in a percentage of total fatty acid amounts, gas-capillary-liquid chromatography (PerkinElmer Clarus 500; PerkinElmer, Waltham, MA) was performed.[23] The intra-assay CVs of LA (18:2n-6) and AA (20:4n-6) in serum n-6 fatty acids were 1.6% and 2.8%, respectively.[23] The CVs for other fatty acids ranged from 2.5% to 9.8%.[23]

Statistical analyses

Log-transformed PAI-1 was used for the analyses, because the distribution of PAI-1 was skewed. To compare descriptive distributions across centers, an analysis of variance (ANOVA) for a continuous variable and a Mantel-Haenszel test for a categorical variable were performed. When a significant difference among the three groups exists, we examined multiple comparison tests using a Bonferroni test. To pool the data, we tested interactions according to centers on associations of PAI-1 with LA as well as AA. We also assessed the center-specific associations of n-6 fatty acids on PAI-1 according to the three study centers (Table 3). After confirming no interaction and the same direction of the associations by centers, we pooled the data. To estimate the association between serum n-6 fatty acids and PAI-1 levels, we performed multivariate linear regression analyses, adjusting for covariates as followings: in the model I, we adjusted for age and center; in the model II, we further adjusted for body mass index (BMI), current smoking, alcohol drinker, hypertension, and diabetes; in the model III, we further adjusted for LDLc, HDLc, triglycerides, and CRP; in the model IV, we further adjusted for marine n-3 and *trans* fatty acids. Because some eicosanoids of n-3 fatty acids were reported to inhibit the production of AA derived eicosanoids,[11] we tested marine n-3 as well as *trans* fatty acids as covariates. The level of significance was considered to be $p<0.05$. All reported p-values were based on two-sided tests. All statistical analyses were performed using SAS 9.2 for Windows (SAS Institute, Inc., Cary, North Carolina, U.S.).

Results

General characteristics of the 915 study participants are shown in Table 1. The average age was 45 years old. In the total study population, participants with hypertension and diabetes

were 24.9% and 7.5%, respectively. Median level (interquartile range) of PAI-1 was 37.4 ng/mL (23.3 to 58.4).

Serum proportions of fatty acids are listed in Table 2. Total n-6, total n-3, saturated, and monounsaturated fatty acids made up 39.0%, 6.5%, 31.2%, and 20.4%, respectively. LA and AA were 28.8% and 8.1%, respectively. A correlation coefficient between LA and AA was 0.06 (p=0.059).

Pooled and center-specific analyses reveal that serum n-6 fatty acids were inversely associated with PAI-1 in the total population as well as in each of three different populations (Table 3). Pooled analyses showed that serum total n-6 fatty acids, LA, and AA had significant inverse associations with PAI-1 levels, even after adjusting for covariates. In center-specific analyses, PAI-1 had significant inverse associations with total n-6 fatty acids and LA over three study populations. These significant associations remained after multivariate adjustments. PAI-1 was inversely associated with AA. No significant interaction existed in the associations of serum n-6 fatty acids, LA, and AA with PAI-1 according to the study centers (p=0.09, 0.08, and 0.24, respectively). Standard parameter estimates indicate a standard deviation (SD) unit change in log-transformed PAI-1 per a 1 SD unit increase in serum n-6 fatty acids.

Discussion

This population-based cross-sectional study found that total serum n-6 fatty acids were inversely and significantly associated with PAI-1 among 915 men, aged 40–49. Additionally, both LA and AA showed significant inverse associations with PAI-1 levels (both, p<0.0001).

Our present study may provide a novel mechanism on the cardioprotective benefits of n-6 fatty acids by improving the fibrinolytic response, such as reducing PAI-1. Our finding of an inverse association between serum n-6 fatty acids and PAI-1 is consistent with the results of several previous studies,[24, 25] but not all.[26] These findings of n-6 fatty acids suggest a favorable fibrinolytic response on vascular thrombosis, including a decrease in platelet aggregation. Fleischman *et al*. found an increased platelet aggregation time (p<0.05) and a decreased disaggregation time (p<0.01) on a dietary LA in each for two weeks from about 2.9% to about 5.0% of energy among 66 subjects.[24] A crossover study by Thijssen *et al*. also demonstrated an increased platelet aggregation time while on a LA diet in comparison to on a saturated fatty acid diet in 18 men (p=0.04).[27] O'Brien *et al*. conducted a clinical trial in 39 healthy men for six weeks with either a PUFA diet (sunflower oil based foods, 65% LA) replaced for saturated fat or a normal diet.[25] They found the decreased platelet count (p=0.01), and the increased bleed time (p=0.05).[25] Further, previous studies, including the Nurses' Health Study,[5] have suggested that n-6 fatty acids lower the CHD risk, through a decrease in blood pressure,[8] a reduction of thrombosis,[9] and an improvement in insulin sensitivity.[10]

Our results of the inverse association between serum n-6 fatty acids and PAI-1 are partially inconsistent with the results of a previous study. Byberg *et al*. showed that PAI-1 activity has a significant inverse association with serum LA but a significant positive association with serum AA in their sub-analysis with 381 men from a population-based cross-sectional sample of 871 men aged 70 years.[26] The discrepancy in the association of PAI-1 with AA may be attributed to different measurements of PAI-1 and fatty acids or to different ages of participants. In measurements of PAI-1 and fatty acids, Byberg *et al*. measured PAI-1 activity (i.e., a free active form) and serum cholesterol ester for fatty acid measurements in older participants (mean average 70 years), whereas we measured total plasma PAI-1 levels

(i.e., free active, free latent, and complex with t-PA forms) and fatty acids in serum cholesteryl ester, phospholipids, and triglycerides, in middle-aged men (ages 40–49). Although the previous study demonstarted a linear association between PAI-1 activity and PAI-1 antigen (r=0.80 in platelet-poor plasma; r=0.88 in platelet-rich plasma), about 66.7% of PAI-1 antigen in plasma was active.[22] Considering very short half-life of PAI-1 levels, and various processes (e.g., temperature, time, or pH) for handling the blood samples, as well as significant diurnal change of PAI-1, the PAI-1 antigen measurement as in our study may have the advantage of detecting comprehensive forms of relatively unstable total plasma PAI-1, rather than measuring only an active form.

Future studies are required in order to elucidate possible reasons of the discrepancy between *in vitro* and the population studies. Several *in vitro* studies have shown that LA increases the secretion of PAI-1 in HepG2 cells.[28, 29] *In vitro* studies reported that AA-produced eicosanoids promoted neutrophil adhesion[30] and IL-1β production by human monocytes[31]. However, more recent studies demonstrated no effect or a beneficial effect. A double-blind placebo-controlled study with an AA supplementation of 840 mg/day for four weeks demonstrated no effect on platelet aggregation in 24 healthy Japanese men who had relatively high levels of fish oil consumption.[32] In another clinical trial of 10 healthy men taking a 200mg/day vs. 1,500mg/day AA regimen, Nelson *et al*. found a borderline significance between higher AA intake and prolonged bleeding time (p=0.06).[33] Although several AA-derived eicosanoids may indeed have a pro-inflammatory role, recent studies suggest that several AA-derived eicosanoids may play an anti-inflammatory role.[34]

Mechanisms responsible for the association of n-6 fatty acids with PAI-1 require future studies. However, two possibilities exist. First, n-6 fatty acids may delay platelet aggregation so that PAI-1, acting as an acute-phase reactant, is decreased within hemodynamic balance and thrombotic response, during vascular injury, in atherosclerosis and CHD. Several previous studies have shown that LA reduces platelet aggregation.[24, 25] Second, LA may reduce PAI-1 levels through its cholesterol lowering effect. A previous study from ERA-JUMP found that higher levels of serum LA and AA were associated with lower levels of LDL and VLDL.[35] An *in vitro* study showed that VLDL led to increased PAI-1.[36] These reduced cholesterol levels may improve or modulate the fibrinolytic response.

We additionally examined the associations of other fatty acids with PAI-1. Serum marine n-3 fatty acids over the three populations showed little overlapped distributions and the lack of consistent associations (supplemental table 1). *Trans* fatty acids of three populations were positively associated with PAI-1 (supplemental table 2).

The strengths of the present study include the following: a) the association was examined in a randomly selected population-based sample; and b) the sample size was relatively large. However, this study also has several limitations: a) the cross-sectional study design could not assess a causality; b) the study population included only men aged 40–49 years, which may limit the generalizability to other populations; and c) as an observational study, this present study may include residual confounding or potentially unmeasured factors, such as total energy intake.[37]

In conclusion, serum n-6 fatty acids were inversely and significantly associated with PAI-1 levels in a population-based cross-sectional study. Total n-6 fatty acids, especially LA and AA, were inversely and significantly associated with PAI-1 levels in both univariate and multivariate models. These findings suggest that n-6 fatty acids may have favorable effects on fibrinolysis. A future study to examine the causality between n-6 fatty acid and PAI-1 is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources

This research was funded by the National Institutes of Health R01 HL68200 and HL071561 and from the Japanese Ministry of Education, Culture, Sports, Science and Technology, B 16790335 and A 13307016

References

1. Ha H, Oh EY, Lee HB. The role of plasminogen activator inhibitor 1 in renal and cardiovascular diseases. Nat Rev Nephrol. 2009 Apr; 5(4):203–211. [PubMed: 19322185]
2. Senno SL, Pechet L. Clinical implications of elevated PAI-1 revisited: multiple arterial thrombosis in a patient with essential thrombocythemia and elevated plasminogen activator inhibitor-1 (PAI-1) levels: a case report and review of the literature. J Thromb Thrombolysis. 1999 Aug; 8(2):105–112. [PubMed: 10436140]
3. Folsom AR, Aleksic N, Park E, et al. Prospective study of fibrinolytic factors and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb Vasc Biol. 2001 Apr; 21(4):611–617. [PubMed: 11304480]
4. Thogersen AM, Jansson JH, Boman K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation. 1998 Nov 24; 98(21):2241–2247. [PubMed: 9826309]
5. Oh K, Hu FB, Manson JE, et al. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. Am J Epidemiol. 2005 Apr 1; 161(7):672–679. [PubMed: 15781956]
6. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis. 2007 Jul; 193(1):1–10. [PubMed: 17507020]
7. Laaksonen DE, Nyyssonen K, Niskanen L, et al. Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. Arch Intern Med. 2005 Jan 24; 165(2):193–199. [PubMed: 15668366]
8. Zhao WS, Zhai JJ, Wang YH, et al. Conjugated linoleic acid supplementation enhances antihypertensive effect of ramipril in Chinese patients with obesity-related hypertension. Am J Hypertens. 2009 Jun; 22(6):680–686. [PubMed: 19300423]
9. Knapp HR. Dietary fatty acids in human thrombosis and hemostasis. Am J Clin Nutr. 1997 May; 65(5 Suppl):1687S–1698S. [PubMed: 9129511]
10. Laaksonen DE, Lakka TA, Lakka HM, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med. 2002 Jun; 19(6):456–464. [PubMed: 12060056]
11. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie. 2009 Jun; 91(6):791–795. [PubMed: 19455748]
12. Nelson GJ, Kelley DS, Emken EA, et al. A human dietary arachidonic acid supplementation study conducted in a metabolic research unit: rationale and design. Lipids. 1997 Apr; 32(4):415–420. [PubMed: 9113630]
13. 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 Feb 17; 119(6):902–907. [PubMed: 19171857]
14. Willett WC. The role of dietary n-6 fatty acids in the prevention of cardiovascular disease. J Cardiovasc Med (Hagerstown). 2007 Sep.8 Suppl 1:S42–S45. [PubMed: 17876199]

15. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. N Engl J Med. 2000 Jun 15; 342(24):1792–1801. [PubMed: 10853003]
16. Levenson J, Giral P, Razavian M, et al. Fibrinogen and silent atherosclerosis in subjects with cardiovascular risk factors. Arterioscler Thromb Vasc Biol. 1995 Sep; 15(9):1263–1268. [PubMed: 7670937]
17. 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 Mar 15; 165(6):617–624. [PubMed: 17244636]
18. Kagan A. The Honolulu Heart Program: an epidemiological study of coronary heart disease and stroke. 1996
19. Abbott RD, Ueshima H, Rodriguez BL, et al. Coronary artery calcification in Japanese men in Japan and Hawaii. Am J Epidemiol. 2007 Dec 1; 166(11):1280–1287. [PubMed: 17728270]
20. Declerck PJ, Collen D. Measurement of plasminogen activator inhibitor 1 (PAI-1) in plasma with various monoclonal antibody-based enzyme-linked immunosorbent assays. Thromb Res Suppl. 1990; 10:3–9. [PubMed: 2315899]
21. Macy EM, Meilahn EN, Declerck PJ, et al. Sample preparation for plasma measurement of plasminogen activator inhibitor-1 antigen in large population studies. Arch Pathol Lab Med. 1993 Jan; 117(1):67–70. [PubMed: 8418765]
22. Declerck PJ, Alessi MC, Verstreken M, et al. Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. Blood. 1988 Jan; 71(1):220–225. [PubMed: 3257145]
23. 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 Aug 5; 52(6):417–424. [PubMed: 18672160]
24. Fleischman AI, Justice D, Bierenbaum ML, et al. Beneficial effect of increased dietary linoleate upon in vivo platelet function in man. J Nutr. 1975 Oct; 105(10):1286–1290. [PubMed: 1159521]
25. O'Brien JR, Etherington MD, Jamieson S. Effect of a diet of polyunsaturated fats on some platelet-function tests. Lancet. 1976 Nov 6; 2(7993):995–996. [PubMed: 62266]
26. Byberg L, Smedman A, Vessby B, et al. Plasminogen activator inhibitor-1 and relations to fatty acid composition in the diet and in serum cholesterol esters. Arterioscler Thromb Vasc Biol. 2001 Dec; 21(12):2086–2092. [PubMed: 11742889]
27. Thijssen MA, Hornstra G, Mensink RP. Stearic, oleic, and linoleic acids have comparable effects on markers of thrombotic tendency in healthy human subjects. J Nutr. 2005 Dec; 135(12):2805–2811. [PubMed: 16317124]
28. Banfi C, Rise P, Mussoni L, et al. Linoleic acid enhances the secretion of plasminogen activator inhibitor type 1 by HepG2 cells. J Lipid Res. 1997 May; 38(5):860–869. [PubMed: 9186904]
29. Ye P, He YL, Wang Q, et al. The alteration of plasminogen activator inhibitor-1 expression by linoleic acid and fenofibrate in HepG2 cells. Blood Coagul Fibrinolysis. 2007 Jan; 18(1):15–19. [PubMed: 17179821]
30. Bates EJ, Ferrante A, Smithers L, et al. Effect of fatty acid structure on neutrophil adhesion, degranulation and damage to endothelial cells. Atherosclerosis. 1995 Aug; 116(2):247–259. [PubMed: 7575780]
31. Sinha B, Stoll D, Weber PC, et al. Polyunsaturated fatty acids modulate synthesis of TNF-[alpha] and Interleukin-1[beta] by human MNC in vitro. Cytokine. 1991; 3(5):457–457.
32. Kusumoto A, Ishikura Y, Kawashima H, et al. Effects of arachidonate-enriched triacylglycerol supplementation on serum fatty acids and platelet aggregation in healthy male subjects with a fish diet. Br J Nutr. 2007 Sep; 98(3):626–635. [PubMed: 17445350]
33. Nelson GJ, Schmidt PC, Bartolini G, et al. The effect of dietary arachidonic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids. 1997 Apr; 32(4):421–425. [PubMed: 9113631]
34. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. Prog Lipid Res. 2008 Mar; 47(2):147–155. [PubMed: 18198131]

35. Choo J, Ueshima H, Curb JD, et al. Serum n-6 fatty acids and lipoprotein subclasses in middle-aged men: the population-based cross-sectional ERA-JUMP study. Am J Clin Nutr. 2010 May; 91(5):1195–1203. [PubMed: 20357040]

36. Nilsson L, Gafvels M, Musakka L, et al. VLDL activation of plasminogen activator inhibitor-1 (PAI-1) expression: involvement of the VLDL receptor. J Lipid Res. 1999 May; 40(5):913–919. [PubMed: 10224160]

37. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997 April 1; 65(4):1220S–1228. 1997. [PubMed: 9094926]

Table 1

General characteristics of the study participants

	Total (n=915)	Caucasian men (n=304)	Japanese men (n=313)	Japanese-American men (n=298)
Age (year)	45.4	45.0	45.1†	46.1‡
BMI (kg/m^2)	26.5	27.9*	23.7†	28.0
Waist circumference (cm)	92.6	98.7*	85.2†	94.0‡
Systolic blood pressure (mmHg)	125.1	122.6	125.0	127.6‡
Diastolic blood pressure (mmHg)	75.8	73.1*	76.5	77.7‡
Hypertension (%)	24.9	15.1*	26.5	33.2‡
LDL cholesterol (mg/dL)	129.5	134.7	132.2†	121.4‡
Triglyceride (mg/dL)§	140.5 (96.0–224.0)	128.0(92.0–185.5)	137(103.0–182.0)	140.5(96.0–224.0)
HDL cholesterol (mg/dL)	50.9	47.8*	54.1†	50.7
Total cholesterol (mg/dL)	212.0	212.1	217.2†	206.5
Glucose (mg/dL)	106.7	101.3*	106.8†	112.0‡
Insulin (μIU/mL)	13.5	15.2*	10.3†	15.1
Diabetes (%)	7.5	3.3	6.1†	13.4‡
Current smoker (%)	23.5	7.2*	49.2†	13.1
Alcohol drinker (%)	49.6	44.1*	67.1†	36.9
C-reactive protein (mg/l)§	0.6 (0.3–1.3)	1.0 (0.5–1.8)	0.3 (0.2–0.7)	0.7 (0.3–1.3)
PAI-1 (ng/mL)§	37.4 (23.3–58.4)	27.4 (15.7–41.3)*	41.2 (24.3–67.3)	45.9 (31.6–61.7)‡

BMI=body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein;

Significance test was based on ANOVA, followed by Bonferroni test if the overall ANOVA was significant.

*Under Bonferroni test, significant difference between Caucasian and Japanese men, p<0.01

†Under Bonferroni test, significant difference between Japanese and Japanese-American men, p<0.01

‡Under Bonferroni test, significant difference between Caucasian and Japanese-American men, p<0.01;

§Median (interquartile range)

Table 2

Distribution of serum fatty acids (%)

	Total (n=915)	Caucasian men (n=304)	Japanese men (n=313)	Japanese-American men (n=298)
Polyunsaturated fatty acids	45.4	45.6*	44.3†	46.5
Total n-6 fatty acids	39.0	41.3*	34.7†	41.1
Linoleic acid	28.8	29.9*	26.5†	30.0
Arachidonic acid	8.1	9.0*	6.6†	8.9
Total n-3 fatty acids	6.5	4.2*	9.6†	5.4‡
Marine n-3 fatty acids	6.0	3.8*	9.3†	4.9‡
α-linolenic fatty acids	0.3	0.3*	0.2†	0.4‡
Monounsaturated fatty acids	20.4	20.3*	21.2†	19.6
Saturated fatty acids	31.2	30.9*	31.7†	30.9
Trans fatty acids	0.8	1.0*	0.6†	0.9‡

Total n-6 fatty acids indicate the sum of linoleic acid (18:2n-6), gamma-linoleic acid (18:3n-6), dihomo-gamma-linolenic acid (20:3n-6) and arachidonic acid (20:4n-6).

Marine-derived n-3 fatty acids were defined as eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3).

Total n-3 fatty acids indicate marine-derived n-3 fatty acids, eicosatetraenoic acid (20:4n-3) and α-linolenic acid (22:18n-3).

Saturated fatty acids indicate the sum of myristic aicd (14:0), palmitic acid (16:0) and stearic acid (18:0).

Monounsaturated fatty acids indicate the sum of palmitoleic acid (16:1n-7), oleic acid (18:1n-9), and cis-vaccenic acid (18:1n-7).

Trans fatty acids indicate the sum of palmitelaidic acid (16n-7:1t), trans 9-octadecanoic acid (18n-9:1t) and linolelaidic acid (18n-6:2tt).

Significance test was based on ANOVA followed by Bonferroni test if the overall ANOVA was significant.

*Under Bonferroni test, significant difference between Caucasian and Japanese men, $p<0.01$

†Under Bonferroni test, significant difference between Japanese and Japanese-American men, $p<0.01$

‡Under Bonferroni test, significant difference between Caucasian and Japanese-American men, $p<0.01$

Table 3

Center-specific and pooled associations between n-6 fatty acids and log(PAI-1)

	Standardized parameter estimates			
	Total (n=915)	**Caucasian men (n=304)**	**Japanese men (n=313)**	**Japanese-American men (n=298)**
Total n-6 Fatty acids				
Univariate	−0.27*	−0.32*	−0.25*	−0.27*
Model I	−0.32*	−0.32*	−0.24*	−0.27*
Model II	−0.24*	−0.18*	−0.20*	−0.21*
Model III	−0.13*	−0.06*	−0.12*	−0.10*
Model IV	−0.14*	−0.09*	−0.12*	−0.09*
Linoleic acid				
Univariate	−0.23*	−0.29*	−0.20**	−0.23*
Model I	−0.24*	−0.29*	−0.19**	−0.23*
Model II	−0.17*	−0.15*	−0.15*	−0.18*
Model III	−0.10*	−0.07*	−0.07*	−0.12*
Model IV	−0.10*	−0.08*	−0.04*	−0.11*
Arachidonic acid				
Univariate	−0.16*	−0.13***	−0.19**	−0.11
Model I	−0.14*	−0.13	−0.18**	−0.11
Model II	−0.10*	−0.08*	−0.17*	−0.07*
Model III	−0.01*	−0.003*	−0.10*	0.04*
Model IV	−0.01*	−0.003*	−0.10*	0.04*

* $p<0.001$;

** $p<0.01$;

*** $p<0.05$

Total n-6 fatty acids indicate the sum of linoleic acid (18:2n-6), gamma-linoleic acid (18:3n-6), dihomo-gamma-linolenic acid (20:3n-6) and arachidonic acid (20:4n-6).

Marine-derived n-3 fatty acids were defined as eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3).

Total n-3 fatty acids indicate marine-derived n-3 fatty acids, eicosatetraenoic acid (20:4n-3) and α-linolenic acid (22:18n-3).

Trans fatty acids indicate the sum of palmitelaidic acid (16n-7:1t), trans 9-octadecanoic acid (18n-9:1t) and linolelaidic acid (18n-6:2tt).

Model I: adjusted for age;

Model II: additionally adjusted for BMI, current smoking, alcohol drinker, hypertension, and diabetes;

Model III: further adjusted for LDL cholesterol, HDL cholesterol, triglycerides, and CRP (C-reactive protein);

Model IV: continuously adjusted for marine n-3 fatty acids, and trans fatty acids.