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

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Abstract

Epidemiological studies suggested that n-6 fatty acids, especially linoleic acid (LA), have beneficial effects on CHD, whereas some in vitro studies have 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). A population-based cross-sectional study recruited 926 randomly selected men aged 40–49 years without CVD during 2002–2006 (310 Caucasian, 313 Japanese and 303 Japanese-American men). Plasma PAI-1 was analysed 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) 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. Among 915 middle-aged men, serum n-6 fatty acids had significant inverse associations with PAI-1.

Key words: Plasminogen activator inhibitor-1: Linoleic acid: Fatty acids

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 CHD or stroke2–4. 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 acids, 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 enhancing platelet aggregation. Thus, AA has been postulated to adversely affect CHD. However, recent studies have 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).

Abbreviations: AA, arachidonic acid; ERA-JUMP, Electron Beam Tomography, Risk Factor Assessment among Japanese and US Men in the Post-World War II Birth Cohort; LA, linoleic acid; PAI-1, plasminogen activator inhibitor-1.

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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. Although PAI-1 is known to be involved in developing atherothrombosis, very few studies have reported their associations with n-6 fatty acids.

The purpose of the present 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 years. 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 years in the Electron Beam Tomography, Risk Factor Assessment among Japanese and US Men in the Post-World War II Birth Cohort (ERA-JUMP) study.

Methods

Study population

The participants were a randomly selected population-based sample of 926 men aged 40–49 years between 2002 and 2006 from three centres: 310 Caucasian men from Allegheny County, Pennsylvania; 313 Japanese men from Kusatsu, Shiga, Japan; 303 Japanese-American men from Honolulu, Hawaii. Those with clinical CVD or other severe diseases were excluded. Detailed descriptions of the study population have been published previously. Our final sample was 915 men (304 Caucasian, 313 Japanese and 298 Japanese-American men) due to eleven 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, to examine the levels of LDL-cholesterol, HDL-cholesterol, TAG, insulin and glucose, as well as systolic blood pressure, diastolic blood pressure, and C-reactive protein; in model IV, we further adjusted for tissue plasminogen activator and PAI-1, which was developed by Dr Collen and colleagues. The inter-assay CV 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, Walhain, MA, USA) was performed. The intra-assay CV of LA (18:2n-6) and AA (20:4n-6) in serum n-6 fatty acids were 1.6 and 2.8%, respectively. The CV for other fatty acids ranged from 2.5 to 9.8%.

Statistical analyses

Log-transformed PAI-1 was used for the analyses, because the distribution of PAI-1 was skewed. To compare descriptive distributions across centres, an ANOVA for a continuous variable and the Mantel–Haenszel test for a categorical variable were performed. When a significant difference among the three groups existed, we examined multiple comparison tests using Bonferroni’s test. To pool the data, we tested interactions according to the centres on associations of PAI-1 with LA as well as with AA. We also assessed the centre-specific associations of n-6 fatty acids on PAI-1 according to the three study centres (Table 3). After confirming no interaction and the same direction of the associations by centres, 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 follows: in model I, we adjusted for age and centre; in model II, we further adjusted for BMI, current smoking, alcohol drinking, hypertension and diabetes; in model III, we further adjusted for LDL-cholesterol, HDL-cholesterol, TAG and C-reactive protein; in 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, 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, NC, USA).

Results

The general characteristics of the 915 study participants are shown in Table 1. The average age of the study participants was 45 years. In the total study population, there were 24.9 and 7.5% participants with hypertension and diabetes, respectively. The median level (interquartile range) of PAI-1 was 37.4 (23.3–58.4) ng/ml.

Serum proportions of fatty acids are listed in Table 2. Total n-6, total n-3, SFA and MUFA made up 39.0, 6.5, 31.2 and 20.4%, respectively. LA and AA were 28.8 and 81.1%.
Pooled analyses showed that serum total fatty acids were inversely associated with PAI-1 in the total population as well as in each of three different populations. The correlation coefficient between LA and AA, LA and AA, had significant inverse associations with PAI-1 levels, even after adjusting for covariates. In centre-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.

### Table 1. General characteristics of the study participants*

(Means or medians with interquartile ranges)

<table>
<thead>
<tr>
<th></th>
<th>Total (n 915)</th>
<th>Caucasian men (n 304)</th>
<th>Japanese men (n 313)</th>
<th>Japanese-American men (n 298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.4</td>
<td>45.0</td>
<td>45.1</td>
<td>46.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5</td>
<td>27.9</td>
<td>27.7</td>
<td>28.0</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>92.6</td>
<td>98.7§</td>
<td>85.2‡</td>
<td>94.0‡</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125.1</td>
<td>122.6</td>
<td>125.0</td>
<td>127.6‡</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.8</td>
<td>73.1§</td>
<td>76.5</td>
<td>77.7†</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>24.9</td>
<td>15.1§</td>
<td>26.5</td>
<td>33.2‡</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/l)</td>
<td>1295</td>
<td>1347</td>
<td>1322†</td>
<td>1214‡</td>
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<tr>
<td>TAG (mg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1405</td>
<td>1280</td>
<td>1370</td>
<td>1405</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>960–2240</td>
<td>920–1855</td>
<td>1030–1820</td>
<td>960–2240</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/l)</td>
<td>509</td>
<td>478§</td>
<td>541†</td>
<td>507</td>
</tr>
<tr>
<td>Total cholesterol (mg/l)</td>
<td>2120</td>
<td>2121</td>
<td>2172†</td>
<td>2065</td>
</tr>
<tr>
<td>Glucose (mg/l)</td>
<td>1067</td>
<td>1013§</td>
<td>1068†</td>
<td>1120‡</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>13.5</td>
<td>15.2§</td>
<td>10.3†</td>
<td>15.1</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7.5</td>
<td>3.3</td>
<td>6.1†</td>
<td>13.4‡</td>
</tr>
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<td>Current smoker (%)</td>
<td>23.5</td>
<td>7.2§</td>
<td>49.2‡</td>
<td>13.1</td>
</tr>
<tr>
<td>Alcohol drinker (%)</td>
<td>49.6</td>
<td>44.1§</td>
<td>67.1†</td>
<td>36.9</td>
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<tr>
<td>CRP (mg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.6</td>
<td>3.1</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.3–1.3</td>
<td>0.5–1.8</td>
<td>0.2–0.7</td>
<td>0.3–1.3</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>37.4</td>
<td>27.4§</td>
<td>41.2</td>
<td>45.9†</td>
</tr>
<tr>
<td>Median</td>
<td>23.3–58.4</td>
<td>15.7–41.3</td>
<td>24.3–67.3</td>
<td>31.6–61.7</td>
</tr>
</tbody>
</table>

### Table 2. Distribution of serum fatty acids (%)*

<table>
<thead>
<tr>
<th></th>
<th>Total (n 915)</th>
<th>Caucasian men (n 304)</th>
<th>Japanese men (n 313)</th>
<th>Japanese-American men (n 298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total n-6 fatty acids§</td>
<td>45.4</td>
<td>45.6†</td>
<td>44.3‡</td>
<td>46.5</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>28.8</td>
<td>29.9†</td>
<td>26.5§</td>
<td>30.0</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>8.1</td>
<td>9.0†</td>
<td>6.6‡</td>
<td>8.9</td>
</tr>
<tr>
<td>Total n-3 fatty acids</td>
<td>6.5</td>
<td>4.2†</td>
<td>9.6§</td>
<td>5.4†</td>
</tr>
<tr>
<td>α-Linolenic fatty acids</td>
<td>6.0</td>
<td>3.8†</td>
<td>9.3§</td>
<td>4.9†</td>
</tr>
<tr>
<td>Marine n-3 fatty acids**</td>
<td>0.3</td>
<td>0.3†</td>
<td>0.2‡</td>
<td>0.4†</td>
</tr>
<tr>
<td>MFUFA††</td>
<td>20.4</td>
<td>20.3†</td>
<td>21.2†</td>
<td>19.6</td>
</tr>
<tr>
<td>SFA‡‡</td>
<td>31.2</td>
<td>30.9†</td>
<td>31.7</td>
<td>30.9</td>
</tr>
<tr>
<td>trans-Fatty acids§§</td>
<td>0.8</td>
<td>1.0†</td>
<td>0.6†</td>
<td>0.9†</td>
</tr>
</tbody>
</table>

*Significance test was based on ANOVA followed by Bonferroni’s test if the overall ANOVA was significant.
† Significant difference between Caucasian and Japanese-American men as determined by Bonferroni’s test (P<0.01).
‡ Significant difference between Japanese and Japanese-American men as determined by Bonferroni’s test (P<0.01).
§ Total n-6 fatty acids indicate 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).
** Marine-derived n-3 fatty acids include marine-derived n-3 fatty acids, eicosatetraenoic acid (20:4n-3) and α-linolenic acid (22:18n-3).
†† Significant difference between Caucasian and Japanese-American men as determined by Bonferroni’s test (P<0.01).
‡‡ SFA indicate the sum of myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0).
§§ trans-Fatty acids indicate the sum of palmitoleic acid (16:1n-7), oleic acid (18:1n-9) and cis-vaccenic acid (18:1n-7).
centres \( (P = 0.09, 0.08 \text{ and } 0.24, \text{ respectively}) \). Standard parameter estimates indicate a standard deviation unit change in log-transformed PAI-1 per 1 sd unit increase in serum n-6 fatty acids.

Discussion

The present 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 years. Additionally, both LA and AA showed significant inverse associations with PAI-1 levels \( (P < 0.0001) \).

The 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. The present 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 favourable fibrinolytic response on vascular thrombosis, including a decrease in platelet aggregation. Fleischman et al.\(^ {24} \) found an increased platelet aggregation time \( (P < 0.05) \) and a decreased disaggregation time \( (P < 0.01) \) on a dietary LA in each participant for 2 weeks from about 29% to about 50% of energy among sixty-six subjects. A cross-over study by Thijsen et al.\(^ {27} \) also demonstrated an increased platelet aggregation time while on a LA diet in comparison with a SFA diet in eighteen men \( (P = 0.04) \). O’Brien et al.\(^ {25} \) conducted a clinical trial in thirty-nine healthy men for 6 weeks with either a PUFA diet (sunflower oil-based foods, 65% LA) replaced for saturated fat or a normal diet. They found decreased platelet count \( (P = 0.01) \) and increased bleed time \( (P = 0.05) \)\(^ {25} \). Furthermore, 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\(^ {9} \), a reduction in thrombosis\(^ {9} \) and an improvement in insulin sensitivity\(^ {10} \).

The present 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.\(^ {26} \) 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. 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 the measurements of PAI-1 and fatty acids, Byberg et al. measured PAI-1 activity (i.e. a free active form) and serum cholesterol esters 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 tissue plasminogen activator forms) and fatty acids in serum cholesterol esters, phospholipids and TAG, in middle-aged men (aged 40–49 years). Although the previous study demonstrated 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 the 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 in PAI-1, the PAI-1 antigen measurement as in the present 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 elucidate possible reasons of the discrepancy between the in vitro and population studies. Several in vitro studies have shown that LA increases the secretion of PAI-1 in HepG2 cells\(^{(28,29)}\). In vitro studies have reported that AA-produced eicosanoids promoted neutrophil adhesion\(^{(30)}\) and IL-1ß production by human monocytes\(^{(30)}\). However, more recent studies have demonstrated no effect or a beneficial effect. A double-blind placebo-controlled study with an AA supplementation of 840 mg/d for 4 weeks demonstrated no effect on platelet aggregation in twenty-four healthy Japanese men who had relatively high levels of fish oil consumption\(^{(32)}\). In another clinical trial of ten healthy men taking a 200 \(\mu\)g 1500 mg/d AA regimen, Nelson et al\(^{(33)}\) found borderline significance between higher AA intake and prolonged bleeding time \((P = 0.06)\). Although several AA-derived eicosanoids may indeed have a pro-inflammatory role, recent studies have suggested 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 the haemodynamic 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 (see Table S1 of the supplementary material, available online http://www.journals.cambridge.org/bjn). Trans-Fatty acids of three populations were positively associated with PAI-1 (see Table S2 of the supplementary material, available online http://www.journals.cambridge.org/bjn).

The strengths of the present study include the following: (1) the association was examined in a randomly selected population-based sample and (2) the sample size was relatively large. However, the present study also has several limitations: (1) the cross-sectional study design could not assess causality; (2) the study population included only men aged 40–49 years, which may limit the generalisability to other populations and (3) as an observational study, the 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 favourable effects on fibrinolysis. A future study to examine the causality between \(n\)-6 fatty acid and PAI-1 is warranted.

Acknowledgements


References


