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A Cross-sectional Study of Endogenous Tissue Plasminogen Activator, Total Cholesterol, HDL Cholesterol, and Apolipoproteins A-I, A-II, and B-100

Paul M. Ridker, Douglas E. Vaughan, Meir J. Stampfer, Frank M. Sacks, Charles H. Hennekens

Elevated levels of endogenous tissue-type plasminogen activator (t-PA) appear to be a marker for preclinical atherosclerosis. At present, however, data describing potential relations between plasma t-PA level and established lipid risk factors for coronary atherosclerosis are sparse. To explore these potential relations, we measured plasma levels of t-PA antigen (t-PA:ag) in 633 apparently healthy men in the Physicians' Health Study as well as total cholesterol, high-density lipoprotein (HDL) cholesterol, HDL2 cholesterol, HDL3 cholesterol, and apolipoproteins A-I, A-II, and B-100. Overall, plasma t-PA:ag levels were inversely correlated with HDL cholesterol ($r=-.1616$, $P<.0005$), HDL2 cholesterol ($r=-.1632$, $P<.0005$), and HDL3 cholesterol ($r=-.0927$, $P=.019$). In stratified analyses, the inverse association between t-PA:ag and HDL cholesterol was present among frequent ($P$ trend=$.002$) and infrequent ($P$ trend=$.004$) consumers of alcohol as well as among the subgroups of frequent exercisers ($P$ trend<$.001$), men in the lower half of body mass index ($P$ trend=$.001$), and nonsmokers ($P$ trend<$.001$). In contrast, there was no association between t-PA:ag and total cholesterol ($r=.0219$, $P=.58$), whereas relations of t-PA:ag with apolipoproteins A-I, A-II, and B-100 were minimal and of borderline significance. These data indicate that plasma levels of endogenous t-PA:ag are inversely related to HDL cholesterol as well as HDL2 and HDL3 cholesterol. Further research will be required to determine whether these modest but highly significant associations are due to a direct effect of lipids on fibrinolytic function or to independent associations of both t-PA:ag and lipids with atherosclerosis or are mediated by a third unmeasured variable. (Arterioscler Thromb. 1993;13:1587-1592.)

KEY WORDS • t-PA • cholesterol • HDL cholesterol • apolipoproteins • epidemiology • atherosclerosis • thrombosis

To explore possible interrelations between fibrinolytic function and lipid fractions, we measured endogenous t-PA antigen (t-PA:ag) levels in 633 apparently healthy men participating in the Physicians' Health Study as well as plasma levels of total cholesterol, HDL cholesterol, HDL2 cholesterol, HDL3 cholesterol, and apolipoproteins A-I, A-II, and B-100.

Methods

Population and Specimen Collection

The Physicians' Health Study is a randomized, double-blind, placebo-controlled 2x2 factorial design trial of aspirin and $\beta$-carotene among 22,071 US male physicians aged 40 to 84 years. Exclusion criteria included a history of myocardial infarction, stroke, or transient ischemic attack; cancer (except nonmelanoma skin cancer); current renal or liver disease; peptic ulcer or gout; contraindication to aspirin consumption; current use of aspirin, other platelet-active drugs, or nonsteroidal anti-inflammatory agents; and current use of vitamin A or $\beta$-carotene supplement.

Before randomization, willing and potentially eligible subjects were given active aspirin and $\beta$-carotene placebo during an 18-week run-in to identify good compli-
ers for long-term follow-up. During the run-in, all participants were asked to provide baseline blood plasma samples. Kits including EDTA Vacutainer tubes and plastic collection vials were sent to each doctor along with instructions for blood drawing. Participants were asked to have their blood drawn and centrifuged in the tubes and return the plasma, accompanied by cold pack, by overnight courier. Upon arrival of the specimens in the laboratory, aliquots were promptly taken and stored at -80°C. Each freezer is equipped with an alarm and a liquid CO₂ backup system. Therefore, no specimen has inadvertently thawed. Approximately 68% of the randomized physicians returned baseline blood sample specimens (14,916 subjects).

This report describes interrelations of endogenous t-PA:ag level with a variety of lipid parameters among 633 healthy men whose samples were selected as controls for those who later developed myocardial infarction, stroke, deep venous thrombosis, pulmonary embolism, or peripheral vascular disease during an average follow-up period of 60.2 months. At that time, the blinded aspirin component of the Physicians’ Health Study was terminated early, principally because of the emergence of a statistically extreme 44% decrease in risk of a first myocardial infarction among those assigned to aspirin.

Laboratory Analysis

Stored plasma was thawed and assayed for t-PA antigen by an enzyme-linked immunosorbent assay (ELISA) with kits purchased from Biopool AB, Umeå, Sweden. Assays were performed in accordance with the manufacturer’s instructions following a procedure described by Ranby et al.9 Briefly, plasma samples were incubated in microtiter plates coated with monoclonal antibodies against the desired antigen, unbound antigens were washed off, and bound antigen was detected by addition of a second specific antibody conjugated to horseradish peroxidase. Standard curves were constructed by use of dilutions of purified antigen in plasma. The amount of t-PA antigen in samples was deduced by comparing the sample absorbance with the calibration curve. At least one pair of specimens from a standard plasma sample was included in each batch of 30 specimens for blinded analysis. The overall coefficient of variation for these standard plasma samples across all assay runs was 6.1%.

Total cholesterol, HDL cholesterol, HDL subfractions, and apolipoproteins A-I, A-II, and B-100 were measured as previously described in the Lipid Research Laboratory of Brigham and Women’s Hospital.10 This laboratory participates in the standardization program for total and HDL cholesterol of the Centers for Disease Control and Prevention (CDC) and the National Heart, Lung, and Blood Institute. In brief, cholesterol in whole plasma, HDL₁, and HDL₂ was measured with enzymatic reagents, and the amount of HDL₃ cholesterol was determined by subtracting HDL₁ cholesterol from HDL cholesterol. HDL subfractions were separated by polyanionic precipitation. Apolipoproteins B-100, A-I, and A-II were measured by radial immunodiffusion; standardized sera for these apolipoproteins were provided by the CDC and used for calibration.

Statistical Analysis

The present analysis includes 633 men who provided an adequate plasma sample and underwent assay for t-PA:ag. With the exception of 176 men for whom apolipoprotein A-II levels were unavailable, all subjects had full lipid evaluations. Pearson correlation coefficients between plasma t-PA:ag levels and each lipid parameter were calculated. Linear regression models were used to assess the relation of t-PA:ag with each lipid fraction. Lipid and lipoprotein levels were treated both as continuous variables and after dividing the sample into five groups according to quintiles of the distribution. Partial correlations between t-PA:ag level and various lipids were computed after adjustment for age using the residuals from multiple linear regression. Stratified analyses were used to assess effect modification by smoking, alcohol consumption, exercise frequency, and body mass. All probability values are two-sided, and all confidence intervals were calculated at the 95% level.

Results

Fig 1 displays the distribution of t-PA:ag level among 633 control subjects in the Physicians’ Health Study. Overall, t-PA:ag levels ranged from 0.5 to 36.0 ng/mL. Mean and median t-PA:ag levels for this group of men were 9.69 and 8.54 ng/mL, respectively.

Unadjusted and age-adjusted correlations of t-PA:ag levels with various lipids are shown in Table 1. There were significant inverse correlations of endogenous t-PA:ag level with HDL cholesterol (Pearson r=-.1616, P<.0005), HDL₂ cholesterol (Pearson r=-.1632, P<.0005), and HDL₃ cholesterol (Pearson r=-.0927, P=.019). In contrast, there were no significant correlations of t-PA:ag with total cholesterol or apolipoproteins A-I, A-II, or B-100. Adjustment for age did not substantially alter these correlations.

Levels of t-PA:ag were calculated by quintile of each lipid fraction as shown in Table 2. The highest levels of endogenous t-PA:ag (mean, 10.62 ng/mL) were found among those men with HDL levels in the lowest quintile (<36.3 mg/dL), and the lowest levels of t-PA:ag (mean,
TABLE 1. Unadjusted and Age-Adjusted Correlation Coefficients Between Plasma t-PA Antigen Levels and Various Lipid Parameters Among a Cross Section of 633 Men Participating in the Physicians’ Health Study

<table>
<thead>
<tr>
<th>Lipid Parameter</th>
<th>Unadjusted</th>
<th>Age Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.0219</td>
<td>0.58</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.1616</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>HDL₂ cholesterol</td>
<td>-0.1632</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>HDL₃ cholesterol</td>
<td>-0.0927</td>
<td>0.19</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>-0.0441</td>
<td>0.27</td>
</tr>
<tr>
<td>Apolipoprotein A-II*</td>
<td>-0.0728</td>
<td>0.12</td>
</tr>
<tr>
<td>Apolipoprotein B-100</td>
<td>.0365</td>
<td>.33</td>
</tr>
</tbody>
</table>

*P<.0005 for HDL and HDL₂ cholesterol, P trend=.004 for HDL₃ cholesterol.

t-PA indicates tissue-type plasminogen activator; HDL, high-density lipoprotein.

8.14 ng/mL) were found among those men with HDL levels in the highest quintile (>55.4 mg/dL). Similar inverse relations were found between endogenous t-PA:ag and quintiles of HDL₂ or HDL₃ cholesterol. Tests for trend in t-PA:ag levels across quintiles of HDL, HDL₂, and HDL₃ cholesterol were all significant (P trend<.0005 for HDL and HDL₂ cholesterol, P trend=.004 for HDL₃ cholesterol). In this analysis, trends of borderline significance were found for t-PA:ag across quintiles of apolipoproteins A-I, A-II, and B-100 (P trend=.07, .046, and .053, respectively). There was no evidence of association between t-PA:ag and quintile of total cholesterol (P trend=.21).

To further evaluate the association of t-PA:ag with HDL cholesterol, we plotted the age-adjusted least-squares linear regression line between the variables along with the upper and lower 95% confidence intervals (Fig 2, top). Similar calculations were performed for the age-adjusted regression between t-PA:ag and HDL₂ cholesterol (Fig 2, middle) and t-PA:ag and HDL₃ cholesterol (Fig 2, bottom).

Finally, to assess whether the relation between t-PA:ag and quintile of HDL cholesterol was affected by factors that might influence HDL level, stratified analyses based on alcohol consumption pattern, exercise frequency, body mass index, and smoking status were performed. As shown in Table 3, the inverse association between t-PA:ag and HDL cholesterol was present among both frequent (P trend=.002) and infrequent (P trend=.004) consumers of alcohol, as well as for the subgroups of frequent exercisers (P trend<.001), those with body mass indices in the lower half of the distribution (P trend=.001), and nonsmokers (P trend <.001).

**Discussion**

In this study of healthy US male physicians, endogenous levels of t-PA:ag were inversely associated with HDL cholesterol levels in the highest quintile (>55.4 mg/dL). Similar inverse relations were found between endogenous t-PA:ag and quintiles of HDL₂ or HDL₃ cholesterol. Tests for trend in t-PA:ag levels across quintiles of HDL, HDL₂, and HDL₃ cholesterol were all significant (P trend<.0005 for HDL and HDL₂ cholesterol, P trend=.004 for HDL₃ cholesterol). In this analysis, trends of borderline significance were found for t-PA:ag across quintiles of apolipoproteins A-I, A-II, and B-100 (P trend=.07, .046, and .053, respectively). There was no evidence of association between t-PA:ag and quintile of total cholesterol (P trend=.21).

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HDL cholesterol as well as HDL₂ and HDL₃ cholesterol fractions. In contrast, there were no associations between t-PA:ag and total cholesterol and minimal associations with apolipoproteins A-I, A-II, and B-100. In stratified analysis, the inverse association between t-PA:ag and HDL cholesterol was highly significant among frequent as well as infrequent consumers of alcohol, frequent exercisers, men in the lower half of body mass index, and nonsmokers.

We are aware of no prior data relating t-PA:ag to HDL subfractions or apolipoprotein levels. With regard to the relation between t-PA:ag and total or HDL cholesterol in apparently healthy men, previous data are sparse and inconsistent. In one study of 26 volunteers, t-PA:ag levels were positively correlated with total but not HDL cholesterol, whereas in a second study of 128 subjects, t-PA:ag levels were positively correlated with total cholesterol and inversely correlated with HDL cholesterol. The present findings of no correlation between t-PA:ag and total cholesterol and an inverse correlation between t-PA:ag and HDL cholesterol are not entirely consistent with previously available data. Although these discrepancies may reflect underlying differences in the populations studied, it is also possible that prior findings simply reflect the play of chance in small samples. In this regard, the current data derive from a sample more than four times that of the two prior studies combined. Further, the present finding of no correlation between t-PA:ag and total cholesterol is consistent with results of the European Concerted Action on Thrombosis study of patients with known coronary artery disease.

The finding of a modest but highly significant inverse relation of t-PA:ag with HDL cholesterol, HDL₂ cholesterol, and HDL₃ cholesterol raises several possibilities concerning potential mechanisms linking HDL cholesterol to thrombosis. First, it is possible that t-PA:ag and HDL are inversely related because of a direct effect of one of these parameters on the other. Although cross-sectional data cannot establish causality or direction of effect, the possibility raised in these data of an interaction between t-PA:ag and HDL cholesterol is consistent with prior work relating HDL cholesterol to other surrogates of fibrinolytic function. Although not assessed in this study, it is also possible that the association between t-PA:ag and HDL is mediated by the primary inhibitor of plasminogen activation, plasminogen activator inhibitor type 1 (PAI-1).

Second, it is possible that t-PA:ag and HDL cholesterol are related to each other because both are associated with the extent of underlying atherosclerosis. In this regard, t-PA:ag level may be a marker of risk for future myocardial infarction primarily because t-PA levels rise in response to an increasing atherosclerotic burden. Thus, if low HDL cholesterol enhances atherogenesis and t-PA secretion is a response to atherosclerotic progression, then a correlation between these two parameters would be expected even if they do not directly interact with each other.

Finally, the inverse association between t-PA:ag and HDL cholesterol may be a result of uncontrolled confounding. That is, if both t-PA:ag and HDL cholesterol are related to a third unknown factor, then controlling for that variable might eliminate or reduce the association between t-PA:ag and HDL. In this analysis, controlling for age did not substantially alter the inverse association between t-PA:ag and HDL cholesterol, and in subgroup analyses the inverse association was apparent in frequent as well as infrequent consumers of alcohol, frequent exercisers, men in the lower half of body mass index, and nonsmokers. It remains unclear
whether such subgroup findings reflect true evidence of effect modification or the play of chance.

Several potential limitations of the present study merit consideration. First, although highly statistically significant, the observed association between t-PA:ag and HDL cholesterol is modest in absolute magnitude. It is important to point out, however, that this modest association appears to have an important biological effect. Specifically, in prospective data from the Physicians' Health Study that demonstrate a strong relation between baseline t-PA:ag and risk of future myocardial infarction, the association was markedly attenuated in analyses controlling for HDL cholesterol (but not total cholesterol), a finding that suggests that the biological interaction between HDL and t-PA:ag is substantial. Second, the results of this study are based on a single plasma sample, a potential limitation because at least two small studies have suggested that t-PA:ag levels display a modest circadian variation. However, this potential limitation cannot account for the observed relation between t-PA:ag and HDL level, since any random misclassification would, if anything, lead to an underestimation of the true effect. Further, in two recent studies of fibrinolytic function, variation in t-PA:ag level between 7 AM and 2 PM was found to be minimal. Finally, because samples in our study were collected in EDTA and stored for a long period of time before assay, it is possible that t-PA:ag concentrations in this study are systematically increased or decreased because of the collection and storage procedures used. This possibility seems highly unlikely, however, since the range, mean, and median t-PA:ag concentrations in the present study are virtually identical to those obtained in other major prospective and cross-sectional studies of men of similar age in which blood samples were collected in citrate rather than EDTA and were assayed soon after collection.

Until recently, data describing potential interactions between fibrinolysis and lipids have focused primarily on PAI-1. For example, high concentrations of PAI-1 are associated with high concentrations of triglycerides and total cholesterol. Because PAI-1 and t-PA complex together in the systemic circulation, short-term changes in PAI-1 concentration can be expected to alter levels of t-PA:ag. Thus, the present finding of an inverse relation of t-PA:ag with HDL cholesterol is particularly intriguing, because recent studies have found that gemfibrozil, a fibric acid derivative that raises HDL, appears to lower PAI-1 synthesis.

In sum, these data indicate that plasma levels of endogenous t-PA:ag are inversely related to HDL cholesterol and HDL cholesterol fractions. Further research will be required to determine whether the observed association is a result of direct effects of lipids on fibrinolytic function or of independent associations of lipids and t-PA:ag with atherosclerosis or is mediated by a third unmeasured variable.

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