Lipid Transfer Protein Activities in Type 1 Diabetic Patients Without Renal Failure and Nondiabetic Control Subjects and Their Association With Coronary Artery Calcification

Helen M. Colhoun,1 Leo M. Scheek,2 Michael B. Rubens,3 Teus Van Gent,2 S. Richard Underwood,3 John H. Fuller,1 and Arie Van Tol2

This study examined the role of cholesteryl ester transfer (CET), cholesteryl ester transfer protein (CETP) activity, and phospholipid transfer protein (PLTP) activity in the increased prevalence of coronary artery calcification (CAC) in diabetic subjects compared with nondiabetic subjects and in the loss of the sex difference in CAC in diabetes. CETP activity, PLTP activity, and CET were measured in 195 type 1 diabetic subjects without renal failure and 194 nondiabetic control subjects of similar age (30–55 years) and sex distribution (50% female). CAC was quantified with electron beam computed tomography. CETP activity was higher in diabetic subjects (mean 84 arbitrary units [AU]) than in nondiabetic subjects (81 AU, \( P < 0.001 \)). PLTP activity was also higher in diabetic subjects (96 AU) than in nondiabetic subjects (81 AU, \( P < 0.001 \)). However, CET was lower in diabetic men (geometric mean 32 nmol \( \cdot \) ml\(^{-1} \cdot h^{-1} \)) than nondiabetic men (37 nmol \( \cdot \) ml\(^{-1} \cdot h^{-1} \), \( P = 0.004 \)) and did not differ between diabetic (30 nmol \( \cdot \) ml\(^{-1} \cdot h^{-1} \)) and nondiabetic (32 nmol \( \cdot \) ml\(^{-1} \cdot h^{-1} \), \( P = 0.3 \)) women. CETP and PLTP activities were not associated with CAC. CETP was positively associated with CAC in both diabetic and nondiabetic subjects (odds ratio per 10 nmol \( \cdot \) ml\(^{-1} \cdot h^{-1} \) increase in CET in all subjects = 1.4, \( P = 0.001 \)). The prevalence of CAC was similar in diabetic (51%) and nondiabetic (54%, \( P = 0.7 \)) men but was much higher in diabetic (47%) than nondiabetic (21%, odds ratio 3.6, \( P < 0.001 \)) women so that there was no sex difference in CAC in diabetic subjects. The odds of CAC in diabetic women compared with nondiabetic women was altered little by adjustment for CETP activity, PLTP activity, or CET (odds ratio on adjustment 3.7, \( P < 0.001 \)). The greater effect of diabetes on CAC in women than in men, i.e., the loss of the sex difference in CAC, was independent of CETP and PLTP activity and CET. In conclusion, among both diabetic and nondiabetic subjects, higher cholesteryl ester transfer is a risk factor for CAC. However, abnormalities in cholesteryl ester transfer or lipid transfer protein activities do not underlie the increased CAC risk in diabetic women compared with nondiabetic women or the loss of the sex difference in CAC in diabetes. Diabetes 50:652–659, 2001

Type 1 diabetic patients have an elevated risk of coronary heart disease (CHD) that is not explained by their coronary risk factor profile. Indeed, in the absence of nephropathy, type 1 diabetic patients often have higher HDL cholesterol (HDL-C) and lower LDL cholesterol (LDL-C) and triglycerides than the general population (1). A particular feature of CHD in type 1 diabetes is that the sex difference in CHD is abolished, and the basis for this is unknown (2).

It has been postulated that the elevated CHD risk in type 1 diabetic patients results from an increase in cholesteryl ester transfer (CET) between HDL particles and triglyceride-rich particles including VLDL, even among apparently normolipidemic patients (3). The resultant compositional changes in HDL might reduce its anti-atherogenic potential in the reverse cholesterol transport pathway, and the resultant cholesterol-rich VLDL may have increased atherogenicity (4,5). Increased CET could be due to altered acceptor lipoproteins or an increase in plasma cholesteryl ester transfer protein (CETP) activity (3). There is some evidence to support these possibilities, but it is far from conclusive. The observation that CET is increased in type 1 diabetes (3) has not been replicated, and the higher HDL-C usually observed in well-controlled type 1 diabetic patients is not consistent with increased CET. The data on CETP activity in type 1 diabetes are conflicting, with decreased, unchanged, and increased activity reported (6–9).

Furthermore, there are no data demonstrating that elevated CET and/or CETP activity is actually associated with the increased atherosclerosis in type 1 diabetic patients. In addition to CETP, phospholipid transfer protein (PLTP) is thought to be important in the reverse cholesterol transport pathway (10). Several actions of PLTP have been reported including facilitation of the transfer of phospholipids from VLDL to HDL particles and remodeling of HDL with the generation of pre-β HDL particles (11).
These activities suggest that PLTP might play a role in protection against atherosclerosis, but there are no data on whether PLTP activity is actually associated with coronary atherosclerosis risk in humans. To date, there are no reports on plasma PLTP activity in type 1 diabetic patients. Investigation of PLTP in type 1 diabetes is warranted because 1) in type 2 diabetic patients, PLTP activity is altered compared with that of nonobese control subjects (12); 2) hyperglycemia-induced hyperinsulinemia results in a decrease in PLTP activity (13); and 3) abnormalities in the phospholipid composition of lipoproteins have been reported in type 1 diabetic patients (14,15).

We carried out a cross-sectional comparison of CETP activity, PLTP activity, and CET in type 1 diabetic men and women and similarly aged nondiabetic subjects randomly selected from the general population. Coronary artery calcification (CAC) was measured as an index of coronary atherosclerosis burden. The type 1 diabetic patients were selected so as not to have renal failure but are otherwise representative of type 1 diabetic patients of this age. The study tested four related hypotheses: 1) CET and lipid transfer protein activities are altered in type 1 diabetic patients even in the absence of renal failure, 2) CET and transfer protein activities are associated with CAC, 3) differences in CET and transfer protein activities contribute to the difference in CAC between diabetic and nondiabetic subjects, and 4) there are sex differences in CET and/or transfer protein activities that are altered in type 1 diabetes, and this underlies the loss of the sex difference in CHD in diabetic patients.

RESEARCH DESIGN AND METHODS

Subjects. A random sample of type 1 diabetic men and women aged 30–55 years was taken from the diabetes registers of five London hospitals. Type 1 diabetes was defined by age of onset <25 years and insulin use within 1 year of diagnosis. A random sample of the general population, stratified to have a similar age and sex distribution to the patients, was drawn from the lists of two London general practices. Patients on renal replacement therapy were excluded. Subjects were included regardless of any history of heart disease. Ethics Committee approval was obtained. Written informed consent was obtained from all subjects after explanation of study procedures. In all, 108 diabetic and 198 nondiabetic subjects were recruited. Three diabetic subjects on lipid-lowering therapy and four hypertriglyceridemic (≥6 mmol/l) nondiabetic subjects were excluded from these analyses.

Examination. Respondents completed a standardized questionnaire. Obesity was defined as a BMI ≥30 kg/m². Being menopausal was defined as not having had a period in the past year without any other known cause. An Ultrafast CT scanner (IMATRON C-150XL, Imatron, San Francisco, CA) was used to quantify coronary calcification. Two sets of 20 transverse tomograms of 3 mm thickness were obtained from the lower margin of the bifurcation of the right branch of the pulmonary artery to the apex of the heart with the subject holding his or her breath. A radiologist placed a region of interest around each potentially calcified lesion (peak density >130 Hounsfield units) within the four coronary arteries: right coronary, circumflex, left anterior descending, and left main. The area and peak density in Hounsfield units of each lesion was measured. A density score of 1–4 was defined based on the peak density of the lesion, and the calcification score was then calculated as the product of the area of the lesion and its density score as described (16). To be included in the calcification score, a lesion had to have an area of at least 0.51 mm², i.e., two contiguous pixels, and a peak density of at least 130 Hounsfield units. A total score for each artery and for the entire heart was calculated by summing the lesion scores. All scans were scored by the same radiologist blinded to the sex and diabetes status of the subject. Based on a small repeatability study (n = 20), the within-observer agreement for the presence of any calcification was high (κ = 0.84).

Laboratory methods. After an overnight fast, blood samples were taken and total cholesterol, HDL-C, and triglycerides were measured using enzymatic colorimetric methods (intra-assay coefficient of variation [CV] = 2.6, 2.6, and 2%, respectively) (17,18). HDL-C was measured directly after stabilization of other lipoproteins (19). LDL-C was calculated as described by Friedewald et al. (20). HbA₁c was measured using a latex enhanced immunoassay (intra-assay CV 1.7%). Respondents completed two timed overnight urine collections, who were menstruating were excluded from urinary albumin analyses (n = 57). Urinary albumin was measured using an immunoturbidimetric method (intra-assay CV 2.3%). All analytes were measured with the laboratory blinded to the sex and diabetes status of the subjects.

Plasma CETP activity level was measured, after removal of VLDL and LDL from each sample, with excess exogenous lipoprotein substrates, as an estimate of CETP mass (between-assay CV 4.5%). CETP activity measured this way is independent of the endogenous lipoproteins present in the measured plasma, and correlates strongly with CETP mass. Plasma CETP activity levels were related to the activity in a reference plasma analyzed in each run and are expressed in arbitrary units (AU), corresponding to the percentage of the activity in the reference plasma. Plasma CET was measured as the rate of CET out of HDL into VLDL plus LDL during incubation of plasma in vitro, i.e., the CETP activity measured in this way. The rate of CET is expressed in nanomoles cholesteryl ester transferred per milliliter plasma per hour and is constant during 3 h of incubation. CET is measured in vitro but is likely to reflect the in vivo CET. Thus, CETP activity is a reflection of CETP mass, whereas CET is a measure of how much cholesteryl ester is actually transferred given that CETP mass of the person or her prevailing lipoprotein profile. The between-assay CV for CET was 7.2%. Plasma PLTP activity was measured with labeled liposome vesicles as the phospholipid donor and excess pooled human HDL as phospholipid acceptor, as described (23). This assay is not influenced by the phospholipid transfer–promoting properties of CETP (25). The between-assay CV was 4.8%. Plasma PLTP activity levels were related to the activity in a human reference plasma and are expressed in AU, corresponding to the percentage of the activity in the reference plasma.

Statistical methods. All analyses were carried out using Stata 6 (Stata, College Station, TX). To adjust for age, we tested whether there are differences in lipid transfer (CET) and lipid transfer protein activities between diabetic and nondiabetic respondents using multiple linear regression. We tested whether the differences in these factors between those with and without diabetes differed by sex by including a diabetes-by-sex interaction term in these regression models. This is the same as testing whether there is a sex difference that is altered by diabetes. The partial correlation coefficients for the association of CET and lipid transfer protein activities with each other and with lipids were examined. The significance levels for these associations should be interpreted conservatively given the number of association tests carried out. We further explored whether any differences between groups in CET and lipid transfer protein activities were explained by differences in associated lipid levels by including these as covariates in the multiple linear regression models. Dependent variables that were positively skewed were log-transformed for regression analyses. Calculations scores (for the total heart) were positively skewed with a high frequency of zero values. Because data transformation would not have normalized this distribution, the odds of having any calcification (a score >0) associated with CET and with lipid transfer proteins was examined using logistic regression, adjusting for age. We examined this association in all subjects adjusting for diabetes, and also examined it in diabetic and nondiabetic subjects separately. Further adjustment was made for HDL-C, LDL-C, and triglycerides in these models. We also noted the effect of adjusting for CET and transfer proteins on the odds ratio for CAC associated with diabetes in the logistic regression model.

RESULTS

Plasma lipid transfer protein activities and lipoproteins in diabetic compared with nondiabetic subjects. Among men, those with diabetes had significantly lower LDL-C and triglycerides and significantly higher HDL-C (Table 1). Among women, those with diabetes had higher HDL-C, but there was little difference in triglycerides or LDL-C. CETP activity was slightly higher in diabetic subjects than in nondiabetic subjects, (Table 1 and Fig. 1). This was a significant difference when both sexes were combined, although not significant within either sex when analyzed separately. CETP activity was positively associated with LDL-C and PLTP activity but not with HDL-C or triglycerides (Table 2). A similar pattern of correlations between CETP activity and related variables was found in
TABLE 1
Lipid levels and lipid transfer protein activity in diabetic and nondiabetic men and women

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>P‡</th>
<th>Women</th>
<th>P‡</th>
<th>Men and women</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>89</td>
<td>101</td>
<td>105</td>
<td>94</td>
<td>194</td>
<td>195</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.8 ± 4</td>
<td>38.0 ± 4</td>
<td>0.7</td>
<td>37.9 ± 4</td>
<td>37.5 ± 4</td>
<td>0.5</td>
</tr>
<tr>
<td>PLTP (AU)</td>
<td>85 ± 15</td>
<td>99 ± 18</td>
<td>&lt;0.001</td>
<td>77 ± 13</td>
<td>93 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CETP (AU)</td>
<td>76 ± 18</td>
<td>82 ± 19</td>
<td>0.06</td>
<td>83 ± 16</td>
<td>86 ± 17</td>
<td>0.3</td>
</tr>
<tr>
<td>CET (nmol · ml⁻¹ · h⁻¹)‡</td>
<td>37 (29–45)</td>
<td>32 (26–39)</td>
<td>0.004</td>
<td>32 (25–39)</td>
<td>30 (26–39)</td>
<td>0.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)†</td>
<td>1.32 (1.0–1.7)</td>
<td>1.05 (0.9–1.5)</td>
<td>0.04</td>
<td>0.96 (0.7–1.3)</td>
<td>0.97 (0.7–1.2)</td>
<td>0.9</td>
</tr>
<tr>
<td>LDL-C (nmol/l)</td>
<td>3.3 ± 1.1</td>
<td>3.0 ± 0.96</td>
<td>0.03</td>
<td>2.9 ± 0.83</td>
<td>2.8 ± 0.87</td>
<td>0.3</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.6 ± 0.34</td>
<td>1.7 ± 0.34</td>
<td>0.01</td>
<td>1.8 ± 0.39</td>
<td>2.0 ± 0.49</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 3.4</td>
<td>25.4 ± 3.2</td>
<td>0.3</td>
<td>25.6 ± 5.6</td>
<td>25.3 ± 3.8</td>
<td>0.6</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.3 ± 0.4</td>
<td>8.4 ± 1.2</td>
<td>&lt;0.001</td>
<td>5.3 ± 0.4</td>
<td>9.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>-</td>
<td>23 ± 7.6</td>
<td>0.001</td>
<td>-</td>
<td>24 ± 7.4</td>
<td>0.001</td>
</tr>
<tr>
<td>On pill or HRT %§</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23 ± 4</td>
<td>19 ± 4</td>
<td>0.3</td>
</tr>
<tr>
<td>Menopausal %§</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7 ± 2</td>
<td>3 ± 2</td>
<td>0.2</td>
</tr>
<tr>
<td>Obese %§</td>
<td>7 ± 3</td>
<td>8 ± 3</td>
<td>0.8</td>
<td>21 ± 4</td>
<td>8 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>With albuminuria %§</td>
<td>3 ± 2</td>
<td>21 ± 4</td>
<td>0.001</td>
<td>3 ± 2</td>
<td>9 ± 3</td>
<td>0.8</td>
</tr>
<tr>
<td>Drinking &gt;21 units/week %§</td>
<td>38 ± 5</td>
<td>25 ± 4</td>
<td>0.04</td>
<td>7 ± 2</td>
<td>11 ± 3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Data are means ± SD or %percent ± SE unless otherwise indicated. *P values are for the difference between individuals with and without diabetes within each sex, adjusted for age using multiple linear regression. The mean values are not adjusted; †adjusted for age, sex, and diabetes; ‡geometric mean and the interquartile range (i.e., 25th and 75th centiles) are shown for variables with a skewed distribution.

Lipid levels and lipid transfer protein activity in diabetic and nondiabetic subjects (Table 2). Correlations were also similar in men and women (data not shown). The higher CETP activity in those with diabetes was independent of triglycerides, HDL-C, LDL-C, and obesity (3.9 units higher on adjustment, P = 0.02). Excluding those with albuminuria slightly increased the difference in CETP activity between diabetic and nondiabetic subjects to 5 AU (P = 0.01).

PLTP activity was substantially elevated in those with diabetes in both sexes (Table 1 and Fig. 2). PLTP activity was positively associated with LDL-C, HDL-C, and CETP activity in all subjects combined (Table 2). Although the association of PLTP activity with HDL-C and LDL-C was not significant among nondiabetic subjects, none of the differences in correlation coefficients between those with and without diabetes shown in Table 2 were statistically significant. The correlations were also similar in men and women (data not shown). PLTP activity was not associated with insulin dose (PLTP activity 2 units higher in those with above vs. below median insulin dose, P = 0.8). The higher PLTP activity in those with diabetes was independent of triglycerides, HDL-C, CETP activity, LDL-C, and obesity (15 units higher on adjustment, P < 0.001). Excluding those with albuminuria did not alter the difference in PLTP activity between diabetic and nondiabetic subjects (14 AU on excluding them, P = 0.01).

PLTP activity and CETP activity did not differ with oral contraceptive pill use, hormone replacement therapy (HRT) use, or menopausal status. Overall, 52 women were either postmenopausal (n = 10), using oral contraceptive pills (n = 37), or using HRT (n = 5) (Table 1), a similar proportion in diabetic and nondiabetic subjects. Accordingly, the difference in both CETP activity and PLTP activity between diabetic and nondiabetic women was unchanged when these 52 women were excluded from the analyses.

Cholesteryl ester transfer in diabetic subjects compared with nondiabetic subjects. CET was lower in diabetic men than in nondiabetic men (Table 1 and Fig. 1), but this difference was not independent of triglycerides (P = 0.05 on adjustment). Among women, the difference in CET between those with and without diabetes was not significant, and this was the case regardless of whether obese subjects were included or excluded. CET did not differ with oral contraceptive pill use, HRT use, or menopausal status. Accordingly, when the 52 menopausal or hormone-using women were excluded from the analyses, there was still no difference in CET between diabetic and nondiabetic women. The apparently greater effect of diabetes on CET in men than women was not significant (P = 0.09 to 0.2 for the diabetes–sex interaction).

Effect of diabetes on sex differences in lipid transfer protein activity and CET. With regard to sex differences, among the nondiabetic group, women had higher CETP activity than men (P = 0.005), and the sex difference in the diabetic group, although nonsignificant (P = 0.06), was of similar magnitude. In the nondiabetic group, women had
lower PLTP activity than men \((P = 0.001)\), and this sex difference was preserved in those with diabetes \((P = 0.01, \text{Table 1})\). Exclusion of those subjects who had albuminuria, were on HRT or the pill, or who were postmenopausal did not alter these results. In nondiabetic subjects, CET was 5 nmol \(\cdot\) mol \(\cdot\) ml \(\cdot\) h \(\cdot\) 1 lower in women than in men adjusted for age \((P = 0.02\) for this sex difference), whereas among diabetic subjects, there was no significant sex difference \((1\) nmol \(\cdot\) mol \(\cdot\) ml \(\cdot\) h \(\cdot\) 1 lower in women, \(P = 0.5)\). However, this effect of diabetes on the sex difference in CET was not statistically significant before \((P = 0.2\) for the interaction) or after \((P = 0.07)\) adjusting for obesity.

**CAC prevalence and severity by diabetes status.** The prevalence of CAC (a score \(>0)\) was similar in diabetic (51%) and nondiabetic men \((54\), odds ratio for diabetic vs. nondiabetic 0.9, 95% CI 0.5–1.6, \(P = 0.7)\) but was much higher in diabetic women \(47\%\) than in nondiabetic women \(21\), odds ratio 3.6, 95% CI 1.9–6.7, \(P < 0.001)\). Among those with calcification, its severity was greater in diabetic patients compared with control subjects in both sexes \((P = 0.04\) for men, \(P = 0.001\) for women). As expected, higher triglycerides \((P = 0.006)\) and lower HDL-C \((0.005)\) were associated with CAC. The association between CAC and LDL-C was not significant \((P = 0.07)\).

**Association of lipid transfer protein activity and CET with CAC.** For all subjects combined, adjusting for age, sex, and diabetes, there was no association between CETP activity and CAC \(\text{odds ratio for CAC per 10 units of CETP} = 1.02, 95\%\) CI 0.9–1.2, \(P = 0.6)\). There was also no any association found within the diabetic \((\text{odds ratio 1.09, 95\%\) CI 0.9–1.3) or nondiabetic \((\text{odds ratio 0.95, 95\%\) CI 0.8–1.4) groups examined separately. Adjustment for HDL-C, LDL-C, triglycerides, and obesity did not alter this. PLTP activity was not associated with CAC in all subjects combined \((\text{odds ratio per 10 units PLTP} = 1.1, 95\%\) CI 0.98–1.26, \(P = 0.09)\) or within either the diabetic \((\text{odds ratio 1.2, 95\%\) CI 0.99–1.4) or nondiabetic \((\text{odds ratio 0.99, 95\%\) CI 0.8–1.24) groups examined separately.

CET was associated with CAC among both diabetic and nondiabetic groups when these were analyzed separately \((\text{Table 3})\). There was no significant difference in the strength of this association between diabetic and nondiabetic subjects \((P = 0.7\) for the diabetes-by-CET interaction). In all subjects combined, this association was independent of triglycerides and HDL-C but not of BMI \((\text{Table 3})\). For comparison, an increase in CET of 1 SD was associated with a similar odds ratio for CAC \((\text{odds ratio of 1.5) as a 1 SD decrease in HDL-C (an odds ratio of 1.45)\). The association was of similar magnitude in men \((\text{odds ratio 1.3) and women (odds ratio 1.5, P = 0.9 for the CET-by-sex interaction)\).**

**Effect of lipid transfer protein activity and CET on the increased prevalence of calcification and loss of sex difference in calcification associated with diabetes.** Because the prevalence of CAC was not increased with diabetes in men, we examined the effect of adjusting for PLTP and CETP activity on the odds of coronary calcification in diabetic women compared with nondiabetic women only. This adjustment reduced the odds ratio in diabetic versus nondiabetic women from 3.6 to 3.1 \((95\%\) CI 1.6–6)\). Adjusting further for CET increased this odds

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**TABLE 2**

Partial correlation coefficients for the association of plasma lipid transfer proteins and CET with other lipid-related variables by diabetes status

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic subjects ((n = 194))</th>
<th>Diabetic subjects ((n = 195))</th>
<th>All subjects ((n = 389))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CETP</td>
<td>PLTP</td>
<td>CET</td>
</tr>
<tr>
<td>BMI</td>
<td>0.05</td>
<td>0.07</td>
<td>0.35*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.007</td>
<td>0.09</td>
<td>0.71*</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.5*</td>
<td>0.09</td>
<td>0.67*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.07</td>
<td>0.07</td>
<td>-0.5*</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.12</td>
<td>0.03</td>
<td>0.17‡</td>
</tr>
<tr>
<td>PLTP</td>
<td>—</td>
<td>—</td>
<td>0.18‡</td>
</tr>
<tr>
<td>CETP</td>
<td>—</td>
<td>0.32*</td>
<td>0.33†</td>
</tr>
</tbody>
</table>

*\(P < 0.001, \dagger P < 0.01, \ddagger P < 0.05\) after adjustment for age and sex. The correlation coefficients are adjusted for sex and age. Those for all subjects are also adjusted for diabetes. Skewed variables \(i.e.,\) CET and triglycerides) were log-transformed before analysis.

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**FIG. 2.** Mean \((95\%\) CI) plasma PLTP activity \((AU)\) by diabetes and sex. DM, diabetic.

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**TABLE 3**

Odds ratio for CAC associated with CET

<table>
<thead>
<tr>
<th></th>
<th>All subjects†</th>
<th>Nondiabetic subjects</th>
<th>Diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>389</td>
<td>194</td>
<td>195</td>
</tr>
<tr>
<td>Adjusted for</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and sex</td>
<td>1.4 ((1.1–1.7)* 1.3 ((1.1–1.6)† 1.5 ((1.1–2.0)† 1.3 ((1.1–1.7)† 1.0 ((0.7–1.4) 1.6 ((1.1–2.2)† 1.3 ((1.0–1.6)† 1.0 ((0.8–1.3) 1.3 ((0.9–1.8) \</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, sex, and triglycerides</td>
<td>1.02, 95% CI 0.9–1.2, (P = 0.6))</td>
<td>1.4 ((1.1–1.7)* 1.3 ((1.1–1.6)† 1.5 ((1.1–2.0)† 1.3 ((1.1–1.7)† 1.0 ((0.7–1.4) 1.6 ((1.1–2.2)† 1.3 ((1.0–1.6)† 1.0 ((0.8–1.3) 1.3 ((0.9–1.8) \</td>
<td></td>
</tr>
<tr>
<td>Age, sex, triglycerides, and HDL-C</td>
<td>1.3 ((1.0–1.6)† 1.0 ((0.7–1.4) 1.4 ((1.0–1.9)† 1.2 ((0.95–1.4) 1.0 ((0.8–1.3) 1.3 ((0.9–1.8) \</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, sex, and BMI</td>
<td>1.2 ((0.95–1.4) 1.0 ((0.8–1.3) 1.3 ((0.9–1.8) \</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are odds ratios for CAC per 10 nmol \(\cdot\) mol \(\cdot\) ml \(\cdot\) h \(\cdot\) 1 of CET \((95\%\) CI). The model for all subjects was also adjusted for diabetes. *\(P < 0.01, \dagger P < 0.05)\, \dagger P < 0.05)\,
Plasma CETP activity. calcification. any major contribution to the loss of the sex difference in the production of CET, but this did not make preserved in diabetes. There was some suggestion of a re-duction in the sex difference in CET in diabetic patients. In type 1 diabetes, PLTP activity was markedly elevated, and CETP activity was mildly elevated. However, despite the higher CETP activity, the actual mass of CET was slightly lower in men with diabetes and was unchanged in women. CETP activity was not associated with CAC, and there was no clear association between PLTP activity and calcification. CET was positively associated with CAC in both diabetic and nondiabetic subjects. CETP activity, PLTP activity, or CET did not explain the increased prevalence of calcification associated with diabetes that we observed in women. We had hypothesized that there may be sex differences in lipid transfer protein activity and/or CET that might be altered in diabetes and that this might contribute to the loss of the sex difference in CAC in diabetic patients. However, sex differences in PLTP and CETP activity were preserved in diabetes. There was some suggestion of a re-duction in the sex difference in CET, but this did not make any major contribution to the loss of the sex difference in calcification.

**Plasma CETP activity.** This is the largest study of CETP activity levels in type 1 diabetic patients to date and serves to clarify conflicting data from other smaller studies. CETP activity has been found to be decreased (6), unchanged (7), and increased (8) in diabetic subjects compared with nondiabetic subjects. We found only a slight increase in CETP activity levels (measured with excess exogenous substrates as an estimate of CETP mass) in diabetic patients. Using CAC as a measure of coronary atherosclerosis, we did not find any support for the hypothesis that increased plasma CETP activity levels per se are athero-genic. Prior data on the effects of elevated CETP activity on atherosclerosis risk are conflicting. In monkeys, higher CETP activity levels were associated with coronary ath-erosclerosis (24). In transgenic mice, CETP expression leads to a moderate increase in atherosclerosis in meta-bolic settings in which clearance of remnants or LDL is severely impaired (25). Other transgenic studies found CETP expression to be pro-atherogenic in the absence of high triglycerides but anti-atherogenic in the presence of high triglycerides (26). In humans, carotid intima-media thickness was positively associated with CETP activity (27). In contrast, genetic CETP deficiency is associated with increased CHD rates despite a concomitant raised HDL-C (28,29). We did not find any association between CETP activity levels and CAC, and this was the case whether stratified by triglyceride level or not. Thus, the data do not support the idea that increased CETP levels per se are the basis for increased atherosclerosis in diabetic patients. Furthermore, because CETP activity is higher in nondia-betic women than men and its increase in type 1 diabetes is of the same magnitude in both sexes, it cannot be the basis for the loss of the sex difference in CHD in diabetic subjects as confirmed in the analysis described above. CET. Despite the slightly higher CETP activity, the mass of cholesteryl esters actually transferred from HDL to other lipoproteins is lower in diabetic men than in nondiabetic men and is not altered by diabetes in women. Although CETP is an important catalyst in CET, CETP activity explains relatively little of the variation in CET (only about 4% in the regression analysis). Thus, CETP activity does not appear to be rate-limiting for CET. By comparison, variation in triglyceride levels explains ~60% of the variation in CET. Not surprisingly, therefore, the fairly small increase in CETP activity (about a quarter of 1 SD) in diabetic versus nondiabetic men is overshadowed by their lower triglyceride levels. The CET results contrast with two smaller studies in which CET was increased in type 1 diabetic patients compared with control subjects (3,30). These authors suggested that sustained activation of the CET system, resulting from peripheral hyperinsulinemia, might be important in the increased atherosclerosis of diabetic patients. We have found no evidence to support this in the present study. The reasons for the different results between our study and these other studies is unclear. The methods differed in that we measured the cumulative transfer of cholesteryl esters over 3 h, whereas the time course of transfer over a 6-h period was the focus of the other studies. However, in the latter studies, the difference in CET with diabetes was maximal at ~3 h; therefore, time of incubation is unlikely to explain the discrepancy in results. Comparison of glycemic control levels between the studies is not possible because different methods (HbA1C and fructosamine) were used. The age range and lipid levels of the subjects were broadly similar (e.g., a mean triglyceride in control subjects of 1.3 mmol/l in our study vs. 1.1 and 1.2 mmol/l in the other studies). However, we have not forced the lipid levels to be similar in patients and control subjects (matching) because our large sample size allows us to control for lipids in the regression analysis of CET. Because we did not match, triglyceride levels were slightly lower in diabetic men than in control subjects in our study, whereas in the other studies, they were virtually the same as those of the control subjects. Nonetheless, even after adjusting for triglyc-erides, we found no suggestion of any increase in CET with diabetes; rather, we found the opposite in men. The source of the healthy control subjects in these early studies is unclear and it is possible that they represented a particularly healthy group with particularly low CET. Probably the most important difference is that our study is substantially larger than the other studies (n = 15–20 diabetic subjects), and it may simply be that sampling variation was responsible for the observation of acceler-ated CET in these smaller studies.

A higher CET (measured with the endogenous plasma lipoprotein substrates) was associated with CAC and this was true in both diabetic and nondiabetic subjects. Thus, differences in CET may be relevant to risk among both diabetic and nondiabetic patients. However, because CET is generally lower in diabetic patients than in nondiabetic patients, clearly it cannot be the cause of the increased...
CAC in diabetic patients compared with nondiabetic patients. Our observation of an association between CET and CAC is important because it adds to the evidence that increased transport of cholesterol from HDL into other lipoprotein particles reflects an atherogenic state. CET and triglyceride levels are highly correlated; therefore, it is not surprising that the association between CET was not independent of triglycerides in nondiabetic subjects. This lack of independence is consistent with the hypothesis that accelerated exchange of lipids between VLDL and HDL is part of the pathway by which triglycerides exert their atherogenic effect (31). With regard to the assay method, CET, measured in vitro during incubation of isolated plasma for relatively short periods (<3 h), is likely to reflect the CET actually occurring in vivo because the changes in lipoprotein composition during incubation are small. It is clear however that lipoprotein turnover is absent in vitro. It cannot be totally excluded that this may have influenced the measured CET.

**PLTP activity.** These are the first data on PLTP in type 1 diabetic patients. We found that PLTP activity is substantially increased in type 1 diabetic patients, with there being a difference of almost 1 SD from the nondiabetic subjects. The basis for this increased activity is unknown. Acute insulin administration downregulates PLTP activity (32), but whether insulin deficiency in type 1 diabetic patients might play a role in their increased PLTP activity is not known. Among the diabetic subjects studied, neither insulin dose per day nor glycemic control were associated with PLTP activity, but longer-term measurement of glycemia or some measure of portal insulin levels might have shown different relationships with PLTP activity. The higher PLTP activity in diabetes was also independent of HDL-C, triglyceride, and LDL-C levels.

We found a significant positive correlation between PLTP and both LDL-C and HDL-C in all subjects combined and within the diabetic group when examined separately. Triglycerides and BMI were not correlated with PLTP. There are few other data on the association of PLTP activity with other lipid parameters in large-scale studies. In 50 premenopausal women, PLTP activity was positively correlated with BMI, HDL-C, and LDL-C (33). The correlation coefficients for HDL-C and LDL-C were greater than those in our study. The assay accuracy was slightly greater in that study (2.2 vs. 4.8%) so that the power to detect a correlation was greater, and this may have contributed to the difference in correlations between the two studies. In that study, there was also no association with triglycerides. In contrast, in a Finnish study of 400 people, nonsmoking PLTP activity was positively associated with triglycerides as well as BMI and LDL-C but not HDL-C (34). These different associations may reflect the fact that the samples were nonfasting. We found that in the general population, there is a large sex difference in PLTP activity with women having lower activity than men, despite their higher HDL-C levels. These data are in contrast to the Finnish Study in which there was no sex difference in PLTP activity.

Remodeling of HDL particles is thought to be an important function of PLTP resulting in the generation of small pre-B HDL-like particles that are the initial acceptors of cell-derived cholesterol (11,35). PLTP knockout mice have reduced plasma HDL concentrations (36,37). Overexpression of the human PLTP gene in mice also decreases plasma HDL cholesterol concentration but increases hepatic uptake of radiolabeled phospholipids and cholesteryl esters from HDL (37–39). Interestingly, PLTP knockout mice have decreased apolipoprotein B100 (40). Together these studies suggest that PLTP might have atherogenic as well as anti-atherogenic properties. We found that PLTP activity was positively associated with LDL-C levels in diabetic subjects (Table 2). This association was independent of their triglyceride and HDL-C levels (data not shown). The effect of PLTP activity on the development of atherosclerosis in transgenic animal models is unknown at present but is expected to become available shortly.

Among all subjects combined, we did not find any association of PLTP activity with CAC. We did not find any evidence that increased PLTP activity is the basis for the increased CAC in diabetic subjects. Because the sex difference in PLTP activity was unchanged in diabetes, PLTP is not a likely cause of the loss of the sex difference in CAC. Nonetheless, the causes and consequences of the increased PLTP activity in type 1 diabetes deserve further exploration.

**Methodologic considerations.** In this study, we used CAC as a measure of atherosclerosis burden rather than a measure of clinical CHD. The usefulness of electron beam–computed tomography (EBCT)-defined CAC as a measure of atherosclerosis burden in the general population is well established (41,42). Although not all atherosclerotic lesions contain calcified foci, autopsy studies have demonstrated that the amount of calcification increases with the amount of atherosclerosis (43–46). For example, the total calcium volume in the coronary arteries is highly correlated with total plaque volume in autopsy specimens (r = 0.87, P < 0.0001) (46). Studies in which excised whole hearts or vessels have been scanned and compared with histomorphometrically defined CAC volume have confirmed that EBCT scoring accurately quantifies CAC ex vivo (47,48). Angiographic studies of symptomatic patients have shown that the CAC score is associated with the extent of luminal stenoses, although slightly less strongly than with atheroma burden in autopsy studies (41). These studies show that CAC score is a sensitive, although not very specific, measure for coronary stenosis (49). This low specificity for stenosis simply means that not all calcified lesions will be stenoic. Of course luminal stenosis is itself not perfectly correlated with atherosclerosis burden, probably because diseased vessels enlarge to preserve lumen size (50), so that the autopsy data are more relevant than the angiography data to the validity of our study. CAC score was associated with carotid intima-media thickness in young men and women (51). Consistent with being a good marker of atherosclerosis burden, the EBCT-defined CAC score is also an important predictor of clinical events (52,53). Nonatherosclerotic calcification can occur in the media of the coronaries, but it is rare. Sporadic reports of extensive medial coronary calcification have been in patients with renal failure, and none of the subjects in this study had renal failure (54,55).

In conclusion, we find no evidence to support the hypothesis that abnormalities of plasma PLTP and CETP activity levels are the basis for increased CAC in diabetes.
or the loss of the sex difference in calcification in diabetes. The causes and consequences of the elevated PLTP activity in type 1 diabetes warrant further investigation, and the data caution against assuming that elevated PLTP activity is anti-atherogenic. The data demonstrate that increased CET is representative of an atherogenic state but is not the cause of the increased calcification in diabetic patients because CET is slightly lower in type 1 diabetic patients than in control subjects. It is possible that a reduction in the sex difference in CET in type 1 diabetes may make some small contribution to the loss of the sex difference in calcification, but we have found only weak evidence to support this hypothesis. Other causes for the increased risk of CHD and the loss of the sex difference in CHD in diabetic patients must therefore be sought.

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