Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism

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Abstract. The mechanisms responsible for the decreased high density lipoprotein (HDL) cholesterol levels associated with obesity and insulin resistance are not well understood. Lecithin: cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) are key factors in the esterification of cholesterol in HDL and the subsequent transfer of cholesterol ester towards apolipoprotein B-containing lipoproteins. Phospholipid transfer protein (PLTP) may be involved in the regulation of HDL particle size. We therefore measured the activities of LCAT, CETP and PLTP using exogenous substrate assays, as well as lipids, lipoproteins, insulin and C-peptide in fasting plasma from eight healthy obese men (body mass index > 27 kg m⁻²) and 24 non-obese subjects. The obese men had lower levels of HDL cholesterol (P<0.05) and higher levels of plasma triglycerides (P<0.05), insulin (P<0.05) and C-peptide (P<0.01), as compared to the quartile of subjects with the lowest body mass index (BMI <22.4 kg m⁻²) and 24 non-obese subjects. The obese men by 35% (P<0.01) and by 15% (P<0.05), respectively. LCAT activity was comparable among the quartiles. Linear regression analysis showed that CETP activity was positively correlated with body mass index (P<0.02), fasting blood glucose (P<0.05) and plasma C-peptide (P<0.05). PLTP activity was positively related to body mass index (P<0.01), waist to hip circumference ratio (P<0.001), as well as to fasting blood glucose (P<0.05) and plasma C-peptide (P<0.05).

It is concluded that the activities of CETP and PLTP are influenced by adiposity and possibly by insulin resistance. Elevated lipid transfer protein activities may provide a mechanism that contributes to alterations in HDL in insulin resistant states.

Keywords. Cholesterol acyltransferase, cholesterol ester transfer protein, high density lipoproteins, insulin resistance, lecithin, phospholipid transfer protein, triglycerides.

Introduction

The obese state is widely recognized to be related to dyslipidaemia, glucose intolerance and hypertension and carries an increased risk of cardiovascular disease [1–6]. Many studies have shown that insulin resistance is a common marker for this cluster of interrelated risk factors [7,8,9]. The pattern of body fat distribution is also an important determinant of lipid abnormalities, since upper body adiposity, as measured by the waist to hip circumference ratio, is associated with high plasma triglycerides and low high density lipoprotein (HDL) cholesterol levels [6,10,11,12].

The mechanisms responsible for the low HDL cholesterol levels associated with obesity and insulin resistance are not well understood. Apart from lipid-protein and hepatic lipase, lecithin: cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) are crucial factors in the intravascular metabolism of HDL cholesterol [13–16]. LCAT is necessary to esterify cholesterol in plasma, whereas CETP catalyzes the subsequent transfer of cholesteryl ester from HDL towards triglyceride-rich lipoproteins. Immunological inhibition of CETP activity increases HDL cholesteryl ester as well as HDL particle size in vitro [17,18], and extremely high HDL cholesterol levels have been observed in human cases of CETP deficiency [19]. Another lipid transfer protein that specifically catalyzes the transfer of phospholipids has been isolated from human plasma [20]. Recent in vitro studies suggest that this phospholipid transfer protein (PLTP) is involved in the regulation of HDL particle size [21].

Virtually no information is available that relates obesity and insulin resistance to the plasma activities of these lipid transfer proteins. Therefore, the present study was undertaken to establish whether the plasma activities of LCAT, CETP and PLTP are associated with parameters of adiposity and insulin resistance as judged by fasting insulin and C-peptide levels.
Methods

Subjects

The obese and non-obese participants, 21–60 years of age, were recruited from hospital personnel and consented to the procedure after explanation of the purpose of the study. The subjects were weighed in light indoor clothing and men with a body mass index (BMI) > 27.0 kg m\(^{-2}\) were considered to be obese. Only male non-smoking subjects were eligible to avoid the effects of the menstrual cycle and cigarette smoking on lipid levels. Apart from obesity the participants were considered to be healthy. They did not suffer from diabetes mellitus as judged by a fasting blood glucose level < 5.6 mmol l\(^{-1}\) and had a normal blood pressure (systolic blood pressure < 160 mmHg and diastolic blood pressure < 95 mmHg). None of the subjects had thyroid or liver dysfunction and they did not use any medication. Fasting plasma total cholesterol and triglycerides were < 8.0 mmol l\(^{-1}\) and < 3.0 mmol l\(^{-1}\), respectively, thus excluding severe hyperlipidaemia. Family history with respect to premature cardiovascular disease (onset before the age of 60 years) was negative. The waist to hip circumference ratio was measured as the ratio of the smallest girth between rib cage and iliac crest, and the largest girth between waist and thigh [12].

Laboratory measurements

Fasting venous blood was collected into EDTA (1.5 mg ml\(^{-1}\)) containing tubes and was placed on ice immediately. Plasma was separated from erythrocytes within 30 min by centrifugation at 3000 rev min\(^{-1}\) for 15 min at 4°C. Plasma samples were frozen at \(-20°C\) until assayed.

LCAT, CETP and PLTP activity assays

LCAT activity was determined using excess exogenous substrate, containing \([\text{H}]\)-cholesterol as described [22]. Plasma samples were incubated for 6 h at 37°C in a total volume of 0.145 ml. The reaction was stopped by addition of 0.3 ml cold ethanol to the incubation mixture. Lipids were extracted twice with 0.4 ml hexane. Free and esterified cholesterol were separated using disposable silica columns, \([\text{H}]\)-cholesterol ester being eluted with 3-0 ml hexane (6:1, vol vol\(^{-1}\)) [23]. The measured LCAT activities were linear with the amount of plasma used.

CETP activity was measured in each plasma after removal of endogenous VLDL+LDL by phosphotungstate/MgCl\(_2\) precipitation [24]. The isotope assay detects the transfer of radioactive cholesteryl ester between exogenous [\(^{1-14}\text{C}\)-oleate]-cholesteryl ester labelled LDL and an excess of unlabelled pooled normal HDL. Dithiobis 2-nitrobenzoic acid was added to the incubation mixture to inhibit CET. Incubations were carried out for 16 h at 37°C. The reaction was stopped by cooling the tubes to 4°C, followed by precipitation of LDL with Mn\(^{2+}\) ions. CETP activity was calculated as the bidirectional transfer of cholesteryl ester between radiolabelled LDL and HDL.

PLTP activity was assayed in each plasma using a phospholipid vesicles-HDL system, as described [25]. Briefly, plasma samples were incubated with \([\text{H}]\)-phosphatidylcholine-labelled vesicles and normal HDL for 1-5 h at 37°C. The reaction was stopped by chilling the tubes on ice. The vesicles were then precipitated with a mixture of NaCl, MgCl\(_2\) and heparin (final concentrations, 230 mmol l\(^{-1}\), 92 mmol l\(^{-1}\) and 200 U ml\(^{-1}\) respectively). The measured PLTP activities were linear with the amount of plasma added to the assay system. The method is specific for PLTP activity and is not influenced by the phospholipid transfer promoting properties of CETP [25].

The assays of LCAT, CETP and PLTP activity were performed in duplicate. The within assay coefficients of variation are 4-5%, 2.7% and 3-5% for LCAT, CETP and PLTP, respectively. The measured activities reflect the activities of the enzyme and lipid transfer proteins as such and are independent of the endogenous lipoproteins present in each plasma. The activities of LCAT, CETP and PLTP were related to the activities of these factors measured in human pool plasma that was included in each run. LCAT activity is expressed in nmol esterified cholesterol per ml plasma h\(^{-1}\), CETP activity is expressed in nmol cholesteryl ester per ml plasma h\(^{-1}\) and PLTP activity is expressed in \(\mu\)mol phosphatidylcholine per ml plasma h\(^{-1}\).

Other laboratory assays

Lipids were measured in whole plasma and in the HDL-containing supernatant fraction after removal of apolipoprotein B-containing lipoproteins with polyethylene glycol-6000 [26]. Very low and low density lipoprotein (VLDL+LDL) lipids were calculated as the difference between plasma and the HDL fraction. Total cholesterol was measured by gaschromatography [27]. Free cholesterol was determined by a modification of this method in which the hydrolysis step was omitted. Cholesteryl ester was calculated as the difference between total and free cholesterol. Triglycerides were measured enzymatically [28]. Phospholipids were measured according to Zilversmit [29]. Apolipoproteins A\(_1\) and B were determined by immu- noturbidimetry using commercially available kits (Boehringer Mannheim, Germany, cat no 726478 and 726494, respectively). Plasma insulin and C-peptide were measured using radioimmunoassays. Blood glucose was measured by the hexokinase method. Free fatty acids were measured according to Mosinger [30].

Statistical analysis

Values are expressed as mean ± SD. Data were divided into quartiles of BMI and compared by Kruskal-Wallis analysis of variance. Duncan's method was used to correct for multiple comparisons. Linear regression analysis was used to calculate correlation...
performed to document the independent relationships
coefficients. Multiple stepwise regression analysis was
performed to document the independent relationships
between data. A two-sided P value < 0.05 was consid-
ered to be significant.

Results

Eight obese and 24 non-obese men were recruited for
the study, which allowed us to divide the subjects into
quartiles of BMI. The clinical characteristics of the
subjects are given in Table 1. The obese men had an
increased waist to hip circumference ratio. In these
subjects plasma insulin and C-peptide levels were also
elevated. Mean age and free fatty acid levels were
increased waist to hip circumference ratio. In these
subjects plasma insulin and C-peptide levels were also
elevated. Mean age and free fatty acid levels were
comparable between quartiles, whereas the differences
in blood glucose and mean arterial pressure were not
significant.

Plasma lipid, lipoprotein and apolipoprotein levels
are shown in Table 2. Plasma triglyceride concentra-
tion was higher, whereas HDL cholesterol was lower in
the obese men as compared to the subjects with the
lowest BMI. The decrease in HDL cholesterol was due
to a reduction in HDL cholesteryl ester. HDL trigly-

Table 1. Clinical characteristics, fasting insulin, C-peptide and free fatty acid levels, according to
quartiles of body mass index

<table>
<thead>
<tr>
<th>Quartiles of body mass index</th>
<th>Q1 (n=8)</th>
<th>Q2 (n=8)</th>
<th>Q3 (n=8)</th>
<th>Q4 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>21.6±0.4</td>
<td>23.1±0.5</td>
<td>24.7±0.8</td>
<td>29.5±1.1</td>
</tr>
<tr>
<td>(21.1–22.3)</td>
<td>(22.4–23.7)</td>
<td>(23.8–26.1)</td>
<td>(28.3–30.9)</td>
<td></td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.86±0.04*</td>
<td>0.82±0.06*</td>
<td>0.85±0.07*</td>
<td>0.98±0.12*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41±10</td>
<td>37±16</td>
<td>36±12</td>
<td>42±15</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>97±9</td>
<td>94±14</td>
<td>95±6</td>
<td>103±10</td>
</tr>
<tr>
<td>Blood glucose (mmol l⁻¹)</td>
<td>4.04±0.33</td>
<td>4.00±0.35</td>
<td>4.09±0.36</td>
<td>4.24±0.53</td>
</tr>
<tr>
<td>Plasma insulin (pmol l⁻¹)</td>
<td>40±23*</td>
<td>30±14</td>
<td>40±25*</td>
<td>80±47</td>
</tr>
<tr>
<td>Plasma C-peptide (mmol l⁻¹)</td>
<td>0.23±0.12†</td>
<td>0.25±0.11*</td>
<td>0.29±0.07</td>
<td>0.57±0.49</td>
</tr>
<tr>
<td>Free fatty acids (mmol l⁻¹)</td>
<td>0.63±0.29</td>
<td>0.51±0.16</td>
<td>0.69±0.36</td>
<td>0.53±0.26</td>
</tr>
</tbody>
</table>

Values are means ± SD and range. *P < 0.05; †P < 0.01 from upper quartile (Q4).

Table 2. Lipid parameters, according to quartiles of body mass index

<table>
<thead>
<tr>
<th>Quartiles of body mass index</th>
<th>Q1 (n=8)</th>
<th>Q2 (n=8)</th>
<th>Q3 (n=8)</th>
<th>Q4 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total cholesterol (mmol l⁻¹)</td>
<td>4.72±0.54</td>
<td>4.91±1.41</td>
<td>5.27±1.32</td>
<td>5.03±1.09</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol l⁻¹)</td>
<td>1.28±0.45*</td>
<td>1.23±0.39*</td>
<td>1.50±0.66</td>
<td>1.78±0.66</td>
</tr>
<tr>
<td>VLDL+LDL cholesterol (mmol l⁻¹)</td>
<td>3.56±0.53</td>
<td>3.80±1.26</td>
<td>4.15±1.37</td>
<td>4.05±1.16</td>
</tr>
<tr>
<td>HDL cholesteryl (mmol l⁻¹)</td>
<td>1.16±0.16*</td>
<td>1.11±0.24</td>
<td>1.13±0.21</td>
<td>0.98±0.21</td>
</tr>
<tr>
<td>HDL cholesteryl ester (mmol l⁻¹)</td>
<td>0.95±0.13*</td>
<td>0.93±0.20</td>
<td>0.90±0.15</td>
<td>0.80±0.17</td>
</tr>
<tr>
<td>HDL triglycerides (mmol l⁻¹)</td>
<td>0.29±0.04</td>
<td>0.27±0.03</td>
<td>0.29±0.02</td>
<td>0.30±0.06</td>
</tr>
<tr>
<td>HDL phospholipids (mmol l⁻¹)</td>
<td>1.20±0.24</td>
<td>1.24±0.26</td>
<td>1.28±0.20</td>
<td>1.10±0.15</td>
</tr>
<tr>
<td>HDL cholesteryl ester/triglycerides</td>
<td>3.30±0.43*</td>
<td>3.53±0.95*</td>
<td>3.20±0.62</td>
<td>2.78±0.87</td>
</tr>
<tr>
<td>Apolipoprotein A₁ (g l⁻¹)</td>
<td>1.59±0.09</td>
<td>1.53±0.21</td>
<td>1.67±0.58</td>
<td>1.45±0.20</td>
</tr>
<tr>
<td>Apolipoprotein B (g l⁻¹)</td>
<td>0.80±0.15</td>
<td>0.76±0.16</td>
<td>0.72±0.23</td>
<td>0.76±0.23</td>
</tr>
</tbody>
</table>

Values are means ± SD. VLDL+LDL, very low and low density lipoproteins; HDL, high density lipoproteins.*P < 0.05 from upper quartile (Q4).
OBESITY AND LIPID TRANSFER PROTEINS

2.5
2.0
1.5
1.0
E
ul
e
L
T
TT

60-50-40-30-20-10-0

0.6 0.7 0.8 0.9 1.0 1.1 1.2

waist/hip ratio

A

B

Figure 1. Relationships between waist to hip circumference ratio, high density lipoprotein (HDL) cholesterol and plasma triglycerides. A. Waist to hip circumference ratio and HDL cholesterol, r = -0.45, P < 0.01. B. Waist to hip circumference ratio and plasma triglycerides, r = 0.56, P < 0.001.

Figure 2. Plasma lecithin: cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) activities according to quartiles of body mass index (BMI). The men from the highest quartile (Q4) are obese (BMI > 27 kg m⁻²). Bars represent means and SEM. * P < 0.05; † P < 0.01 from Q4.

subjects in the third quartile of BMI. The univariate correlation analyses of plasma LCAT, CETP and PLTP activities with the various clinical parameters are given in Table 3. LCAT activity was only correlated with age. CETP activity was positively correlated with BMI (Fig. 3A), blood glucose and plasma C-peptide, but not with waist to hip circumference ratio. PLTP activity was positively correlated with BMI (Fig. 3B) and waist to hip circumference ratio (Fig. 3C), as well as with blood glucose and plasma C-peptide. Remarkably, neither CETP nor PLTP activity was significantly correlated to plasma insulin. Multiple stepwise regression analysis with BMI, waist to hip circumference ratio, blood glucose and log fasting C-peptide as independent variables demonstrated that the relationship of CETP with BMI remained significant (P < 0.02). PLTP activity was independently related to waist to hip circumference ratio (P < 0.001) and blood glucose (P < 0.05). The multiple r-values of the models were r = 0.43, P < 0.02 for CETP activity and r = 0.70, P < 0.001 for PLTP activity, respectively. LCAT activity was correlated with plasma triglycerides (r = 0.67, P < 0.001) and with VLDL + LDL cholesterol (r = 0.45, P < 0.01), but only the relationship with triglycerides remained significant (P = 0.001) in the multiple regression analysis. CETP activity was weakly correlated with VLDL + LDL cholesterol (r = 0.35, P = 0.05) and not with plasma triglycerides (r = 0.28, NS). PLTP activity was correlated with plasma triglycerides (r = 0.40, P < 0.05), as well as with VLDL + LDL cholesterol (r = 0.38, P < 0.05). The relationship between PLTP activity and plasma triglycerides was significant (P < 0.05) in the multiple regression analysis. Neither HDL cholesterol ester nor HDL phospholipids were significantly correlated with the plasma activities of LCAT, CETP and PLTP. Finally, CETP and PLTP activities were interrelated (r = 0.40, P < 0.05).
Discussion

Our results support the well-known association between (upper body) adiposity, high triglyceride and low HDL cholesterol concentrations [1,2,5,6,8,10,11,12]. Interestingly, the plasma levels of CETP and PLTP activity were found to be related to various parameters of adiposity. Moreover, the activities of CETP and PLTP were positively correlated to fasting blood glucose and C-peptide levels, raising the possibility of a link between insulin resistance and the activity of these lipid transfer proteins.

Both experimental and clinical observations support that CETP is a key factor in the intravascular metabolism of HDL [14,15,16]. Intravenous injection of CETP into rats, a species that lacks this lipid transfer protein, decreases HDL cholesterol [31] and transgenic mice expressing human CETP show lowered HDL cholesterol levels [32]. In human subjects elevated CETP activity is associated with low levels of HDL cholesterol as well [33,34,35]. Conversely, increases in HDL cholesterol are related to low levels of CETP activity in hypothyroidism [36]. Therefore, it is likely that elevations in CETP activity can also contribute to the low levels of HDL cholesterol, HDL cholesteryl ester and the low HDL cholesteryl ester/triglyceride ratio observed in obesity.

The CETP-catalysed transfer of cholesteryl ester from HDL towards apolipoprotein B-containing lipoproteins is not only dependent on the activity of CETP as such, but also on the concentration and composition of the lipoproteins involved [15,33,37]. Importantly, the rate of cholesteryl ester mass transfer out of HDL is well correlated with VLDL triglyceride concentration [38]. In normotriglyceridaemia the rate of cholesteryl ester mass transfer from HDL is determined by the amount of VLDL triglyceride in the incubations, while in hypertriglyceridaemia the amount of CETP becomes the rate limiting factor [39]. The recently described net mass transfer of cholesteryl ester from LDL towards HDL may also influence HDL cholesterol levels [40]. In the present study which included mildly hyperlipidaemic subjects HDL cholesterol was not correlated with CETP activity, but instead with plasma triglycerides.

Besides CETP several other factors, including lipases, LCAT and PLTP, have been implicated in the
metabolism of HDL [13–16,21]. The actions of lipoprotein lipase and LCAT result in an increase in HDL cholesterol levels and in HDL particle size [13,14]. Lipoprotein lipase activity in adipose tissue is not decreased in obesity, although the stimulation of this enzyme by insulin is impaired [9]. Moreover, lipoprotein lipase, measured in post-heparin plasma, is not significantly decreased in insulin resistant individuals with low HDL cholesterol and high triglyceride levels [41]. In our study, LCAT activity was comparable between obese and non-obese subjects. Therefore, it is not very likely that impaired activities of plasma lipoprotein lipase or LCAT as such play an important role in the low levels of HDL cholesterol associated with obesity and insulin resistance. In vitro studies suggest that PLTP can act as an HDL conversion factor, since the protein has the ability to convert HDL₃, the dense HDL subfraction, into populations of larger and smaller HDL particles [21]. In the absence of LCAT and CETP, PLTP could facilitate the formation of pre-beta-migrating HDL, particles [42]. Thus it is possible that concomitant elevations in plasma CETP and PLTP can influence HDL subfraction levels. Although PLTP activity was only modestly elevated in obese subjects, the correlations with plasma triglycerides, VLDL + LDL cholesterol, adiposity parameters, blood glucose, and plasma C-peptide were stronger for PLTP than for CETP activity. This suggests that subtle variations in PLTP activity levels may be important in adiposity-related changes in lipoprotein metabolism.

The mechanisms responsible for the relationships between lipid transfer protein activities, adiposity, fasting blood glucose and C-peptide levels remain to be established. It is likely that elevated levels of CETP activity are due to an increase in CETP mass [43], although a decrease in putative inhibitor activity cannot be excluded [44]. Liver, adipose tissue, and muscle are possible sources of CETP [16,45], but the site of PLTP synthesis is unknown. In animal tissues elevated CETP messenger-RNA levels have been found after cholesterol feeding, suggesting that high levels of apolipoprotein B-containing lipoproteins can stimulate CETP synthesis [45,46]. The positive correlations between VLDL + LDL cholesterol and plasma lipid transfer protein activities are in agreement with the contention that there is a metabolic interrelationship between the regulation of lipid transfer proteins and that of apolipoprotein B-containing lipoproteins. PLTP but not CETP activity was correlated with plasma triglycerides. In accordance, plasma CETP mass is not increased in subjects with moderate hypertriglyceridemia [43]. The relationship between LCAT activity and plasma triglycerides has been described before [47], but in contrast to these previous studies we did not observe a correlation with adiposity parameters. Apparently, different mechanisms are involved in the regulation of LCAT, CETP, and PLTP.

There is evidence that insulin action is impaired in obesity [8,48]. Furthermore, the insulin-mediated glucose disposal rate has been shown to be a more important clinical determinant of HDL cholesterol and plasma triglycerides than BMI [8,49] and waist to hip circumference ratio [8]. Therefore, it is likely that insulin resistance plays a major role in the adiposity-associated dyslipidemia. Despite insulin resistance, the obese subjects showed normal fasting free fatty acid levels, but in obesity the flux of free fatty acids is increased and is less suppressable by exogenous insulin [50]. As a result, an increased fuel delivery to the liver ensues that will lead to enhanced hepatic VLDL triglyceride synthesis in insulin resistant states [9,51]. In vitro studies in a model system of cultured hepatocytes show that insulin itself can exert a direct inhibitory effect on hepatic lipoprotein synthesis [52]. Of interest, the present study showed a positive correlation of lipid transfer protein activities with C-peptide levels, but not with insulin. Obviously, further experiments are needed to establish the effects of insulin resistance, hyperinsulinemia, increased free fatty acid flux and hepatic lipoprotein synthesis on lipid transfer protein regulation.

In conclusion, the present study demonstrates that the plasma activities of CETP and PLTP are influenced by adiposity and possibly by insulin resistance. Elevated lipid transfer protein activities may provide a mechanism that contributes to alterations in HDL in insulin-resistant states.

Acknowledgments

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