Fibroblast cholesterol efflux to plasma from metabolic syndrome subjects is not defective despite low high-density lipoprotein cholesterol

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Abstract
Objective: We tested whether in metabolic syndrome (MetS) subjects the ability of plasma to stimulate cellular cholesterol efflux, an early step in the anti-atherogenic reverse cholesterol transport pathway, is maintained despite low high-density lipoprotein (HDL) cholesterol.
Design: In 76 subjects with and 94 subjects without MetS based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria, we determined plasma (apo)lipoproteins, pre-β-HDL formation, phospholipid transfer protein (PLTP) activity, cholesterol esterification (EST), cholesteryl ester transfer (CET), adiponectin, and the ability of plasma from each subject to stimulate cholesterol efflux out of cultured fibroblasts obtained from a single donor.
Results: Apo E, PLTP activity, EST, and CET were higher (P<0.04 to <0.001), whereas adiponectin was lower in MetS subjects (P<0.01). Pre-β-HDL and pre-β-HDL formation were not different between subjects with and without MetS. Cellular cholesterol efflux to plasma from MetS subjects was slightly higher versus plasma from subjects without MetS (8.8±1.0 vs 8.5±0.9%, P=0.05), but the difference was not significant after age, sex, and diabetes adjustment. Cellular cholesterol efflux was positively related to pre-β-HDL formation, EST, PLTP activity, and apo E (P<0.05 for all by multiple linear regression analysis), without an independent association with MetS and diabetes status.
Conclusions: The ability of plasma from MetS subjects to promote fibroblast cholesterol efflux is not defective, although HDL cholesterol is decreased. Higher cholesterol esterification, PLTP activity, and apo E levels may contribute to the maintenance of cholesterol efflux in MetS.

Introduction
The metabolic syndrome (MetS) represents a cluster of cardiovascular risk factors, including (central) obesity, high blood pressure, high plasma triglycerides, low high-density lipoprotein (HDL) cholesterol, and hyperglycemia (1). In recent years, several expert groups have proposed (and revised) their criteria for MetS (2–5) with the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria, originally appeared in 2001 (3) and being widely used in the literature. The existence of such a syndrome has been criticized, and it has been disputed whether resistance to insulin-stimulated glucose uptake should be regarded as the unifying pathophysiological abnormality (1). Nonetheless, a systemic review has shown that cardiovascular risk is increased by 65% in subjects fulfilling the NCEP ATP III criteria for MetS (6), an observation that was recently extended by the DECODE study group (7).

The reverse cholesterol transport (RCT) process directs cholesterol via HDL from the peripheral tissues to the liver for subsequent excretion in the bile, and is considered to represent a pathway that protects against atherosclerosis development (8–10). Efflux of cholesterol from peripheral cells to extracellular acceptors is an important early step in RCT (11, 12). Cellular cholesterol removal is governed by several still incompletely understood mechanisms, including aqueous diffusion, transport of cholesterol via the ATP-binding cassette (ABC) transporters, ABCA1, and ABCG1, as well as via scavenger receptor class B type 1 (SR-BI) (11–14). Small, lipid-poor, or lipid-free apo A-I-containing particles, commonly designated pre-β-HDL, are considered to be the initial acceptors of cell-derived cholesterol via the ABCA1 transporter (8, 10, 15, 16). Among other factors, the metabolism of these particles is governed in a complex way by phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP), which contribute to pre-β-HDL generation, and by lecithin:cholesterol acyltransferase (LCAT), which...
decreases pre-β-HDL (8, 10, 16, 17). SR-BI and the recently discovered ABCG1 transporter are able to mediate cholesterol efflux to mature α-HDL particles (13, 14).

In MetS, increased catabolism of HDL particles contributes to low plasma levels of its major apolipoproteins (apos), apo A-I and A-II, and a drop in lipoprotein A-I:A-II, i.e., HDL particles that contain both apos (18, 19), but little is known about possible abnormalities in the RCT pathway. One study in MetS subjects has demonstrated a normal potential of individual skin fibroblasts, a cell system that expresses ABCA1 but hardly any SR-BI (11, 12, 20, 21), to transport cholesterol to purified apo A-I, acting as a cholesterol acceptor (22). On the other hand, cholesterol efflux from monocyte-derived macrophages to apo A-I is defective in a considerable number of subjects with isolated low HDL cholesterol, even in the absence of mutations in ABCA1 (23). Using Fu5AH cells, which express SR-BI but no ABCA1 (11, 12), cholesterol efflux to serum or plasma is maintained in hypertriglyceridemic (24) and insulin-resistant subjects (25).

We questioned whether plasma from MetS subjects maintains the ability to promote cholesterol efflux out of cultured human fibroblasts despite its low HDL concentration. In the present study, we compared the ability of plasma to promote cellular cholesterol removal, as well as plasma pre-β-HDL, PLTP activity, CET, and cholesterol esterification (EST) between subjects with MetS and without MetS.

### Subjects and methods

The study was approved by the medical ethics committee of the University Medical Center Groningen, the Netherlands. Participants were recruited by advertisement in local newspapers, and written informed consent was obtained from all of them. All participants were aged > 18 years. Smoking, use of lipid-lowering drugs, clinically manifest cardiovascular disease, renal disease (elevated serum creatinine or urinary albumin > 20 mg/l), thyroid disease (abnormal serum thyrotrophin level, or use of thyroxine or antithyroid drugs), and alcohol use > 3 beverages per day were the exclusion criteria. Diabetic patients were included, except when using insulin or thiazolidinediones. The use of antihypertensive medication was allowed.

MetS was defined according to the NCEP ATP III 2001 criteria (3). Three or more of the following criteria were required for categorization of subjects with MetS: waist circumference > 102 cm for men and > 88 cm for women; plasma triglycerides ≥ 1.7 mmol/l; HDL cholesterol < 1.0 mmol/l for men and < 1.3 mmol/l for women; hypertension (blood pressure ≥ 130/85 mmHg or use of antihypertensive medication); fasting glucose ≥ 6.1 mmol/l or known diabetes. Type 2 diabetes mellitus had been previously diagnosed by primary care physicians using blood glucose cut-off values as defined by the WHO (2), and patients were treated with either diet alone or in combination with oral glucose-lowering agents.

All participants were evaluated after an overnight fast. BMI was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured on bare skin between the tenth rib and the iliac crest. Blood pressure was measured after a 15-min rest at the left arm in the sitting position using a sphygmomanometer. Homeostasis model assessment was used as a measure of insulin sensitivity (HOMAIR) using the equation: fasting plasma insulin (mU/l) × glucose (mmol/l)/22.5 (26).

### Laboratory measurements

After blood collection, EDTA-containing plasma samples were prepared at 4 °C. The samples were directly stored at −80 °C until shortly before the various analyses, except for plasma glucose and glycated hemoglobin (HbA1c), which were measured directly after blood collection.

Plasma cholesterol and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi catalogue numbers 11876023 and 11875540 respectively; Roche Diagnostics GmbH). HDL cholesterol was determined with a homogeneous enzymatic colorimetric test (Roche/Hitachi cat. no. 03030024), and its measurement is unchanged after a prolonged period of storage at −80 °C. Non-HDL cholesterol was calculated as the difference between plasma total cholesterol and HDL cholesterol. Apolipoproteins (apos) A-I and B were assayed by immunoturbidimetry (Roche/Cobas Integra Tinaquant cat. no. 03032566 and 03032574 respectively; Roche Diagnostics). Apo E was measured using an immunoturbidimetric assay (cat. no. 417-35906; Wako Inc., Osaka, Japan).

Plasma pre-β-HDL concentration was measured by crossed immunoelectrophoresis, essentially as described elsewhere (27), except that anti-human apo A-I was used. Briefly, plasma samples were thawed while kept on ice, and 0.9 µmol/l Pefabloc SC (Boehringer-Roche) and 1.8 µg/l Trasylol (Bayer) were added to inhibit proteolysis (both final concentrations). The crossed immunoelectrophoresis consisted of agarose electrophoresis in the first dimension for the separation of lipoproteins with pre-β- and α-mobility. Antigen migration from the first agarose gel into the second agarose gel, containing goat anti-human apo A-I antiserum (0.66% vol/vol; Midland Bioproducts Corporation, Boone, IA, USA, cat. no. 71101), was used to quantitatively precipitate apo A-I. The antiserum was monospecific for human apo A-I antiserum (0.66% vol/vol; Midland Bioproducts Corporation, Boone, IA, USA, cat. no. 71101), was used to quantitatively precipitate apo A-I. The antiserum was monospecific for human apo A-I using an immunodiffusion assay. Lipoprotein electrophoresis was carried out in 1% (weight/vol) agarose gels in Tris (80 mmol/l)–Tricine (24 mmol/l) buffer, 5% (vol/vol) polyethylene glycol 300 (pH 8.6), and run in an LKB 2117 system (4 °C for 3 h, 210 V). Plasma was applied at 3 µl/well. The track of the first agarose gel was excised and annealed with melted agarose to a gel containing 0.66% (vol/vol) goat anti-human apo A-I anti-serum (Midland Bioproducts corporation) and 0.01% m/V Tween 20, which was cast on GelBond film.
with DMEM, they were loaded with \[ 3 \text{H} \] cholesterol in 10% vol/vol fetal calf serum (FCS). After washing them, the cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% (vol/vol) FCS. The ability of plasma to generate pre-\( \beta \)-HDL, was assessed using the same procedure, but after 24-h incubation of plasma at 37 °C under conditions of LCAT inhibition. To this end, iodoacetate (final concentration 1.0 mmol/l) was added directly after thawing the samples. Pre-\( \beta \)-HDL and pre-\( \beta \)-HDL formation were calculated using the total plasma apo A-I concentration and were expressed in apo A-I.

Plasma PLTP activity was assayed with a phospholipid vesicles–HDL system, using \([14 \text{C}]\)-labeled dipalmitylphosphatidylcholine (28). This method is specific for the PLTP activity, and the PLTP promoting property of CETP does not interfere with the assay. Plasma PLTP activity is related to the activity in human reference pool plasma and is expressed in arbitrary units (AU: 100 AU corresponds to 13.6 \( \mu \)mol phosphatidylcholine transferred, ml/h). Plasma CET was determined as described previously (29). Briefly, \([3 \text{H}]\) cholesterol was equilibrated for 24 h at 37 °C with plasma cholesterol followed by incubation of plasma at 37 °C. Thereafter, apo B-containing lipoproteins were precipitated and the labeled cholesteryl esters were separated from the labeled unesterified cholesterol on silica columns. Plasma EST was measured as the formation of cholesteryl esters after the addition of \([3 \text{H}]\) cholesterol to plasma as described (29). Equilibration of added \([3 \text{H}]\) cholesterol was obtained after incubation at 4 °C for 24 h. Subsequently, the plasma was incubated at 37 °C.

Cholesterol efflux to plasma was determined using human fibroblasts as cholesterol donor, essentially as described (20, 30). In brief, fibroblasts were obtained from a normolipidemic control and were cultured (until passages 5–15) in 24-well culture plates to full confluency. The cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% vol/vol fetal calf serum (FCS). After washing them with DMEM, they were loaded with \([3 \text{H}]\) cholesterol (0.5 \( \mu \)Ci/ml) during 24 h in the presence of added unlabelled cholesterol (30 \( \mu \)g/ml), thereby inducing ABCA1 (20). After cholesterol loading, cells were washed three times with 0.2% PBS/BSA (weight/vol). The efflux assay was started by the addition of the individual plasma sample, diluted to 1% in efflux medium. Heparin (1.25 U/ml) was added to prevent clotting. After 4-h incubation at 37 °C, the medium was collected and centrifuged. \([3 \text{H}]\) cholesterol was quantified by liquid scintillation counting. Total cellular \([3 \text{H}]\) cholesterol was determined after extraction of the cells with 2-propanol. The percentage efflux was calculated by dividing the radioactive counts in the efflux medium by the sum of the counts in the medium and the cell extract. All plasma samples were analyzed in duplicate, and values were corrected for radioactivity appearing in the culture medium in the absence of plasma. To be able to normalize results between series of experiments and to correct for day-to-day variation, efflux to human plasma pool was determined in quadruplicate. Experiments with \([14 \text{C}]\) cholesterol were carried out, demonstrating that influx of \([14 \text{C}]\) cholesterol from the plasma to the cells was consistently <10% (triplicate measurements using three different plasma samples). Therefore, the assumption is valid that efflux is almost exclusively from the cells to the medium under the current assay conditions. Moreover, the ability of plasma to stimulate cellular cholesterol efflux was unaffected by prolonged storage of the samples at −80 °C (data not shown).

Plasma insulin was measured with a microparticle enzyme immunoassay (AxSYM insulin assay; Abbott Laboratories). Free fatty acids (FFAs) were assayed by a commercially available kit (Wako Chemicals, Neuss, Germany, cat. no. 999-75406). High-sensitive C reactive protein (CRP) was assayed by nephelometry with a threshold of 0.175 mg/l (BNII N; Dade Behring, Marburg, Germany). Plasma adiponectin was assayed using Luminex xMAP technology with a commercially available kit, according to the manufacturer’s instructions (Linco Research Inc., St Charles, MO, USA; Lincoplex panel A cat. no. HADK1-61K-A, purchased from Nuclilab Inc. Ede, the Netherlands). Adiponectin, as measured with this technology, is strongly correlated (\( r^2 = 0.95 \)) with assay results obtained by enzyme-linked immunoassays from Linco Inc. (data provided by the manufacturer). Glucose was measured with an APEC glucose analyzer (APEC Inc., Danvers, MA, USA) and HbA1c by HPLC (Bio-Rad; reference ranges 4.6–6.1%).

Statistical analysis

Data are shown as mean ± s.d. or in the case of a skewed distribution as geometric mean (95% confidence intervals, CI). \( \chi^2 \) analysis was used to evaluate the differences in proportions among the groups. Between-group differences were evaluated by Student’s \( t \)-tests or ANOVA where appropriate. When variables had a skewed distribution, logarithmically transformed values were used. Univariate correlations were assessed by linear regression analysis, using Pearson’s correlation coefficients. Multiple linear regression analysis was used to reveal independent relationships between variables. Two-sided \( \text{P} \) values <0.05 were considered to be statistically significant.

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**Results**

A total of 170 subjects (99 men, 71 women) were included in the study. Out of these, 76 subjects fulfilled the criteria for MetS, whereas 94 did not. Age and sex distribution were not significantly different between subjects with and without MetS (Table 1). Diabetes mellitus ($P < 0.001$) was found in 55 subjects with and 26 without MetS. Out of these diabetic subjects, 24 were treated with diet alone, 16 used biguanides alone, 17 used sulfonylurea alone, and 24 used both glucose-lowering drugs. Twenty-eight of the MetS subjects and seven of the subjects without MetS used antihypertensive drugs. Seven of the subjects without MetS used antihypertensive drugs alone, and 24 used both glucose-lowering drugs. Two women in each group used estrogens.

BMI, waist circumference, blood pressure, plasma glucose, insulin, HOMA$_{IR}$, FFA, and CRP levels were higher in subjects with MetS compared with subjects without MetS, and these differences remained significant after adjustment for age, sex, and diabetes status (Table 1). Plasma adiponectin, HDL cholesterol, and apo A-I were decreased, whereas non-HDL cholesterol, triglycerides, apo B, and apo E levels were increased in MetS subjects (Table 1).

As shown in Table 2, PLTP activity, EST, and CET were higher in MetS subjects and remained so after age, sex, and diabetes status adjustment. Plasma pre-$\beta$-HDL and pre-$\beta$-HDL formation were similar in subjects with and without MetS. The ability of plasma to promote cholesterol efflux out of fibroblasts was slightly higher in subjects with MetS compared with subjects without MetS, but the ability of plasma to promote cholesterol efflux was not significant after controlling for age, sex, and diabetes.

We also compared non-diabetic subjects with ($n = 21$) and without MetS ($n = 68$), diabetic subjects with MetS ($n = 55$), and diabetic subjects without MetS ($n = 26$; Table 3). Pre-$\beta$-HDL and pre-$\beta$-HDL formation were similar in these four groups, whereas PLTP activity, EST, and CET were the highest in subjects with both MetS and diabetes. Cellular cholesterol efflux was somewhat higher in diabetic subjects with MetS compared with diabetic subjects without MetS, but was similar between non-diabetic subjects with and without MetS.

In univariate regression analysis, cellular cholesterol efflux to plasma was positively correlated with plasma apo A-I, and pre-$\beta$-HDL cholesterol, plasma apo A-I, and adiponectin in either group.

Multiple linear regression analysis was done to discern the independent relationships between HDL-related variables and cellular cholesterol efflux to plasma. In this analysis, only those HDL-related variables that were significantly correlated with cellular cholesterol efflux in univariate analysis were included. In the combined subjects (Table 4). Cellular cholesterol efflux was not significantly correlated with HDL cholesterol, plasma apo A-I, and adiponectin in either group.

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Discussion

Our study shows that plasma apo B, apo E, EST, CET, as well as PLTP activity are increased, whereas plasma pre-β-HDL and pre-β-HDL formation are unahtlered in the MetS subjects. Furthermore, using cultured human skin fibroblasts as cell system, we demonstrate for the first time that cholesterol efflux to plasma from the MetS subjects is maintained despite low HDL cholesterol.

Essentially similar findings were observed when comparing non-diabetic subjects with and without the MetS. In diabetic subjects, cholesterol efflux was slightly higher among those who fulfilled the criteria for the MetS. Cellular cholesterol efflux to plasma was found to be related to plasma EST, pre-β-HDL formation, PLTP activity, and apo E, without an independent association with the presence of the MetS and the diabetic state. Taken together, our data thus suggest that, as far as the ability of plasma to promote cholesterol efflux out of fibroblasts is concerned, this step in the RCT pathway is not impaired in the MetS.

Cholesterol efflux from cells to extracellular acceptors is governed by a number of known and even unknown pathways, and is influenced by the capacity and the quality of extracellular acceptor particles (11, 12, 21). In the present study, human skin fibroblasts, obtained from a single normolipemic donor, were used to assess the ability of plasma to promote cholesterol efflux. These cells abundantly express ABCA1 (20), and probably also ABCG1 (31), but contain negligible SR-BI (21). Thus, it is most likely that several pathways including ABCA1-mediated cholesterol transport contribute to fibroblast cholesterol efflux, although their relative contribution is still unknown. It is well established that pre-β-HDL act as primary acceptors of cellular cholesterol via ABCA1 (10, 12, 15, 16), and the relevance of this pathway is emphasized by the observation that efflux from fibroblasts that lack functional ABCA1 is strongly diminished (22, 30). In our study, cholesterol efflux was positively related to pre-β-HDL formation and PLTP activity in multiple linear regression analysis, in agreement with the proposed important role of ABCA1–pre-β-HDL interaction to promote cholesterol efflux (10, 12, 14), as well as with previous data showing that PLTP is able to stimulate cholesterol efflux directly via ABCA1 (32). It has been suggested that ABCA1 and ABCG1 act in a coordinate fashion in cellular cholesterol removal by first promoting cholesterol efflux to pre-β-HDL and subsequently to more mature α-HDL (14). LCAT activity may induce net cholesterol efflux via a non-specific cholesterol exchange mechanism (33). Very recently, it has been demonstrated that the presence of LCAT and apo E on α-HDL particles is important for cholesterol efflux out of macrophages (34). In our study, cholesterol efflux from fibroblasts was independently related both to plasma EST and to apo E.

Clearly, the absence of a defect in the ability of plasma from MetS subjects to promote cellular cholesterol

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Table 2 Plasma pre-β-high-density lipoprotein (HDL; formation), phospholipid transfer protein (PLTP) activity, cholesterol esterification (EST), cholesteryl ester transfer (CET), and cholesterol efflux from cultured fibroblasts to plasma from subjects without and with metabolic syndrome.

<table>
<thead>
<tr>
<th></th>
<th>Metabolic syndrome absent</th>
<th>Metabolic syndrome present</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=94)</td>
<td>(n=76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-β-HDL (apo A-I, g/l)</td>
<td>0.052±0.020</td>
<td>0.052±0.019</td>
<td>0.90</td>
<td>0.68</td>
</tr>
<tr>
<td>Pre-β-HDL formation (apo A-I, g/l)</td>
<td>0.312±0.069</td>
<td>0.396±0.065</td>
<td>0.13</td>
<td>0.24</td>
</tr>
<tr>
<td>PLTP activity (AU)</td>
<td>94±10</td>
<td>104±11</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EST (nmol/ml per h)</td>
<td>56.4±15.6</td>
<td>63.8±17.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CET (nmol/ml per h)</td>
<td>19.6±7.1</td>
<td>25.4±8.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol efflux (%/4 h)</td>
<td>8.5±0.9</td>
<td>8.8±1.0</td>
<td>0.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Data are shown as mean±S.D. or geometric mean (95% confidence intervals). *P value, adjusted for age, sex, and diabetes status.

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Table 3 Plasma pre-β-high-density lipoprotein (HDL; formation), phospholipid transfer protein (PLTP) activity, cholesterol esterification (EST), cholesteryl ester transfer (CET), and cholesterol efflux from cultured fibroblasts to plasma according to metabolic syndrome and diabetes status.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>(n=68)</td>
<td>(n=21)</td>
<td>(n=26)</td>
<td>(n=55)</td>
<td></td>
</tr>
<tr>
<td>Pre-β-HDL (apo A-I, g/l)</td>
<td>0.051±0.019</td>
<td>0.050±0.017</td>
<td>0.054±0.023</td>
<td>0.052±0.020</td>
<td>0.79</td>
</tr>
<tr>
<td>Pre-β-HDL formation (apo A-I, g/l)</td>
<td>0.317±0.068</td>
<td>0.298±0.070</td>
<td>0.299±0.069</td>
<td>0.295±0.063</td>
<td>0.27</td>
</tr>
<tr>
<td>PLTP activity (AU)</td>
<td>93±10</td>
<td>98±10</td>
<td>97±10</td>
<td>107±11†††‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EST (nmol/ml per h)</td>
<td>55.0±16.0</td>
<td>60.8±12.8</td>
<td>52.3±14.7</td>
<td>69.1±18.0††</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CET (nmol/ml per h)</td>
<td>19.8±7.4</td>
<td>23.1±5.9*</td>
<td>19.2±6.1</td>
<td>26.2±8.9‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol efflux (%/4 h)</td>
<td>8.5±0.9</td>
<td>8.6±1.1</td>
<td>8.3±0.8</td>
<td>8.8±0.9‡</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Subjects are categorized into four groups: no metabolic syndrome and no diabetes (MetS – DM –), metabolic syndrome without diabetes (MetS + DM –), diabetes without metabolic syndrome (MetS – DM +), and metabolic syndrome and diabetes (MetS + DM +). Data are shown as mean±S.D. P values by ANOVA are shown: *P<0.05 versus MetS – DM –; †P<0.05 versus MetS + DM –; ‡P<0.05 versus MetS – DM +.
removal is complementary to another report showing that the cellular cholesterol removal capacity of fibroblasts from individual MetS subjects is not impaired (22), and to the unchanged cholesterol efflux capacity out of SR-BI-expressing Fu5AH cells observed in hypertriglyceridemia and insulin resistance (24, 25). Obviously, it will be important to replicate these findings using cholesterol-loaded macrophage cell lines, such as THP1 or J774. It is generally appreciated that cellular cholesterol removal should be regarded as an anti-atherogenic process (8–10). Thus, the previous and present findings would suggest that the increased cardiovascular risk in MetS subjects (6, 7) is unlikely to be attributable to abnormalities in the early steps in the RCT pathway. This hypothesis needs to be tested in prospective studies.

Plasma PLTP activity was increased in MetS subjects after controlling for age, sex, and diabetes status, and this higher PLTP activity coincided with higher triglycerides, larger waist circumference, and insulin resistance in the present study. Such a higher PLTP activity agrees with previous reports from several research groups, in which a positive relationship of PLTP activity with hypertriglyceridemia, insulin resistance, and obesity with PLTP activity was observed (20, 35, 36). In marked contrast, plasma PLTP activity was observed to be strongly decreased in one study comprising of a small cohort of non-diabetic MetS subjects, albeit in that study plasma PLTP activity was also very low in lean healthy subjects (18). We also documented increased plasma EST and CET in the whole group of MetS subjects. Plasma EST and subsequent CET are considered to be integrated processes (37). These abnormalities are, therefore, most likely explained at least in part by higher plasma triglycerides in MetS, reflecting increased very low-density lipoproteins that are able to accept cholesteryl esters from HDLs (17).

We assayed pre-β-HDL concentrations by crossed immunoelectrophoresis. The presently reported values in subjects without MetS or diabetes, being 0.051 g apo A-I/l on average, are similar to those in several other publications using a variety of assay methods, e.g., quantitative electrophoretic transfer followed by solid-phase RIA (38), crossed immunoelectrophoresis (39), immunoblotting with chemiluminescent detection (40), non-denaturing two-dimensional gradient gel electrophoresis (41), and filtration and isotope dilution (42). In the healthy subjects, substantially higher pre-β-HDL concentrations have also been reported (24, 43–45). These discrepancies could be due to differences in assay methodology and to different treatment of plasma samples during and after blood collection and plasma isolation, since pre-β-HDL may be formed in vitro.

In the present study, pre-β-HDL and pre-β-HDL formation were found to be unchanged in MetS. We therefore propose that increased activities of plasma lipid transfer proteins contribute to the maintenance of plasma pre-β-HDL concentration and formation in MetS, in spite of lower total plasma apo A-I and HDL cholesterol levels. The positive relationships of cellular cholesterol efflux with plasma PLTP activity, EST, and CET shown here may be interpreted to reflect potentially anti-atherogenic mechanisms. Accordingly, an inverse correlation of pre-β-HDL with intima media thickness, an established marker of subclinical atherosclerosis, was found recently (43). Nonetheless, the net effect of PLTP (46, 47) and CETP action may be pro-atherogenic (48, 49), much depending on metabolic circumstances like elevated plasma triglycerides (50).

In conclusion, this study demonstrates that cholesterol efflux out of fibroblasts to plasma from the MetS subjects is not defective, despite low HDL concentrations.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Metabolic syndrome absent (n=94)</th>
<th>Metabolic syndrome present (n=76)</th>
<th>Combined subjects (n=170)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>−0.03</td>
<td>0.02</td>
<td>−0.08</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>0.12</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Apo E</td>
<td>0.35‡</td>
<td>0.27*</td>
<td>0.33‡</td>
</tr>
<tr>
<td>Pre-β-HDL</td>
<td>0.14</td>
<td>0.31†</td>
<td>0.21†</td>
</tr>
<tr>
<td>Pre-β-HDL formation</td>
<td>0.38‡</td>
<td>0.33†</td>
<td>0.33†</td>
</tr>
<tr>
<td>PLTP activity</td>
<td>0.24*</td>
<td>0.35†</td>
<td>0.33†</td>
</tr>
<tr>
<td>EST</td>
<td>0.37‡</td>
<td>0.39‡</td>
<td>0.40‡</td>
</tr>
<tr>
<td>CET</td>
<td>0.37‡</td>
<td>0.28*</td>
<td>0.35†</td>
</tr>
<tr>
<td>Ln adiponectin</td>
<td>0.02</td>
<td>−0.07</td>
<td>−0.06</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients are shown: *P<0.05; †P<0.01; ‡P<0.001.

### Table 5

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-β-HDL formation</td>
<td>0.234</td>
<td>0.001</td>
</tr>
<tr>
<td>EST</td>
<td>0.226</td>
<td>0.005</td>
</tr>
<tr>
<td>PLTP activity</td>
<td>0.166</td>
<td>0.024</td>
</tr>
<tr>
<td>Apo E</td>
<td>0.153</td>
<td>0.047</td>
</tr>
</tbody>
</table>

β, standardized regression coefficient; PLTP, phospholipid transfer protein; EST, cholesterol esterification.
cholesterol. An increase in plasma EST, CET, and PLTP activity are responsible for part of the abnormalities associated with the MetS, and could modulate cardiovascular risk.

Acknowledgements

This study was supported by a grant from the Dutch Diabetes Research Foundation (grant no. 2001.00.012). Dr L D Dikkeschei, PhD (Laboratory of Clinical Chemistry, Isala Clinics, Zwolle, the Netherlands) performed the lipid and apolipoprotein assays. A Zonneveld and L van Vark-van der Zee (Laboratory for Vascular Medicine, Erasmus University Medical Center, Rotterdam) and M J A van Wijland (Department of Experimental Hepatology, Academic Medical Center, Amsterdam, the Netherlands) carried out the PLTP activity measurements and cellular cholesterol efflux studies respectively. F Perton, PhD (Laboratory Center, University Medical Center, Groningen, the Netherlands) is acknowledged for the assay of pre-β-HDL, cholesterol esterification, and cholesteryl ester transfer.

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Received 10 September 2007
Accepted 16 October 2007