Relationships between changes in plasma lipid transfer proteins and apolipoprotein B-100 kinetics during fenofibrate treatment in the metabolic syndrome

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ABSTRACT

The aim of the present study was to investigate the association between changes in apoB (apolipoprotein B-100) kinetics and plasma PLTP (phospholipid transfer protein) and CETP (cholesteryl ester transfer protein) activities in men with MetS (the metabolic syndrome) treated with fenofibrate. Eleven men with MetS underwent a double-blind cross-over treatment with fenofibrate (200 mg/day) or placebo for 5 weeks. Compared with placebo, fenofibrate significantly increased the FCRs (fractional catabolic rates) of apoB in VLDL (very-low-density lipoprotein), IDL (intermediate-density lipoprotein) and LDL (low-density lipoprotein) (all \(P < 0.01\)), with no significant reduction (–8%; \(P = 0.131\)) in VLDL-apoB PR (production rate), but an almost significant increase (+15%, \(P = 0.061\)) in LDL-apoB PR. Fenofibrate significantly lowered plasma TG [triacylglycerol] (triglyceride); \(P < 0.001\), the VLDL-TG/apoB ratio (\(P = 0.003\)) and CETP activity (\(P = 0.004\)), but increased plasma HDL (high-density lipoprotein)-cholesterol concentration (\(P < 0.001\)) and PLTP activity (\(P = 0.03\)). The increase in PLTP activity was positively associated with the increase in both LDL-apoB FCR (\(r = 0.641, P = 0.034\)) and PR (\(r = 0.625, P = 0.040\)), and this was independent of the fall in plasma CETP activity and lathosterol level. The decrease in CETP activity was positively associated with the decrease in VLDL-apoB PR (\(r = 0.615, P = 0.044\)), but this association was not robust and not independent of changes in PLTP activity and lathosterol levels. Hence, in MetS, the effects of fenofibrate on plasma lipid transfer protein activities, especially PLTP activity, may partially explain the associated changes in apoB kinetics.

Key words: apolipoprotein B-100 (apoB), cholesteryl ester transfer protein (CETP), fenofibrate, fractional catabolic rate, metabolic syndrome, peroxisome-proliferator-activated receptor-α (PPAR-α), plasma phospholipid transfer protein (PLTP).

Abbreviations: apoB, apolipoprotein B-100; apoC-III, apolipoprotein C-III; CETP, cholesteryl ester transfer protein; CV, coefficient of variation; CVD, cardiovascular disease; FCR, fractional catabolic rate; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; HOMA, homoeostasis model assessment; IDL, intermediate-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; MetS, metabolic syndrome; NEFA, non-esterified fatty acid; PLTP, phospholipid transfer protein; PPAR-α, peroxisome-proliferator-activated receptor-α; PR, production rate; TG, triacylglycerol; VLDL, very-low-density lipoprotein.

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INTRODUCTION

MetS (the metabolic syndrome), a disorder characterized by central obesity, insulin resistance and dyslipoproteinaemia, accelerates the risk of CVD (cardiovascular disease) and Type II diabetes in all populations [1]. Lipoprotein kinetic studies indicate that MetS is associated with increased production and/or delayed catabolism of apoB (apolipoprotein B-100)-containing lipoproteins [2,3]. Therefore decreasing production and enhancing catabolism of apoB-containing lipoproteins is a major therapeutic aim.

PLTP (phospholipid transfer protein) plays an important role in lipoprotein metabolism [4]. Experimentally, PLTP has anti-atherogenic properties that may relate to favourable remodelling of HDL (high-density lipoprotein) particles with enhancement of HDL-mediated cellular cholesterol efflux [5,6]. However, by facilitating apoB secretion, PLTP may also be atherogenic [7]. The pro-atherogenicity of PLTP is supported by one, but not all, reports in patients with CVD [8–10]. Insulin resistance has also been associated with both low and high plasma PLTP activities [11–13], our own data showing consistently low plasma PLTP activity in men with MetS [14]. CETP (cholesteryl ester transfer protein) also plays an important role in lipoprotein metabolism by promoting the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins in exchange for TGs [triacylglycerols (triglycerides)] [15]. Increased CETP activity has been reported to be associated with low HDL-C (HDL-cholesterol) level and increased cardiovascular risk [16,17]. This is particularly pertinent to diabetes and MetS and may primarily be consequent on expansion in the TG-rich VLDL (very-low-density lipoprotein) pool. Accordingly, therapeutic inhibition of CETP increases HDL-C and this is kinetically attributable to a reduction in the catabolism of HDL particles [18]. The role of CETP in the regulation of apoB metabolism in MetS is less clear, although there is evidence that genetic mutations in CETP may be related to hepatic apoB secretion in obesity [19]. As with PLTP, the precise role of CETP in atherogenesis remains unclear.

The PPAR-α (peroxisome-proliferator-activated receptor-α) agonist fenofibrate decreases the incidence of cardiovascular events in Type II diabetes [20,21], and this may partly relate to its effects in regulating apoB kinetics [22]. Fenofibrate increases the expression of PLTP in experimental animals [23] and decreases plasma CETP activity in subjects with combined hyperlipidaemia [24]. Whether changes in the activities of these lipid transfer proteins contribute to the improvements in apoB kinetics in MetS with fenofibrate remains conjectural. In an extension to a previous intervention study [22], we investigated the relationships between changes in apoB kinetics and changes in both PLTP and CETP activities in subjects with MetS treated with fenofibrate. Because fibrates have been shown to decrease cholesterol synthesis and substrate availability [25], which may impact on apoB metabolism [22], we also studied the associations of apoB kinetics with plasma lipid transfer protein activities in relation to changes in plasma lathosterol, a surrogate marker of in vivo cholesterologenesis [26].

MATERIALS AND METHODS

Subjects

Eleven men with MetS, defined according to National Cholesterol Education Program Adult Treatment Panel III criteria [27], but excluding diabetes, were recruited, as described previously [22]. Exclusions included cholesterol > 7 mmol/l, TGs > 4.5 mmol/l, consumption of > 30 g of alcohol/day, use of lipid regulators, apolipoprotein E2/E2, macroproteinuria, creatinaemia (> 120 µmol/l) and hypothyroidism. The study was approved by the Ethics Committee of the South Eastern Sydney Area Health Service, in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and all subjects provided informed written consent.

Study design and clinical protocols

This was a randomized double-blind placebo-controlled cross-over trial. Eligible patients entered a 4-week run-in period at the end of which they were randomized to a 5-week treatment period of either micronized fenofibrate (200 mg/day), atorvastatin (40 mg/day) or placebo, separated by 2-week-washout periods. In the present study, we report on the associations between changes in variables in the fenofibrate relative to the placebo arm of the study. Apolipoprotein kinetic studies were conducted at the end of each treatment by primed infusion of [³H]leucine, as described previously [22].

Isolation and measurement of isotopic enrichment of apoB

VLDL, IDL (intermediate-density lipoprotein) and LDL (low-density lipoprotein) were isolated from 3 ml of plasma by sequential ultracentrifugation, followed by isopropanol precipitation, delipidation, HCl hydrolysis and oxazolinone derivatization [28]. Plasma-free leucine was also isolated by cation-exchange chromatography. Isotopic enrichment was determined using GC/MS with selected ion monitoring of samples at a mass to charge ratio (m/z) of 212 and 209 and negative-ion chemical ionization. Tracer to tracee ratios were derived from isotopic ratios for each sample.

Quantification of apoB and other biochemical analyses

ApoB in VLDL, IDL and LDL was precipitated with isopropanol and quantified using the modified Lowry
Effects of fenofibrate on plasma lipid and lipoprotein levels

Table 1  Effects of fenofibrate on plasma lipid and lipoprotein levels

<table>
<thead>
<tr>
<th></th>
<th>Fenofibrate</th>
<th>Placebo</th>
<th>Group differences</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.58 ± 0.15</td>
<td>5.87 ± 0.17</td>
<td>−0.29 ± 0.20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TGs (mmol/l)</td>
<td>1.72 ± 0.29</td>
<td>2.43 ± 0.31</td>
<td>−0.72 ± 0.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.73 ± 0.18</td>
<td>3.94 ± 0.22</td>
<td>−0.22 ± 0.20</td>
<td>0.146</td>
</tr>
<tr>
<td>Non-HDL-C (mmol/l)</td>
<td>4.56 ± 0.16</td>
<td>4.94 ± 0.13</td>
<td>−0.39 ± 0.22</td>
<td>0.013</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.02 ± 0.07</td>
<td>0.94 ± 0.04</td>
<td>0.08 ± 0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>0.97 ± 0.04</td>
<td>1.11 ± 0.03</td>
<td>−0.14 ± 0.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ApoC-III (mg/l)</td>
<td>115.0 ± 14.6</td>
<td>150.5 ± 10.5</td>
<td>−35.6 ± 11.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VLDL-TG/apoB ratio</td>
<td>12.1 ± 1.3</td>
<td>16.3 ± 1.3</td>
<td>−4.23 ± 1.10</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL-C/apoB ratio</td>
<td>3.86 ± 0.10</td>
<td>3.67 ± 0.10</td>
<td>0.18 ± 0.05</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

with fenofibrate were examined by univariate and partial correlation analyses (SPSS 11.5).

RESULTS

Effects of fenofibrate on lipid and lipoprotein concentrations

Compared with placebo, fenofibrate significantly decreased plasma cholesterol, TGs, non-HDL-C, apoB-100 and apoC-III concentrations, as well as the VLDL-TG/apoB ratio, indicative of a reduction in VLDL size (Table 1). Fenofibrate also significantly increased HDL-C and the LDL-C/apoB ratio, indicative of an increase in LDL size (Table 1).

Effects of fenofibrate on lipid transfer protein activities and other metabolic indices

Compared with placebo, fenofibrate significantly increased plasma PLTP activity, while significantly decreasing plasma CETP activity and lathosterol levels, but did not significantly change plasma LCAT activity, NEFAs and HOMA score (Table 2).

Effects of fenofibrate on apoB kinetics

Table 3 shows the effects of fenofibrate on VLDL, IDL and LDL metabolism. Compared with placebo, fenofibrate significantly increased the FCRs (fractional catabolic rates) of apoB in VLDL (+33 %), IDL (+34 %) and LDL (+26 %). Fenofibrate did not affect the PRs (production rates) of apoB, although there was an almost significant increase (P = 0.06) in LDL-apoB PR (+15 %). The accelerated FCRs of apoB on fenofibrate treatment accounted for the significant decreases in plasma VLDL, IDL and LDL-apoB pool sizes (Table 3).

Associations between changes in apoB kinetics and changes in plasma PLTP and CETP activities and lathosterol levels

These associations are summarized in Table 4 and Figure 1. On fenofibrate, the increase in plasma PLTP method [28]. Plasma cholesterol, TG, HDL-C and glucose concentrations were determined by enzymatic methods, with LDL-C (LDL-cholesterol) calculated by using the Friedewald equation and non-HDL as total cholesterol − HDL-C. VLDL-TG/apoB and LDL-C/apoB ratios were also calculated as indicators of lipoprotein particle size. Plasma total apoB concentration was determined by immunonephelometry (Dade Behring BN2 Nephelometer), plasma apoC-III (apo-lipoprotein C-III) concentration was determined by immuno-inhibition (Daichi), NEFAs (non-esterified fatty acids) were determined by an enzymatic kit (Randox), and insulin was determined by RIA (Diasorin). Insulin resistance was estimated by HOMA (homeostasis model assessment) score. Plasma lathosterol concentration, an index of in vivo cholesterol synthesis [26], was assayed by GC/MS [28]. Interassay CVs (coefficients of variation) for foregoing analyses were all < 6 %.

Plasma PLTP activity was determined by measuring the transfer of radiolabelled phosphatidylcholine ([14C]DPPC) from small unilamellar vesicles to isolated HDL [29,30]; the inter-assay CV was <10 %. Plasma CETP activity was analysed by measuring the transfer of radiolabelled cholesteryl ester ([2H3]CE) from exogenous donor (HDL3) to acceptor (LDL) [31,32], a different method from our previous study [22]; the interassay CV was < 5 %. LCAT (lethithin:cholesterol acyltransferase) activity was measured using a fluorescence kit (Wako-Chemie Medical); the interassay CV was < 10 %.

Kinetic analyses

A multicompartmental model was used to describe VLDL-, IDL- and LDL-apoB leucine tracer/tracee ratios. The SAAMII program (SAAM Institute, Seattle) was used for modelling the data. The details and assumptions of the model have been described previously [28].

Statistical analyses

Skewed variables were logarithmically transformed. Data at the end of each treatment period were compared using a mixed effects model (SAS Proc Mixed; SAS Institute). Associations between changes in variables with fenofibrate were examined by univariate and partial correlation analyses (SPSS 11.5).
activity was significantly and positively associated with the increase in both LDL-apoB FCR (Figure 1A) and LDL-apoB PR (Figure 1B), with no significant association with other changes in apoB kinetics. After adjusting for changes in both lathosterol level and CETP activity, the associations between changes in PLTP activity and the FCR ($r = 0.764, P = 0.017$) and PR ($r = 0.624, P = 0.05$) of LDL-apoB remained statistically significant. The decrease in plasma CETP activity on fenofibrate was significantly and positively associated with the decrease in VLDL-apoB PR (Figure 1D) and both VLDL- and IDL-apoB pool sizes, with no significant association with other changes in apoB metabolism (Table 4 and Figure 1C); the significant association
Associations of PLTP and CETP activities and apoB-100 kinetics in the metabolic syndrome

**DISCUSSION**

We present new findings showing that in subjects with MetS fenofibrate significantly increases plasma PLTP activity with a reciprocal reduction in the activity of plasma CETP, and that the changes in lipid transfer proteins are correlated with changes in apoB kinetics. We have reported previously on the effects of atorvastatin on apoB kinetics in MetS [22]. Changes in apoB kinetics with fenofibrate reported in the present study agree with findings in other sample populations [33–35].

The effect of fenofibrate in enhancing plasma PLTP activity concurs with a previous animal study [23] showing significant increases in plasma PLTP activity and hepatic PLTP mRNA. Two other studies in hypertriglyceridaemic patients have, however, shown that gemfibrozil [36] and bezafibrate [37] reduce plasma PLTP activity by 7% and 8% respectively. Discrepancies between these and our present study may be due to differences in study population, specificity of PPAR-α activation and assay characteristics. The significant reduction in plasma CETP activity with fenofibrate is in line with a previous study in combined hyperlipidaemia [24].

Fenofibrate enhanced plasma PLTP activity probably as a result of up-regulation of PLTP gene expression through a PPAR-α-dependent mechanism [23]. Both mouse and human PLTP gene promoter regions have been found to contain PPREs (peroxisome-proliferator-response elements) [38,39]. Although fenofibrate may increase PLTP activity in mice [23], it reduces human PLTP promoter activity in HepG2 cells [39]. Lipolysis induced by fenofibrate could enhance PLTP-mediated transfer of phospholipids from VLDL to HDL, accounting for the negative association between PLTP activity and VLDL-TG/apoB ratio in our present study. Conversely, but less likely, elevated PLTP activity with fenofibrate could also increase the transfer of phospholipids from VLDL to HDL, thereby accelerating lipolysis of VLDL-TGs and formation of smaller VLDL particles [40]. As a consequence of such intravascular remodelling of VLDL, the formation of more buoyant LDL particles, which have high affinity for LDL receptors, could be increased [33]. This mechanism, as well as ‘balancing feedback’, could explain the significant positive association we report between the changes in plasma PLTP activity and LDL-apoB kinetics.
Reduction in plasma CETP activity with fenofibrate could be due to down-regulation of CETP gene expression [37]. By decreasing plasma CETP activity, fenofibrate limits the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins and the absolute mass uptake of cholesterol by the liver, which could impact on hepatic VLDL-apoB secretion [41]. This could explain the significant positive association between the fall in CETP activity and the fall in VLDL-apoB PR and associated changes in both VLDL- and IDL-apoB pool sizes.

Beyond changes in plasma PLTP and CETP activities, reductions in plasma lathosterol (reflecting inhibition of cholesterogenesis) and apoC-III concentrations may play a significant role in the regulation of apoB metabolism with fenofibrate [22,25,42–44]. That we found no significant correlation between changes in apoB kinetics and changes in lathosterol or apoC-III may partially reflect the lower variation of changes in these variables compared with those in lipid transfer protein activities in response to fenofibrate.

Fenofibrate can decrease progression of coronary atherosclerosis and cardiovascular events in subjects with Type II diabetes [20,21] and, by implication, MetS. Our present data suggest that associated changes in plasma PLTP and CETP activities may contribute to these benefits by regulating apoB metabolism. Further studies should examine whether these lipid transfer proteins could regulate a wider spectrum of lipoprotein subclasses in response to fenofibrate.

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