Atorvastatin and Fenofibrate Have Comparable Effects on VLDL–Apolipoprotein C-III Kinetics in Men With the Metabolic Syndrome

Dick C. Chan, Gerald F. Watts, Esther M.M. Ooi, Juying Ji, Anthony G. Johnson, P. Hugh R. Barrett

**Objectives**—The metabolic syndrome (MetS) is characterized by insulin resistance and dyslipidemia that may accelerate atherosclerosis. Disturbed apolipoprotein (apo) C-III metabolism may account for dyslipidemia in these subjects. Atorvastatin and fenofibrate decrease plasma apoC-III, but the underlying mechanisms are not fully understood.

**Methods and Results**—The effects of atorvastatin (40 mg/d) and fenofibrate (200 mg/d) on the kinetics of very-low density lipoprotein (VLDL)-apoC-III were investigated in a crossover trial of 11 MetS men. VLDL–apoC-III kinetics were studied, after intravenous d3-leucine administration using gas chromatography-mass spectrometry and compartmental modeling. Compared with placebo, both atorvastatin and fenofibrate significantly decreased (P<0.001) plasma concentrations of triglyceride, apoB, apoB-48, and total apoC-III. Atorvastatin, not fenofibrate, significantly decreased plasma apoA-V concentrations (P<0.05). Both agents significantly increased the fractional catabolic rate (+32% and +30%, respectively) and reduced the production rate of VLDL–apoC-III (~20% and ~24%, respectively), accounting for a significant reduction in VLDL–apoC-III concentrations (~41% and ~39%, respectively). Total plasma apoC-III production rates were not significantly altered by the 2 agents. Neither treatment altered insulin resistance and body weight.

**Conclusions**—Both atorvastatin and fenofibrate have dual regulatory effects on VLDL–apoC-III kinetics in MetS; reduced production and increased fractional catabolism of VLDL–apoC-III may explain the triglyceride-lowering effect of these agents. *Arterioscler Thromb Vasc Biol. 2008;28:1831-1837*

**Key Words:** lipoprotein metabolism ■ obesity ■ insulin resistance ■ cardiovascular disease ■ statin ■ fenofibrate

Insulin resistance is a heterogeneous metabolic disorder of complex etiology. It underpins dyslipoproteinemia, a key feature of the metabolic syndrome (MetS) that independently predicts cardiovascular disease (CVD). Hypertriglyceridemia, the most consistent lipid disorder in subjects with obesity and type 2 diabetes mellitus, is chiefly a consequence of overproduction and delayed fractional clearance of triglyceride-rich lipoproteins (TRLs). Although the precise mechanisms involved are incompletely understood, experimental and clinical evidence suggests that elevated apolipoprotein (apo) C-III may play a crucial role in the dysregulation of TRL metabolism.

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ApoC-III inhibits lipoprotein lipase (LPL) activity, an enzyme that hydrolyzes triglyceride in TRL and facilitates their clearance from the circulation. ApoC-III also impairs the hepatic uptake of TRLs, possibly by interfering with the interaction of apoE on the lipoproteins with the LDL receptors. Using stable isotope techniques, we have previously reported that in viscerally obese men plasma apoC-III concentration was positively associated with VLDL-apoB secretion rate, and inversely with VLDL-apoB fractional catabolism and conversion of VLDL to LDL. We and others also demonstrated that elevated VLDL–apoC-III is a consequence of elevated VLDL–apoC-III production in subjects with obesity and hypertriglyceridemia. Under normal physiological circumstances, apoC-III expression is downregulated, in part, by insulin via the promoter insulin response element on the APOC3 gene. Insulin resistance may blunt the sensitivity to the normal insulin-mediated suppression of apoC-III gene expression. The overproduction of VLDL–apoC-III in these subjects may also relate to the effect of insulin resistance in decreasing the transcription of peroxisome proliferator-activated receptor alpha (PPAR-α), a negative regulator of apoC-III transcription.

Several clinical trials support the use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) and PPAR-α to treat dyslipidemia in insulin resistance and obesity. Both statins and fibrates regulate...
lipoprotein metabolism and decrease the incidence of CVD in high-risk subjects including those with MetS. Statins competitively inhibit HMG-CoA reductase, thereby decreasing cholesterol biosynthesis, reciprocally upregulating hepatic apoB-containing LDL receptors, and enhancing the clearance of apoB-containing lipoproteins. In animal models, statins decreased both the expression and concentration of apoC-III. Fibrates are ligands that bind to and activate PPAR-α, chiefly in the liver. Via PPAR-α activation, fibrates reduce triglyceride substrate availability in the liver by stimulation of peroxisomal and mitochondrial β-oxidation, thereby decreasing hepatic secretion of VLDL. Fibrates also promote intravascular VLDL lipolysis by inducing and repressing the genetic expression of LPL and apoC-III, respectively. We previously reported that both atorvastatin and fenofibrate effectively lowered plasma triglycerides owing to enhanced fractional catabolism of TRLs. These findings may relate to the lowering effects of atorvastatin and fenofibrate on plasma apoC-III concentrations. However, the precise mechanisms of action of these agents on VLDL–apoC-III kinetics in MetS subjects have not yet fully been investigated.

We previously carried out a randomized crossover study to examine the kinetic effect of atorvastatin and fenofibrate on apoB metabolism in subjects with MetS. We extend that study by investigating the effects of these agents on VLDL–apoC-III kinetics in subjects with MetS, and allow to go home. Additional fasting blood samples were collected in the morning on the following 4 days of the same week (24, 48, 72, and 96 hours). All the procedures were repeated at the end of each treatment period.

Isolation and Measurement of Isotopic Enrichment of VLDL–ApoC-III

The methods used for the isolation of apoC-III in VLDL have been described previously. Briefly, 3 mL of plasma was used for isolation of 1 mL VLDL (<1.006 kg/L) fractions by ultracentrifugation at 40,000 rpm in a Ti 50.4 rotor (Optima LE-80K, Beckman Coulter). The VLDL samples were then prepared for isoelectric focusing (IEF) gel electrophoresis. VLDL (200 μL) from each time point was delipidated and reconstituted in 50 μL of IEF sample buffer (8 mol/L urea; 0.001%wt/vol bromphenol blue). ApoC-III was isolated by preparative IEF gel electrophoresis (8 mol/L urea; 7.5% acrylamide; 1.5% ampholytes pH 4 to 6; 16 hour; 200 V; 4°C), transferred to polyvinyllidine fluoride (PVDF) membranes (Immobilon, Millipore) and stained with Coomassie Brilliant Blue R250. IEF resolves apoC-III into three isoforms, apoC-III, apoC-III, and apoC-III. ApoC-III is the most abundant isoform, and radiotracer studies support that the kinetics of apoC-III isoforms are similar. ApoC-III in the present study refers to the apoC-III isoform. The apoC-III protein bands were excised from the PVDF membranes, hydrolyzed, and derivatised using a modified oxazolimine method, and analyzed by negative ion chemical ionisation GCMS. The isotopic enrichment was determined as the tracer to trace ratio of monitored selected ions at mass to charge (m/z) ratio of 212 (derived from -l-leucine) and 209 (derived from unlabeled leucine).

Quantification of Biochemical Analytes

Plasma aliquots were combined to yield 5 pooled VLDL samples per patient study, as described previously. ApoB was isolated in each lipoprotein fraction using isopropanol and quantitated with the Lowry method.

Plasma cholesterol and triglyceride concentrations were determined by enzymatic methods using a Hitachi 917 Biochemical Analyser (Hitachi Ltd). HDL cholesterol was measured enzymatically (Boehringer Mannheim). LDL cholesterol was calculated by Friedewald equation, and non-HDL cholesterol as total cholesterol minus HDL cholesterol. Total plasma apoB, apoAl concentrations were determined by immunonephelometry (Dade Behring BN2, Behring) and plasma apoC-III concentration by immunoturbidity (Daichi); VLDL–apoC-III and HDL–apoC-III were determined by electro-immuno diffusion using a Hydragel LP CIII Electro-immunodiffusion kit (Sebia). Plasma apoA-V and apoB-48 levels were measured by enzyme immunoassay kits (Millipore; Fujirebio). Plasma nonesterified fatty acids (NEFAs) were measured commercially by an enzymatic method kit (Randox, Co). Plasma insulin was measured by radioimmunoassay (Diazorini s.r.l.). Insulin resistance was estimated using the HOMA score. Plasma glucose, alanine, and asapate transaminases, alkaline phosphatase, and creatinine kinase were analyzed on a Hitachi 917 Biochemical Analyser.

Model of VLDL–apoC-III Metabolism and Calculation of Kinetic Parameters

Figure 1 shows a compartment model developed, using the SAAM II program (Resource Facility for Population Kinetics, University of Washington, Seattle), to describe VLDL–apoC-III tracer data. Part of the model consists of a 4-compartment leucine subsystem (compartments 1 to 4) that describes plasma leucine tracer data. Component
Compared with placebo, atorvastatin significantly decreased plasma HDL cholesterol (+11%) and apoA-I (+6%) concentrations. No significant differences were observed in body weight, blood pressures, plasma nonesterified fatty acids, HDL–apoC-III, glucose, insulin, and HOMA score. Nutrient and alcohol intake did not differ significantly during the study (data not shown).

Table 1. Clinical and Biochemical Characteristics of the 11 Subjects at Baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>46.3 ± 6.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>97.4 ± 11.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.5 ± 2.6</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>102.1 ± 1.8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128.5 ± 3.1</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83.8 ± 2.3</td>
</tr>
<tr>
<td>Nonesterified fatty acids, mmol/L</td>
<td>0.54 ± 0.10</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.68 ± 0.46</td>
</tr>
<tr>
<td>Fasting insulin, mu/L</td>
<td>19.91 ± 6.74</td>
</tr>
<tr>
<td>HOMA score, mean ± SD</td>
<td>5.10 ± 2.05</td>
</tr>
</tbody>
</table>

Figure 1. Compartmental model describing VLDL–apoC-III tracer kinetics. Compartments 1 to 4 represent plasma leucine tracer data; compartments 5 and 6 are 2 delay compartments accounting for the time associated with the synthesis and secretion of apoC-III; compartment 7 represents plasma VLDL–apoC-III, and compartment 8 represents an extravascular exchange compartment.

1 is connected to 2 delay compartments (compartments 5 to 6) that account for the time required for the synthesis and secretion of apoC-III into plasma. The kinetics of apoC-III in VLDL is described by a plasma compartment (compartment 7) and an extravascular exchange compartment (compartment 8). The fractional catabolic rate (FCR) of plasma apoC-III was derived from the model parameters giving the best fit. The production rates (PR) for apoC-III in VLDL was derived as the product of VLDL–apoC-III FCR and the corresponding pool size, and expressed as mg/kg/d. Pool size was calculated as the product of apoC-III concentration (mg/L) and plasma volume (0.045 L/kg). Given that VLDL- and HDL–apoC-III FCRs (or total plasma apoC-III) are equivalent as we previously reported,10,24 the production rates (PR) for apoC-III in total plasma and HDL–apoC-III were also derived as the product of VLDL–apoC-III FCR and their corresponding pool sizes, and expressed as mg/kg/d.

Statistical Analysis

Skewed variables were logarithmically transformed. Because of the study design, we first tested for carry-over and time-dependent effects, but this proved negative. Data at the end of the 3 treatment periods were compared using a mixed effects model (SAS Proc Mixed, SAS Institute). Using Tukey-Kramer test, adjusted probability values were reported to account for multiple comparisons, for a given variable across the 3 treatment periods. The level of statistical significance was set at 5%.

Results

The pretreatment clinical and biochemical characteristics of the subjects are shown in Table 1. On average, they were middle-aged, centrally obese, normotensive, and insulin resistant. Tablet and capsule counts confirmed that compliance with active treatments was 100%.

The effects of intervention on plasma lipid, lipoprotein, and apolipoprotein concentrations are shown in Table 2. Compared with placebo, atorvastatin significantly decreased total cholesterol (−40%), triglyceride (−38%), LDL-cholesterol (−53%), non-HDL cholesterol (−48%), apoB-100 (−42%), VLDL-apoB (−30%), apoB-48 (−27%), apoC-III (−20%), apoA-V (−30%), and lathosterol (−68%) concentrations. Compared with placebo, fenofibrate significantly decreased plasma concentrations of total cholesterol (−6%), triglyceride (−32%), non-HDL cholesterol (−6%), apoB-100 (−13%), VLDL-apoB (−26%), apoB-48 (−17%), apoC-III (−23%), and lathosterol (−20%). It also significantly increased plasma HDL cholesterol (+11%) and apoA-I (+6%) concentrations. No significant differences were observed in body weight, blood pressures, plasma nonesterified fatty acids, HDL–apoC-III, glucose, insulin, and HOMA score. Nutrient and alcohol intake did not differ significantly during the study (data not shown).

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Compared with nonobese, nondiabetic subjects,10 the MetS subjects had significantly higher VLDL–apoC-III concentration (96±14 versus 42±7 mg/L, P<0.01) and production rate (3.42±0.39 versus 1.56±0.29 mg/kg/d) without significant difference in the corresponding FCR (0.84±0.06 versus 0.82±0.05 pools/d). The effects of the interventions on VLDL–apoC-III metabolism were shown in Table 3. As shown in Figure 2, both atorvastatin and fenofibrate significantly increased VLDL–apoC-III FCR (+32% and +30%, respectively). This was paralleled by a significant decrease in the corresponding production rates (−20% and −24%, respectively), accounting for a significant reduction in plasma VLDL–apoC-III concentrations (−41% and −39%, respectively). The effects of atorvastatin and fenofibrate on VLDL–apoC-III kinetics were not statistically different from each other (P>0.05 in all). There was a significant effect of both atorvastatin and fenofibrate on increasing HDL–apoC-III FCR (P<0.05 in both). This was coupled to a significant increase in the corresponding production rates (placebo: 2.30±0.28 mg/kg/d, atorvastatin 3.50±0.39 mg/kg/d and fenofibrate: 3.07±0.35 mg/kg/d), accounting for a lack of treatment effect on plasma HDL–apoC-III concentrations. Moreover, the production rates of total plasma apoC-III were not significantly altered by the 2 drugs (placebo: 5.72±0.33 mg/kg/d, atorvastatin: 6.25±0.61 mg/kg/d and fenofibrate: 5.67±0.53 mg/kg/d).

In univariate analysis, changes in plasma triglycerides with atorvastatin or fenofibrate were both significantly (P<0.05) associated with corresponding changes in VLDL–apoC-III (r=0.882 and 0.695, respectively). Similarly, changes in VLDL-apoB concentrations were also associated (P<0.05 for both) with changes in VLDL–apoC-III with these agents (r=0.815 and 0.662, respectively). Reduction in plasma VLDL–apoC-III with fenofibrate was associated with reduction in VLDL–apoC-III production (r=0.665, P<0.05). However, the change in VLDL–apoC-III concentrations was not associated with changes in VLDL–apoC-III production or fractional catabolism with atorvastatin. Moreover, reductions
in VLDL–apoC-III concentration with atorvastatin and fenofibrate were significantly correlated \((r=0.603, P<0.05)\). There were no significant associations between corresponding changes in production \((r=-0.110, P=0.748)\) or fractional catabolic rates \((r=-0.372, P=0.260)\), respectively.

**Discussion**

This is the first study to compare the effects of statins and fibrates on VLDL–apoC-III metabolism in subjects with MetS. We showed that dysregulation of VLDL–apoC-III in these subjects respond similarly to treatment with atorvastatin and fenofibrate. Specifically, both treatments significantly reduce VLDL–apoC-III concentration by decreasing the production and enhancing the fractional catabolic rate. These effects were achieved with no significant alteration in insulin resistance and body weight.

**ApoC-III Model**

We have previously developed several mechanistic models to better understand the metabolism of apoC-III.\(^{10,24}\) The most general compartment model of apoC-III includes the secretion of apoC-III into the VLDL and HDL fractions, the exchange of apoC-III between VLDL and HDL, and the removal of apoC-III from plasma via both VLDL and HDL.

Using Intralipid extraction method of apoC-III, we found that the isotopic enrichments of apoC-III in VLDL and HDL are similar in both obese and normolipidemic subjects, suggesting a rapid exchange of apoC-III between VLDL and HDL fractions. As such, the kinetic parameters of this complex model including VLDL and HDL compartments could not be determined with any degree of precision. Because the kinetics of apoC-III in the VLDL and HDL fractions are indistinguishable and hence the exchange between VLDL and HDL cannot be determined, we developed a simple model that describes apoC-III kinetics in VLDL, HDL, and total plasma (Figure 1). This model fitted the tracer data well, allowing the FCRs and hence production rate to be determined with high precision.

**Previous Kinetic Studies**

Hypertriglyceridemia in insulin-resistant subjects, including patients with obesity and type 2 diabetes, results from overproduction and decreased fractional catabolism of triglyceride-rich VLDL particles.\(^{4,5}\) Other studies have demonstrated that these abnormalities may be associated with disturbances in VLDL–apoC-III metabolism.\(^{9,10,24}\) Furthermore, our data also confirmed that in-insulin-resistant obese subjects, elevated VLDL–apoC-III is a consequence of ele-

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**Table 3. Kinetic Estimates of the Metabolism of VLDL–ApoC-III After Treatment With Atorvastatin, Fenofibrate, and Placebo**

<table>
<thead>
<tr>
<th></th>
<th>Atorvastatin (A)</th>
<th>Fenofibrate (F)</th>
<th>Placebo (P)</th>
<th>A vs P</th>
<th>F vs P</th>
<th>A vs F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool size, mg</td>
<td>245±38</td>
<td>256±55</td>
<td>422±61</td>
<td>0.001</td>
<td>0.001</td>
<td>0.962</td>
</tr>
<tr>
<td>Fractional catabolic rate, pools/day</td>
<td>1.11±0.07</td>
<td>1.09±0.07</td>
<td>0.84±0.06</td>
<td>0.033</td>
<td>0.026</td>
<td>0.990</td>
</tr>
<tr>
<td>Production rate, mg/kg/day</td>
<td>2.75±0.46</td>
<td>2.60±0.42</td>
<td>3.42±0.39</td>
<td>0.006</td>
<td>0.004</td>
<td>0.988</td>
</tr>
</tbody>
</table>

Mean±SEM.

Adjusted \(P\) values were reported to account for multiple comparisons using Tukey–Kramer test.
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tein lipase and decreased hepatic apoC-III synthesis with
fibrates could contribute to improvements in the metabolism
of TRLs. However, our data found that atorvastatin and
fibrates could be secondary to enhanced fractional catabolism of VLDL particles, resulting in a shift of apoC-III to HDL. In the absence of HOMA changes, it is unlikely that improved insulin signaling, possibly via FOXO1, would lower apoC-III gene expression and secretion. Therefore, the potential inhibitory effect of atorvastatin and fenofibrate on apoC-III expression may be diminished in the presence of persistent insulin resistance. Consistent with this, Nasgashima et al reported that pioglitazone, an insulin sensitizing agent, was shown to significantly decrease apoC-III concentration in type 2 diabetes by reducing its production rate. The mechanism responsible for the regulation of apoC-III distribution between VLDL and HDL by atorvastatin and fenofibrate in plasma remains unclear.

In univariate analysis, we found that change in VLDL–apoC-III concentration with fenofibrate was associated with change in VLDL–apoC-III production. This observation was consistent with our data that off-treatment plasma concentration of VLDL–apoC-III was significantly associated with its production rate ($r = 0.874, P < 0.001$). The lack of significant association between plasma VLDL–apoC-III concentration and its kinetic parameters with atorvastatin treatment, however, suggests that the impact of atorvastatin on both production and fractional catabolism are crucial to determine VLDL–apoC-III concentrations.

**Fractional Catabolic Rate**
Both atorvastatin and fenofibrate treatment also significantly increased the fractional catabolism of VLDL–apoC-III. Given that apoC-III is attached to VLDL particles and that the catabolism of VLDL-apoB was upregulated by both atorvastatin and fenofibrate, one would anticipate that the FCR of VLDL–apoC-III increases in parallel. Both agents decreased plasma concentration of apoB-48, a marker of intestinally-derived triglyceride-rich lipoprotein metabolism, probably owing to decreased competition with VLDL for common removal pathways.

Using apoB kinetic data from the same subjects, we found that the increase in VLDL–apoC-III FCR with fenofibrate was significantly associated with increased VLDL–apoB FCR ($r = 0.612, P < 0.05$). However, this coupling effect was not observed with atorvastatin. The precise mechanism accounting for this observation remains unclear, but may relate to their divergent effect on plasma apoA-V concentrations. Given that apoA-V was shown to counteract the effect of apoC-III on TRL metabolism in animal models, it is possible that reduction in plasma apoA-V with atorvastatin

**Data Interpretation**
Previous studies have only examined the effect of statins and fibrates on apoB and apoA-I kinetics. The present report extends previous studies to VLDL–apoC-III kinetics in MetS subjects.

**Production Rate**
We demonstrate that both treatments reduced the production of VLDL–apoC-III. The precise mechanism of action of statin on VLDL–apoC-III production is unclear. HMG-CoA reductase inhibitors may possibly stimulate PPAR-α, thereby repressing apoC-III expression. Schoonjans et al showed that in animal models simvastatin decreased the expression of apoC-III mRNA. Fibrates also activate PPAR-α in the liver with a variety of consequences that may impact on lipoprotein metabolism. In vitro, increased expression of lipoprotein lipase and decreased hepatic apoC-III synthesis with fibrates could contribute to improvements in the metabolism of TRLs. However, our data found that atorvastatin and fenofibrate reduced total plasma apoC-III concentration chiefly by increasing total plasma apoC-III FCR, with no significant changes to total plasma apoC-III production. Conversely, there was a significant effect of both atorvastatin and fenofibrate on increasing HDL–apoC-III FCR. This was coupled to a significant increase in the corresponding production rates, accounting for a lack of treatment effect on plasma HDL–apoC-III concentrations. Taken together, these findings suggest that the reduction in VLDL–apoC-III PR (ie, transport rate of apoC-III through the VLDL pool) may be a function of redistribution of apoC-III between VLDL and HDL particles with both treatments, rather than an effect on apoC-III gene expression and hence, hepatic or intestinal apoC-III synthesis. The reduction in transport of apoC-III in VLDL with atorvastatin and fenofibrate could be secondary to enhanced fractional catabolism of VLDL particles, resulting in a shift of apoC-III to HDL. In the absence of HOMA changes, it is unlikely that improved insulin signaling, possibly via FOXO1, would lower apoC-III gene expression and secretion. Therefore, the potential inhibitory effect of atorvastatin and fenofibrate on apoC-III expression may be diminished in the presence of persistent insulin resistance. Consistent with this, Nasgashima et al reported that pioglitazone, an insulin sensitizing agent, was shown to significantly decrease apoC-III concentration in type 2 diabetes by reducing its production rate. The mechanism responsible for the regulation of apoC-III distribution between VLDL and HDL by atorvastatin and fenofibrate in plasma remains unclear.

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may modulate the regulatory effect of apoC-III on VLDL catabolism. In addition, we previously showed that atorvastatin, but not fenofibrate, decreased cholesteryl ester transfer protein (CETP) activity in these subjects. The reduction in CETP activity with atorvastatin may also contribute to an altered distribution of apoC-III between VLDL and HDL particles and hence, changes to its kinetics. This speculation merits further investigation. The lack of significant association between corresponding changes in VLDL–apoC-III production or FCR with atorvastatin and fenofibrate also suggests that the regulation by the two agents is mediated by different mechanisms.

Limitations

Our study is limited by its small sample size. Although the comparative effects of atorvastatin and fenofibrate on apoC-III kinetics were not statistically different, this does not imply these agents have therapeutically equivalent effects on apoC-III metabolism. A formal noninferiority or equivalence trial would require formally a different statistical approach and greater sample size, noting that the percentage difference between atorvastatin and fenofibrate on VLDL–apoC-III production was <4%. Whether our results also apply to women with MetS require investigation. Furthermore, VLDL metabolism is dependent on the activity of LPL and CETP, which are also regulated by both atorvastatin and fenofibrate. Therefore, measurement of LPL activities, together with free fatty acid and triglyceride kinetics, may help to clarify the precise relationship between apoC-III and TRL metabolism.

Implications and Conclusions

The recognition of hypertriglyceridaemia as an independent predictor of CVD in the metabolic syndrome requires identifying factors that regulate TRL metabolism. ApoC-III could offer a new approach to the management of hypertriglyceridaemia. Moreover, the results of large clinical studies have indicated that plasma apoC-III concentration (particularly in apoB-containing lipoproteins) is a stronger predictor of cardiovascular risk than plasma triglycerides. Dysregulation of apoC-III metabolism is a feature of dyslipidemia in insulin resistance, and our results explain how these 2 commonly used lipid-regulating agents can improve this abnormality. Although the production of total plasma apoC-III is unchanged in the present study, further investigations should explore the incremental effect of weight reduction or insulin sensitizers added to a statin or fibrate on the kinetics of apoC-III in these subjects.

Acknowledgments

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Disclosures

None.

References

22. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on...


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Dick C. Chan, Gerald F. Watts, Esther M.M. Ooi, Juying Ji, Anthony G. Johnson and P. Hugh R. Barrett

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