# Differential Regulation of Lipoprotein Kinetics by Atorvastatin and Fenofibrate in Subjects With the Metabolic Syndrome

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**The metabolic syndrome is characterized by insulin resistance and abnormal apolipoprotein AI (apoAI) and apolipoprotein B-100 (apoB) metabolism that may collectively accelerate atherosclerosis. The effects of atorvastatin (40 mg/day) and micronised fenofibrate (200 mg/day) on the kinetics of apoAI and apoB were investigated in a controlled cross-over trial of 11 dyslipidemic men with the metabolic syndrome. ApoAI and apoB kinetics were studied following intravenous $d_3$-leucine administration using gas-chromatography mass spectrometry with data analyzed by compartmental modeling. Compared with placebo, atorvastatin significantly decreased ($P < 0.001$) plasma concentrations of cholesterol, triglyceride, LDL cholesterol, VLDL apoB, intermediate-density lipoprotein (IDL) apoB, and LDL apoB. Fenofibrate significantly decreased ($P < 0.001$) plasma triglyceride and VLDL apoB and elevated $HDL_2$ cholesterol ($P < 0.001$), $HDL_3$ cholesterol ($P < 0.01$), apoAI ($P = 0.01$), and apoAII ($P < 0.001$) concentrations, but it did not significantly alter LDL cholesterol. Atorvastatin significantly increased ($P < 0.002$) the fractional catabolic rate (FCR) of VLDL apoB, IDL apoB, and LDL apoB but did not affect the production of apoB in any lipoprotein fraction or in the turnover of apoAI. Fenofibrate significantly increased ($P < 0.01$) the FCR of VLDL, IDL, and LDL apoB but did not affect the production of VLDL apoB. Relative to placebo and atorvastatin, fenofibrate significantly increased the production ($P < 0.001$) and FCR ($P = 0.016$) of apoAI. Both agents significantly lowered plasma triglycerides and apoCIII concentrations, but only atorvastatin significantly lowered ($P < 0.001$) plasma cholesteryl ester transfer protein activity. Neither treatment altered insulin resistance. In conclusion, these differential effects of atorvastatin and fenofibrate on apoAI and apoB kinetics support the use of combination therapy for optimally regulating dyslipoproteinemia in the metabolic syndrome. *Diabetes* 52:803–811, 2003**

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apoAI, apolipoprotein AI; apoB, apolipoprotein B-100; CETP, cholesteryl ester transfer protein; FCR, fractional catabolic rate; GCMS, gas chromatography; HOMA, homeostasis model assessment; HMG, hydroxymethylglutaryl; IDL, intermediate-density lipoprotein; LCAT, lecithin cholesterol acyltransferase; PPAR, peroxisome proliferator–activated receptor; PR, production rate.

Insulin resistance underpins the spectrum of abnormalities of the metabolic syndrome (1). Insulin resistance typically induces dyslipoproteinemia owing to abnormal metabolism of lipoproteins containing apolipoprotein B-100 (apoB) and apolipoprotein AI (apoAI). ApoB is the main protein of endogenously synthesized lipoproteins, including VLDL, intermediate-density lipoprotein (IDL), and LDL, and apoAI is the main protein of HDL. Elevated plasma concentrations of apoB and depressed plasma apoAI are both proatherogenic (2,3), and may account for increased incidence of cardiovascular disease in subjects with the metabolic syndrome and diabetes (1,4).

Hepatic insulin resistance increases hepatic glucose production and lipogenesis and may ultimately be the consequence of adipose tissue insulin resistance and increased free fatty acid flux to the liver (5). Increased fatty acid release from adipose tissue also impairs the peripheral uptake of glucose by skeletal muscle (5). Both hepatic and peripheral insulin resistance result from impaired insulin receptor signaling. Insulin resistance increases hepatic secretion of apoB by several mechanisms (1,5–8). These include increased fatty acid flux to the liver, increased de novo lipogenesis related to increased expression of SREBP-lc, and decreased expression of peroxisome proliferator–activated receptors (PPARs), increased triglyceride availability owing to increase expression of microsomal triglyceride transfer protein, and resistance to a direct inhibitory effect of insulin on apoB secretion. Chronic hyperinsulinemia may additionally channel hepatic fatty acids from storage triglyceride pools into a secretory pool that directly enhances secretion of VLDL (8). Insulin resistance also down-regulates LDL receptor expression and activity via a direct mechanism and by regulating cholesterol biosynthesis, thereby delaying hepatic clearance of all apoB-containing lipoproteins from plasma (9). Furthermore, in insulin resistance hepatic overproduction of VLDL together with decreased lipoprotein lipase activity results in expansion in the VLDL triglyceride pool (8); this in turn enhances cholesteryl ester transfer protein (CETP)-mediated hetero-exchange of neutral lipids among lipoproteins, thereby increasing HDL triglyceride content. Subsequent hydrolysis by hepatic lipase results in a thermodynamically unstable HDL apoAI particle that is catabolized rapidly by the liver (10,11).

Statins regulate lipoprotein metabolism (12,13) and decrease the incidence of cardiovascular disease in high-risk subjects including those with the metabolic syndrome (14). Statins competitively inhibit hydroxymethylglutaryl (HMG) CoA, thereby decreasing cholesterol biosynthesis, reciprocally upregulating hepatic LDL receptors, and enhancing the clearance of apoB-containing lipoproteins (15). Inhibition of cholesterogenesis by statins reduces hepatic output of apoB in insulin sensitive subjects (16). By decreasing plasma triglyceride levels, statins may also alter the metabolic fate of HDL particles (17) and perhaps increase the expression of apoAI in vitro, but these mechanisms have not been yet explored in vivo (18).

Fibrates regulate lipid metabolism (19) and may also diminish cardiovascular events in high-risk subjects with the metabolic syndrome (20,21). Fenofibrate was recently shown to decrease progression of coronary atherosclerosis in type 2 diabetes (22). Fibrates activate PPAR-α in the liver with a variety of consequences that may favor the metabolism of both apoB- and apoAI-containing lipoproteins (19,21). Fibrates increase acyl-coenzyme A synthase and fatty acid transporter protein; this facilitates intracellular transport, acylation, and β-oxidation of fatty acids with the net effect of decreasing the availability of fatty acids for triglyceride synthesis and hepatic apoB secretion (19,21). In vitro, fibrates can induce the expression of genes encoding lipoprotien lipase (23), apoAI (24), apoAII (25), and ABCAI (26), a transporter that controls apoAI-mediated cholesterol efflux from macrophages. In addition, fibrates decrease the expression of apoCIII (27), thereby contributing to improvements in the metabolism of triglyceride-rich lipoproteins. The kinetic effects of fibrates on apoB and apoAI metabolism in insulin-resistant obese subjects have not yet been investigated, however.

Despite reports of the relative effects of atorvastatin and fenofibrate on plasma lipid and lipoprotein concentrations (21,28), no studies have compared their effects on the flux of lipoproteins, particularly in the metabolic syndrome. In the present metabolic study, we hypothesized that the atorvastatin and fenofibrate would principally improve apoB and apoAI kinetics, respectively, in the metabolic syndrome.

## RESEARCH DESIGN AND METHODS

**Subjects.** A total of 11 men with the metabolic syndrome were recruited. This was defined as the presence of at least three of the following: waist circumference >102 cm, triglycerides >1.7 mmol/l, HDL cholesterol <1.05 mmol/l, blood pressure ≥130/≥85 mmHg, and fasting glucose >6.1 mmol/l (29), while consuming an ad libitum weight-maintenance diet. We excluded subjects with plasma cholesterol >7 mmo/l, triglycerides >4.5 mmo/l, diabetes (defined by oral glucose tolerance test), cardiovascular disease, consumption of >30 g alcohol/day, use of agents affecting lipid metabolism, apolipoprotein E2/E2 genotype, macroproteinuria, creatinemia (>120 μmol/l), hypothyroidism, and abnormal liver and muscle enzymes. For comparison, we also studied a group of five age-matched normolipidemic lean men (mean ± SD: aged 53.1 ± 9 years, BMI 24.9 ± 2.9 kg/m$^2$, cholesterol 4.3 ± 0.3 mmol/l, triglyceride 0.8 ± 0.2 mmol/l, HDL cholesterol 1.28 ± 0.3 mmol/l, glucose 5.3 ± 0.2 mmol/l, and insulin 6.0 ± 0.6 mU/l) not on any treatment on one occasion. All subjects provided written consent, and the study was approved by the Ethics Committee of the South Eastern Sydney Area Health Service.

**Study design and clinical protocols.** This was a randomized, double-blind, placebo-controlled, cross-over trial. Eligible patients entered a 4-week run-in diet-stabilizing period at the end of which they were randomized to a 5-week treatment period of either atorvastatin (40 mg/day), micronized fenofibrate (200 mg/day), or placebo. Advice was given to continue isocaloric diets and maintain physical activity constant. Compliance with study medication was checked by tablet count at the end of each treatment period.

All subjects were admitted to the metabolic ward in the morning after a 12-h fast. They were studied in a semirecumbent position and allowed to drink only water. Venous blood was collected for biochemical measurements. Plasma volume was determined by multiplying body weight by 0.045 (9). Arterial blood pressure was recorded after 3 min in the supine position using a Dinamap1846 SX/P monitor (Critikon, Tampa, FL). Dietary intake was assessed for energy and major nutrients using at least two 24-h dietary diaries during run-in and treatment periods. Diets were analyzed using DIET four Nutrient Calculation Software (Xyris Software, Qld, Australia).

A primed infusion of [$d_3$]-leucine (1 mg/kg bolus and 1 mg · kg$^{-1}$ · h$^{-1}$ infusion) was administered intravenously for 6 h into an antecubital vein via a Teflon cannula. Blood samples were taken at baseline and at 15, 30, and 45 min and at 1, 2, 4, 6, 8, 10, 12, and 16 h after isotope injection. Subjects were then given a snack and allowed 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 h). All of the procedures were repeated at the end of each treatment period.

**Isolation and measurement of isotopic enrichment of apoB and apoAI**

***ApoB.*** VLDL, IDL, and LDL were isolated from 3 ml plasma by sequential ultracentrifugation (Optima XL-100K; Beckman Coulter, Fullerton, Australia) at densities of 1.006, 1.019, and 1.063 g/ml, respectively. The procedures for isopropranol precipitation, delipidation, hydrolysis, and derivatization of apoB to the oxazolinone derivative were described previously (9,30). Plasma-free leucine was also isolated by cation-exchange chromatography using AG 50 W-X8 resin (BioRad, Richmond, CA) after removing plasma proteins with 60% perchloric acid. Isotopic enrichment was determined using gas chromatography (GCMS) 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.

***ApoAI.*** ApoB was precipitated from 250 μl plasma using heparin (25 μl) and 12.5 μl of 2.0 mol/l $MnCl_2$. Then, 60 μl of 64% CsCl was added to 200 μl heparin/manganese-treated plasma to adjust the density to 1.21 g/ml. HDL was subsequently isolated from 230 μl of this sample by ultracentrifugation (Centrikon T-1,190; Kontron Instruments, Milano, Italy). ApoAI was isolated using PAGE and transferred to polyvinylidine fluoride membrane. The apoAI bands were excised from the membrane, hydrolyzed with 200 μl 6 mol/l HCl at 110°C for 16 h, and dried for derivatization as described above.

**Quantification of apoB, apoAI, and other analytes.** Plasma aliquots were combined to yield five pooled VLDL, IDL, and LDL samples per patient study, as described previously (30). ApoB was isolated in each lipoprotein fraction using isopropanol and quantitated with the Lowry method (9,30). Four aliquots of plasma from 4 separate days were pooled to assay HDL apoAl concentration, which was measured as plasma apoAl concentration, assuming that >90% of apoAl resides in the HDL fraction (31).

Plasma cholesterol and triglyceride concentrations were determined by enzymatic methods using a Hitachi 917 Biochemical Analyser (Hitachi, Tokyo, Japan). HDL cholesterol was measured enzymatically (Boehringer Mannheim, Mannheim, Germany). $HDL_2$ (1.063 g/ml $HDL_2$ 1.125 g/ml) and $HDL_3$ (1.125 g/ml $HDL_3$ 1.21 g/ml) were isolated by ultracentrifugation from 0.5 ml plasma, and cholesterol concentrations were measured. LDL cholesterol was calculated by Friedewald equation, and non-HDL cholesterol as total cholesterol minus HDL cholesterol. Total plasma apoB, apoAl, and apoAll concentration were determined by immunonephrelometry (Dade Behring $BN_2$ Nephelometer) and plasma apoCIII concentration by immunoturbidity (Daichi); interassay CVs were <4.3%. Plasma nonesterified fatty acids were measured commercially by an enzymatic method kit (Randox, Antrim, U.K.). Plasma insulin was measured by radioimmunoassay (DiaSorini, Saluggia, Italy). Insulin resistance was estimated as before using the homeostasis model assessment (HOMA) score (30). Plasma lathosterol concentration was assayed by GCMS (9,30). CETP activity was determined using a kit (Roar Biochemical, New York, NY). Lecithin cholesterol acyltransferase (LCAT) activity was measured using a fluorescence kit (Wak-Chemie Medical, Frankfurt, Germany). Plasma glucose, alanine and asparate transaminases, alkaline phosphatase, and creatinine kinase were analyzed on a Hitachi 917 Biochemical Analyzer.

**Model of apoAI and apoB metabolism and calculation of kinetic parameters.** Figure 1 shows the multicompartmental model used to describe VLDL-, IDL-, and LDL-apoB leucine tracer-to-tracee ratios. The SAAMII program (SAAM Institute, Seattle, WA) was used for modeling the data. The details and assumptions of the model were described previously (30). Briefly, compartments 1–4 describe plasma leucine kinetics. These are connected to an intrahepatic compartment (compartment 5) that accounts for synthesis and secretion of apoB into plasma. Compartments 6–10 describe the kinetics of VLDL-apoB. Compartments 6–9 represent a delipidation cascade. Plasma IDL

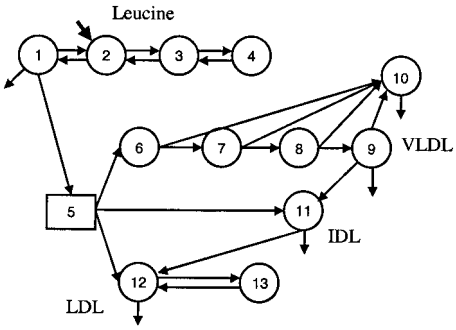


**FIG. 1. Compartment model describing apoB tracer kinetics. Leucine tracer is injected into plasma, represented by compartment 2. Compartments 1, 3, and 4 represent nonplasma leucine compartments. Compartment 5 represents an intrahepatic pool that accounts for the time associated with the synthesis, assembly, and secretion of apoB into the VLDL, IDL, and LDL fractions. VLDL apoB is described by five compartments (6–10). VLDL apoB is converted to IDL (compartment 11) or is cleared directly from plasma, presumably via the LDL receptor. IDL apoB is converted to LDL, compartment 12, or is cleared from plasma. LDL apoB is cleared from this compartment and exchanges with an extravascular LDL pool, compartment 13.**

kinetics are described by compartment 11. Compartment 12 describes plasma LDL, and compartment 13 is an extravascular LDL compartment. VLDL, IDL, and LDL apoB metabolic parameters (fractional catabolic rate [FCR], production rate [PR], percent conversion, and direct synthesis) were derived following a fit of the model to the plasma leucine, VLDL, IDL, and LDL apoB tracer-to-tracee ratio data.

Figure 2 shows the multicompartmental model used to describe HDL apoAI leucine tracer-to-tracee ratios (32). Compartments 1–4 describe plasma leucine kinetics, as for the apoB model. This subsystem is connected to an intrahepatic delay compartment (compartment 5) that accounts for the synthesis and secretion of apoAI into plasma. Compartments 6 and 7 describe the kinetics of apoAI in the plasma HDL fraction and in a nonplasma compartment, respectively. HDL apoAI metabolic parameters (FCR and PR) were derived following a fit of the model to the plasma leucine and apoAI tracer-to-tracee ratio data.

**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 three treatment periods was compared using a mixed effects model (SAS Proc Mixed; SAS Institute). Nominal $P$ values are reported in RESULTS; to adjust for multiple comparisons for a given variable across the three treatment periods, we defined statistical significance at the 1.7% level. Comparisons between obese and lean subjects were performed using ANOVA, with the statistical significance set at 5%.

## RESULTS

Table 1 shows the clinical characteristics of the subjects at entry into the study. On average they were middle aged, overweight/obese, normotensive, and insulin resistant. Plasma cholesterol, triglyceride, LDL cholesterol, and total apoB concentrations, as well as HOMA scores, were significantly higher ($P < 0.001$), and HDL cholesterol ($P < 0.003$) and apoAI ($P < 0.05$) concentrations were significantly lower in the experimental compared with the lean group. Mean daily dietary intake during the run-in phase was: energy 8,095 ± 20 kJ, total fat 73.5 ± 3.1 g, (saturates 35%, polyunsaturates 37%, and monounsaturates 18%), total carbohydrate 230 ± 9.4 g, sugars 87.6 ± 12.2 g, protein 84.5 ± 4.5, cholesterol 256 ± 19 g, and alcohol 2.7 ± 1.7 g.

Table 2 shows the plasma lipid, lipoprotein, apolipoprotein, lathosterol, glucose, and insulin concentrations during the atorvastatin, fenofibrate, and placebo treatment phases. Compared with placebo, atorvastatin significantly decreased plasma concentrations of total cholesterol, triglyceride, LDL cholesterol, non-HDL cholesterol, total apoB, apoCIII, and lathosterol; the ratios of LDL cholesterol–to–apoB and lathosterol-to-cholesterol and CETP activity also fell significantly. Compared with placebo, fenofibrate significantly decreased plasma concentrations of triglycerides, total apoB, apoCIII, and lathosterol, as well as the VLDL triglyceride–to–apoB and lathosterol-to-cholesterol ratios. Fenofibrate also significantly increased plasma HDL, $HDL_2$, and $HDL_3$ cholesterol, apoAl, and apoAll concentrations, and the LDL cholesterol–to–apoB ratio.

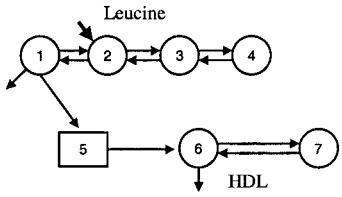


**FIG. 2. Compartment model describing HDL apoAI tracer kinetics. Leucine tracer is injected into plasma, represented by compartment 2. Compartments 1, 3, and 4 represent nonplasma leucine compartments. Compartment 5 represents an intrahepatic pool that accounts for the time associated with the synthesis, assembly, and secretion of apoAI HDL fraction. HDL apoAI is represented as a single plasma compartment, compartment 6. ApoAI is cleared from this compartment and exchanges with an extravascular HDL pool, compartment 7.**

As shown in Table 2, with atorvastatin the reductions in total cholesterol, non-HDL cholesterol, LDL cholesterol, total apoB, lathosterol, lathosterol-to-cholesterol ratio, and CETP activity were significantly greater than with fenofibrate. By contrast, the increase in plasma HDL, $HDL_2$, and $HDL_3$ cholesterol, apoAl and apoAll concentrations, and LDL cholesterol–to–apoB ratio were significantly greater with fenofibrate than with atorvastatin.

There were no significant alterations in body weight, blood pressure, or dietary intake (data not shown) during drug treatment and placebo phases. Drugs were well tolerated with no symptoms reported and no significant increases in plasma transaminases or creatine kinase; alkaline phosphatase, however, fell significantly ($P < 0.01$) with fenofibrate treatment compared with placebo and atorvastatin. Capsule counts confirmed 100% compliance with treatments.

Figure 3 shows isotopic tracer curves for VLDL apoB, IDL apoB, LDL apoB, and HDL ApoAI after the administration of [$d_3$]-leucine in a representative subject during treatment with atorvastatin, fenofibrate, and placebo. Plasma leucine tracer curves did not differ significantly among treatment periods. ApoB tracer curves were of similar contour and demonstrated a precusor-product relationship between VLDL, IDL, and LDL apoB. On treatment the rate of appearance of tracer within the VLDL and LDL apoB fractions was increased, consistent with a reduced VLDL apoB pool and increased catabolism of VLDL and LDL apoB. HDL apoAI tracer curves showed no consistent changes with treatment.

Table 3 compares the metabolic parameters for lean subjects and obese subjects on placebo. The obese subjects had significantly increased concentrations of VLDL apoB, IDL apoB, and LDL apoB, related to an increased VLDL apoB secretion rate and decreased IDL apoB and LDL apoB FCR. Compared with lean subjects, obese subjects had a lower HDL apoAI concentration that was associated with a significantly increased apoAI FCR.

TABLE 1
Clinical and biochemical characteristics of the 11 subjects at recruitment

| | |
|---|---|
| Age (years) | 46.3 ± 6.9 |
| Weight (kg) | 97.4 ± 11.7 |
| BMI (kg/m$^2$) | 30.5 ± 2.6 |
| Systolic BP (mm/Hg) | 128.5 ± 3.1 |
| Diastolic BP (mm/Hg) | 83.8 ± 2.3 |
| Glucose (mmo/l) | 5.68 ± 0.46 |
| Insulin (mμ/l) | 19.91 ± 6.74 |
| HOMA score | 5.10 ± 2.05 |
| Total cholesterol (mmol/l) | 5.88 ± 0.50 |
| Triglyceride (mmol/l) | 2.43 ± 1.04 |
| HDL cholesterol (mmol/l) | 0.94 ± 0.14 |
| non-HDL cholesterol (mmol/l) | 4.94 ± 0.44 |
| LDL cholesterol (mmol/l) | 3.94 ± 0.69 |
| Apolipoprotein B-100 (g/l) | 1.11 ± 0.10 |
| Apolipoprotein AI (g/l) | 1.13 ± 0.16 |
| Apolipoprotein AII (g/l) | 0.31 ± 0.04 |

Data are means ± SD.

Table 4 gives the metabolic parameters of VLDL, IDL, and LDL apoB and HDL ApoAI after treatment with atorvastatin, fenofibrate, and placebo. Figure 4 also shows the percentage change in pool size, FCR, and PR of VLDL apoB, IDL apoB, LDL apoB, and HDL apoAI after each treatment period. Compared with placebo, atorvastatin significantly decreased the pool sizes of VLDL, IDL, and LDL apoB and significantly increased the FCR of all of these apolipoproteins. Atorvastatin also significantly increased the percentage conversion rate of VLDL to IDL apoB but did not alter the PRs or direct syntheses of VLDL, IDL, or LDL apoB nor did it alter the pool size or kinetics of HDL apoAI. Compared with placebo, fenofibrate significantly decreased the pool size of VLDL, IDL, and LDL apoB. These changes were accompanied by a significant increase in the FCR of VLDL, IDL, and LDL apoB. However, there was no significant change in the PR of VLDL, IDL, or LDL apoB, and no change in the conversion rates of VLDL to IDL and IDL to LDL. Fenofibrate also significantly increased the pool size, FCR, and production rate of HDL apoAI.

As shown in Table 4, with atorvastatin the reductions in LDL apoB pool size and direct synthesis of VLDL and IDL apoB, as well as the increase in FCR of LDL apoB, were all significantly greater than with fenofibrate. By contrast, with fenofibrate the increase in HDL apoAI pool size, FCR, and PR, as well as the fall in direct synthesis of VLDL apoB, was significantly greater than with atorvastatin. Compared with the lean control subjects, atorvastatin normalized all kinetic estimates of apoB metabolism, except for VLDL apoB secretion, which tended to remain elevated; apoAI concentration also remained significantly low ($P = 0.02$) and apoAI FCR high ($P = 0.01$). Relative to the lean group, fenofibrate normalized the HDL apoAI concentration, related to a higher PR ($P < 0.04$) and FCR ($P = 0.002$) of apoAI; fenofibrate did not normalize the FCR of IDL apoB ($P = 0.017$), and as with atorvastatin, the PR of VLDL apoB tended to remain elevated ($P = 0.098$).

## DISCUSSION

In this direct comparison of the effects of a statin and fibrate on lipoprotein metabolism, we have demonstrated

TABLE 2
Plasma lipid, lipoprotein, apolipoprotein, and lathosterol concentrations, insulin resistance score, and LCAT and CETP activities after treatment with atorvastatin, fenofibrate, and placebo

| | | | | Group differences ($P$) | |
|---|---|---|---|---|---|
| | Atorvastatin | Fenofibrate | Placebo | Atorvastatin vs. placebo | Fenofibrate vs. placebo | Atorvastatin vs. fenofibrate |
| Total cholesterol (mmol/l) | 3.5 ± 0.17 | 5.58 ± 0.15 | 5.87 ± 0.17 | −2.38 ± 0.14 (<0.001) | −0.29 ± 0.20 (0.036) | −2.01 ± 0.22 (<0.001) |
| Triglycerides (mmol/l) | 1.51 ± 0.20 | 1.72 ± 0.29 | 2.43 ± 0.31 | −0.93 ± 0.18 (<0.001) | −0.72 ± 0.18 (<0.001) | −0.18 ± 0.15 (0.145) |
| LDL cholesterol (mmol/l) | 1.88 ± 0.17 | 3.73 ± 0.18 | 3.94 ± 0.22 | −2.06 ± 0.13 (<0.001) | −0.22 ± 0.20 (0.146) | −1.87 ± 0.19 (<0.001) |
| non-HDL cholesterol (mmol/l) | 2.55 ± 0.16 | 4.56 ± 0.16 | 4.94 ± 0.13 | −2.39 ± 0.14 (<0.001) | −0.39 ± 0.22 (0.013) | −1.93 ± 0.24 (<0.001) |
| HDL cholesterol (mmol/l) | 0.94 ± 0.05 | 1.02 ± 0.07 | 0.94 ± 0.04 | 0.01 ± 0.03 (0.664) | 0.10 ± 0.03 (<0.001) | −0.08 ± 0.03 (0.001) |
| $HDL_2$ cholesterol (mmol/l) | 0.26 ± 0.02 | 0.32 ± 0.02 | 0.25 ± 0.02 | 0.02 ± 0.02 (0.256) | 0.07 ± 0.01 (<0.001) | −0.05 ± 0.02 (0.002) |
| $HDL_3$ cholesterol (mmol/l) | 0.66 ± 0.03 | 0.74 ± 0.04 | 0.67 ± 0.04 | −0.01 ± 0.03 (0.719) | 0.08 ± 0.03 (0.007) | −0.08 ± 0.03 (0.003) |
| Apolipoprotein B-100 (g/l) | 0.64 ± 0.04 | 0.97 ± 0.04 | 1.11 ± 0.03 | −0.47 ± 0.03 (<0.001) | −0.14 ± 0.04 (<0.001) | −0.33 ± 0.05 (<0.001) |
| Apolipoprotein A-I (g/l) | 1.11 ± 0.04 | 1.20 ± 0.06 | 1.13 ± 0.05 | −0.01 ± 0.04 (0.597) | 0.07 ± 0.03 (0.010) | −0.09 ± 0.03 (0.003) |
| Apolipoprotein A-II (g/l) | 0.29 ± 0.01 | 0.38 ± 0.02 | 0.31 ± 0.01 | −0.01 ± 0.01 (0.156) | 0.07 ± 0.01 (<0.001) | −0.08 ± 0.01 (<0.001) |
| Apolipoprotein CIII (mg/l) | 123.2 ± 8.6 | 115.0 ± 14.6 | 150.5 ± 10.5 | −27.2 ± 8.1 (0.001) | −35.6 ± 11.7 (<0.001) | 4.64 ± 8.98 (0.527) |
| VLDL triglyceride/apoB | 16.6 ± 1.6 | 12.1 ± 1.3 | 16.3 ± 1.3 | 0.25 ± 2.11 (0.851) | −4.23 ± 1.10 (0.003) | 4.47 ± 1.51 (0.002) |
| LDL cholesterol/apoB | 3.49 ± 0.09 | 3.86 ± 0.10 | 3.67 ± 0.10 | −0.18 ± 0.06 (<0.001) | 0.18 ± 0.05 (<0.001) | −0.37 ± 0.05 (<0.001) |
| Nonesterified fatty acids (mmol/l) | 0.53 ± 0.03 | 0.54 ± 0.05 | 0.54 ± 0.03 | −0.01 ± 0.04 (0.889) | 0.01 ± 0.06 (0.835) | −0.01 ± 0.05 (0.728) |
| Insulin resistance (HOMA score) | 6.13 ± 1.05 | 6.48 ± 1.71 | 5.10 ± 0.62 | 1.03 ± 0.60 (0.169) | 1.38 ± 1.16 (0.069) | −0.36 ± 0.90 (0.626) |
| Lathosterol (μmol/l) | 1.43 ± 0.15 | 5.93 ± 0.68 | 7.72 ± 0.65 | −6.28 ± 0.55 (<0.001) | −1.78 ± 0.51 (<0.001) | −4.50 ± 0.59 (<0.001) |
| Lathosterol to cholesterol ratio × $10^{-3}$ | 0.42 ± 0.04 | 1.12 ± 0.09 | 1.33 ± 0.12 | −0.91 ± 0.09 (<0.001) | −0.26 ± 0.10 (0.003) | −0.69 ± 0.08 (<0.001) |
| LCAT activity (units/8 h) | 4.67 ± 0.13 | 4.32 ± 0.13 | 4.48 ± 0.07 | 0.19 ± 0.17 (0.145) | −0.16 ± 0.12 (0.219) | 0.36 ± 0.19 (0.011) |
| CETP activity (pmol/3 h) | 51.1 ± 3.2 | 69.5 ± 2.8 | 64.9 ± 2.9 | −13.8 ± 3.03 (<0.001) | 4.59 ± 2.89 (0.055) | −18.4 ± 2.72 (<0.001) |

Data are means ± SEM.

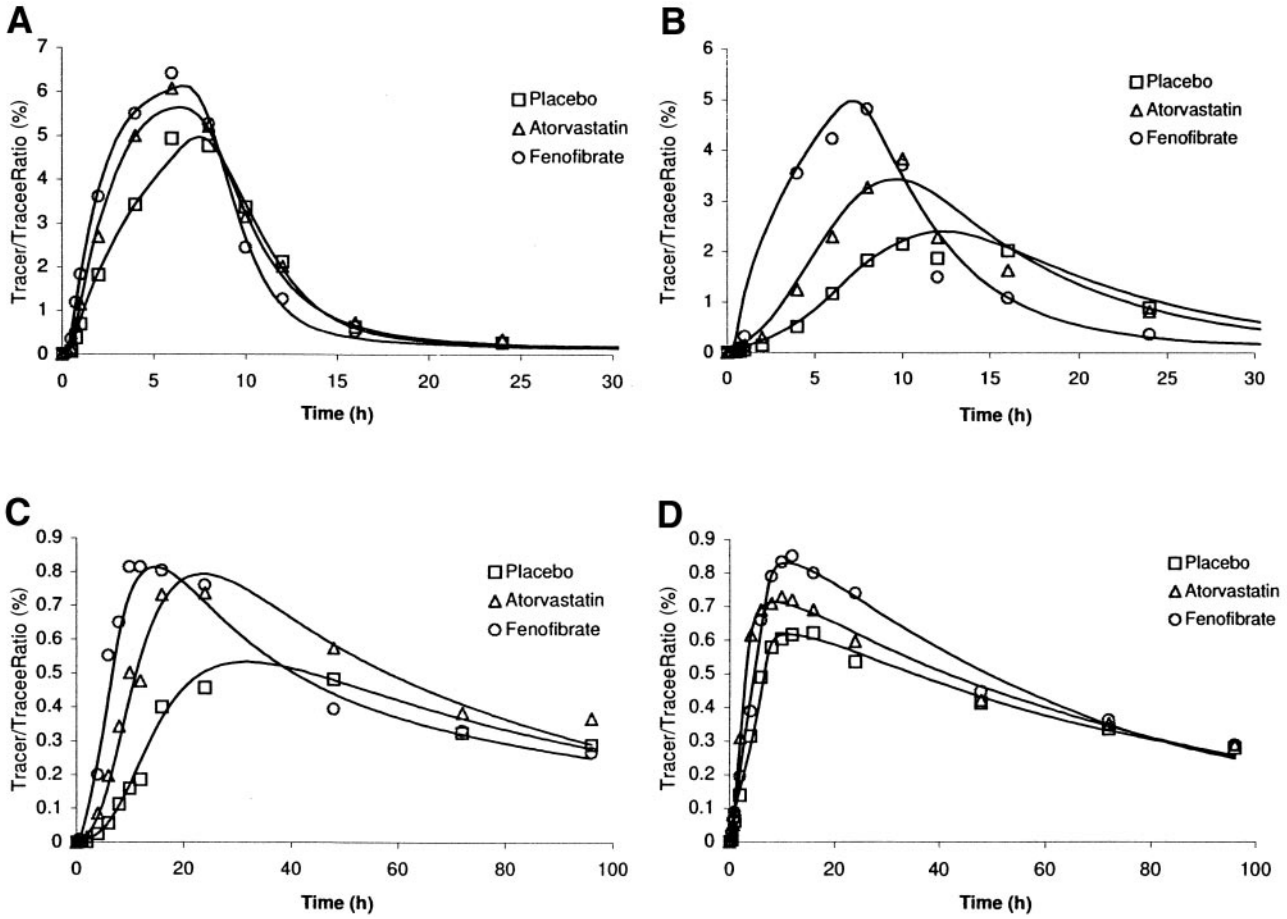


**FIG. 3. Isotopic enrichment for VLDL (*A*), IDL (*B*), and LDL (*C*) apoB and for HDL apoAI (*D*) with $d_3$-leucine in a representative subject on placebo, atorvastatin, or fenofibrate therapy.**

for the first time important differences between atorvastatin and fenofibrate on the kinetics of apoB-containing lipoproteins and HDL apoAI in subjects with the metabolic syndrome. We showed that atorvastatin improved dyslipoproteinemia by increasing the catabolism of VLDL, IDL, and LDL apoB without significantly altering synthesis or the turnover of HDL apoAI. Fenofibrate also increased the catabolism of all apoB-containing lipoproteins, but it increased LDL apoB FCR less than atorvastatin, and the associated increase in LDL apoB production rate resulted in an unchanged pool size of LDL apoB. In contrast to atorvastatin, fenofibrate also increased the synthetic rate of HDL apoAI, and this accounted for a net increase in apoAI pool size, given that it also stimulated the HDL apoA1 FCR. The kinetic effects occurred in the absence of changes in dietary intake, body weight, and insulin resistance.

The kinetic characteristics of our sample population agree with previous studies. We previously showed that centrally obese subjects with the metabolic syndrome have elevated hepatic secretion of VLDL apoB with decreased fractional catabolism of IDL and LDL apoB (30,33). These kinetic defects are due to increased lipid supply to the liver and direct hepatic effects of insulin resistance on the metabolism of apoB-containing lipoproteins (8,34). We also demonstrated elsewhere that delayed catabolism of triglyceride-rich lipoproteins is related to increased plasma apoCIII concentration (33). Other studies in insulin-resistant and type II diabetic patients have shown increased FCR of apoAI and occasionally increased apoAI production (11,35,36). As suggested by others (10,36), the consistent increase in apoAI FCR seen in hypertriglyceridemic subjects could be consequence of remodeling of the HDL particle due to increased cholesterol ester transfer protein activity and decrease in the ratio of the activities of lipoprotein lipase to hepatic lipase.

Previous kinetic studies have focused on the effects of statins and fibrates as monotherapies in patients with diverse lipoprotein phenotypes. These studies have generally used radioisotopes, small sample sizes, and uncontrolled designs and have focused on the kinetics of a single lipoprotein subclass. Variable effects have, hence, been reported with statins on the production and catabolism of apoB-containing lipoproteins (13,37,38). Consistent with our present findings, we have previously shown that in centrally obese subjects, atorvastatin decreases the plasma concentration of all apoB-containing particles by increasing their catabolism and not by altering their production or conversion rates (30). Only one publication has previously addressed the effect of a statin on HDL turnover (39): the data suggested that pravastatin increased apoAI production, but the study was in normolipidemic subjects and was not placebo controlled. Fibrates have been shown to decrease VLDL apoB production and increase VLDL apoB catabolism in markedly hypertriglyceridemic subjects (40,41). In volunteers with comparable characteris-

TABLE 3
Comparison of VLDL, IDL, and LDL apolipoprotein B-100 and HDL apolipoprotein AI metabolic parameters between lean subjects and obese subjects on placebo

| | Lean | Obese on placebo | *P* |
|---|---|---|---|
| Plasma concentrations (mg/l) | | | |
| VLDL apoB | 50.0 ± 8.9 | 92.3 ± 9.8 | 0.034 |
| IDL apoB | 36.0 ± 6.0 | 67.1 ± 5.7 | 0.019 |
| LDL apoB | 390 ± 27 | 565 ± 35 | 0.005 |
| HDL apoAI | 1358 ± 99 | 1132 ± 48 | 0.007 |
| Fractional catabolic rate (pools/day) | | | |
| VLDL apoB | 4.65 ± 0.87 | 3.77 ± 0.30 | 0.245 |
| IDL apoB | 6.23 ± 1.00 | 2.86 ± 0.21 | <0.001 |
| LDL apoB | 0.56 ± 0.10 | 0.35 ± 0.02 | 0.036 |
| HDL ApoAI | 0.20 ± 0.03 | 0.30 ± 0.01 | <0.001 |
| PR ($mg \cdot kg^{-1} \cdot day^{-1}$) | | | |
| VLDL apoB | 10.20 ± 1.42 | 14.75 ± 1.38 | 0.037 |
| IDL apoB | 9.53 ± 1.84 | 8.32 ± 0.57 | 0.427 |
| LDL apoB | 9.74 ± 1.98 | 8.78 ± 0.58 | 0.548 |
| HDL ApoAI | 12.01 ± 1.96 | 15.48 ± 0.84 | 0.037 |

Data are means ± SEM.

tics to our population, bezafibrate increased the production and catabolism of LDL apoB (42). The same group later showed that fenofibrate increases the catabolism of $VLDL_1$, reducing its conversion of $VLDL_2$, and also accelerates the production and catabolism of $LDL_2$, consistent with a distribution of LDL particles to a larger less dense type (43). Fibrates have also been demonstrated to increase the synthesis of apoAI in familial hyperlipidemias (44,45), but not all reports have been consistent.

Both in vitro and in vivo studies confirm that inhibition of cholesterol synthesis by HMGCoA reductase inhibitors upregulates LDL receptor activity (13,15). Predictably, we found that atorvastatin decreased plasma lathosterol levels and reciprocally increased the FCR of VLDL, IDL, and LDL apoB. Statins also enhance the in vitro effects of PPAR-α activity (18), thereby potentially stimulating lipoprotein lipase activity and inhibiting apoCIII expression. The elevated plasma apoCIII concentrations in our patients at baseline could contribute to inhibition of lipolysis of VLDL by lipoprotein lipase, as well as to decreased hepatic uptake of triglyceride-rich remnants by the LDL receptor (33). Hence, reduction in plasma apoCIII levels with atorvastatin may partly explain the increase in FCR of VLDL and IDL apoB (30). The lack of inhibition of hepatic secretion of apoB with atorvastatin could be due to the effects of persistent insulin resistance hyperinsulinemia that drive both hepatic lipogenesis and apoB synthesis by multiple mechanisms (1,6–8). Hence, although statins may decrease hepatic cholesterol availability, triglyceride synthesis, and the expression of apoB mRNA (46), in the setting of insulin resistance these effects may not affect apoB secretion. The significant fall in plasma CETP activity in our study with atorvastatin concurs with another report (17) and could be due to decreased expressed of CETP (17) and the effect of the marked reduction in the pool of apoB-containing lipoproteins that are acceptors for cholesteryl esters from HDL. The fact that the fall in CETP activity was insufficient to significantly increase plasma HDL cholesterol levels could be explained by both the

TABLE 4
Kinetic estimates of the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 and HDL apolipoprotein AI after treatment with atorvastatin, fenofibrate, and placebo

| | | | | Group differences (*P*) | | |
|---|---|---|---|---|---|---|
| | Atorvastatin | Fenofibrate | Placebo | Atorvastatin vs. placebo | Fenofibrate vs. placebo | Atorvastatin vs. fenofibrate |
| Pool size (mg) | | | | | | |
| VLDLapoB | 251 ± 32 | 302 ± 46 | 408 ± 47 | −156 ± 28 (<0.001) | −106 ± 35 (<0.001) | −50 ± 24 (0.041) |
| IDL apoB | 181 ± 23 | 232 ± 18 | 294 ± 26 | −112 ± 18 (<0.001) | −62 ± 32 (0.006) | −50 ± 26 (0.021) |
| LDL apoB | 1306 ± 116 | 2222 ± 129 | 2452 ± 143 | −1145 ± 93 (<0.001) | −230 ± 123 (0.017) | −915 ± 122 (<0.001) |
| HDL apoAI | 4951 ± 311 | 5307 ± 398 | 5007 ± 346 | −57 ± 153 (0.629) | 300 ± 133 (0.017) | −357 ± 57 (0.006) |
| Fractional catabolic rate (pools/day) | | | | | | |
| VLDL apoB | 5.69 ± 0.52 | 5.00 ± 0.49 | 3.77 ± 0.30 | 1.92 ± 0.34 (0.001) | 1.25 ± 0.31 (<0.001) | 0.69 ± 0.48 (0.032) |
| IDL apoB | 5.96 ± 1.25 | 3.84 ± 0.40 | 2.86 ± 0.21 | 3.10 ± 1.17 (0.002) | 0.98 ± 0.31 (0.007) | 2.12 ± 1.17 (0.079) |
| LDL apoB | 0.76 ± 0.11 | 0.44 ± 0.02 | 0.35 ± 0.02 | 0.41 ± 0.12 (<0.001) | 0.09 ± 0.02 (<0.001) | 0.33 ± 0.12 (0.011) |
| HDL ApoAI | 0.30 ± 0.01 | 0.33 ± 0.02 | 0.30 ± 0.01 | 0.00 ± 0.01 (1.000) | 0.03 ± 0.01 (0.016) | −0.03 ± 0.02 (0.016) |
| PR ($mg \cdot kg^{-1} \cdot day^{-1}$) | | | | | | |
| VLDL apoB | 13.4 ± 1.19 | 13.4 ± 1.09 | 14.8 ± 1.38 | −1.31 ± 0.79 (0.098) | −1.19 ± 1.22 (0.131) | −0.12 ± 0.84 (0.876) |
| IDL apoB | 9.21 ± 1.06 | 8.72 ± 0.75 | 8.32 ± 0.57 | 0.88 ± 1.00 (0.263) | 0.39 ± 0.77 (0.615) | 0.49 ± 1.14 (0.529) |
| LDL apoB | 9.78 ± 1.13 | 10.1 ± 0.70 | 8.78 ± 0.58 | 1.00 ± 0.97 (0.148) | 1.31 ± 0.47 (0.061) | −0.32 ± 1.01 (0.637) |
| HDL ApoAI | 15.4 ± 0.7 | 18.1 ± 1.6 | 15.5 ± 0.84 | −0.13 ± 0.36 (0.845) | 2.59 ± 0.99 (0.001) | −2.72 ± 1.08 (<0.001) |
| Direct synthesis (%) | | | | | | |
| VLDL apoB | 83 ± 2 | 74 ± 4 | 80 ± 3 | 3.5 ± 3.1 (0.228) | −6.1 ± 4.7 (0.044) | 9.7 ± 3.0 (0.003) |
| IDL apoB | 13 ± 2 | 19 ± 2 | 18 ± 2 | −5.0 ± 2.9 (0.038) | 1.2 ± 3.4 (0.619) | −6.2 ± 2.3 (0.013) |
| LDL apoB | 4 ± 1 | 8 ± 3 | 3 ± 1 | 1.5 ± 1.8 (0.401) | 5.0 ± 2.8 (0.009) | −3.5 ± 2.0 (0.058) |
| Conversion rate (%) | | | | | | |
| VLDL to IDL apoB | 56 ± 8 | 39 ± 7 | 36 ± 5 | 20 ± 7 (0.008) | 2.7 ± 10 (0.700) | 17 ± 9.1 (0.020) |
| IDL to LDL apoB | 97 ± 3 | 100 | 100 | 2.5 ± 2.5 (0.131) | 0 ± 0 (1.000) | −2.51 ± 2.51 (0.131) |

Data are means ± SEM.

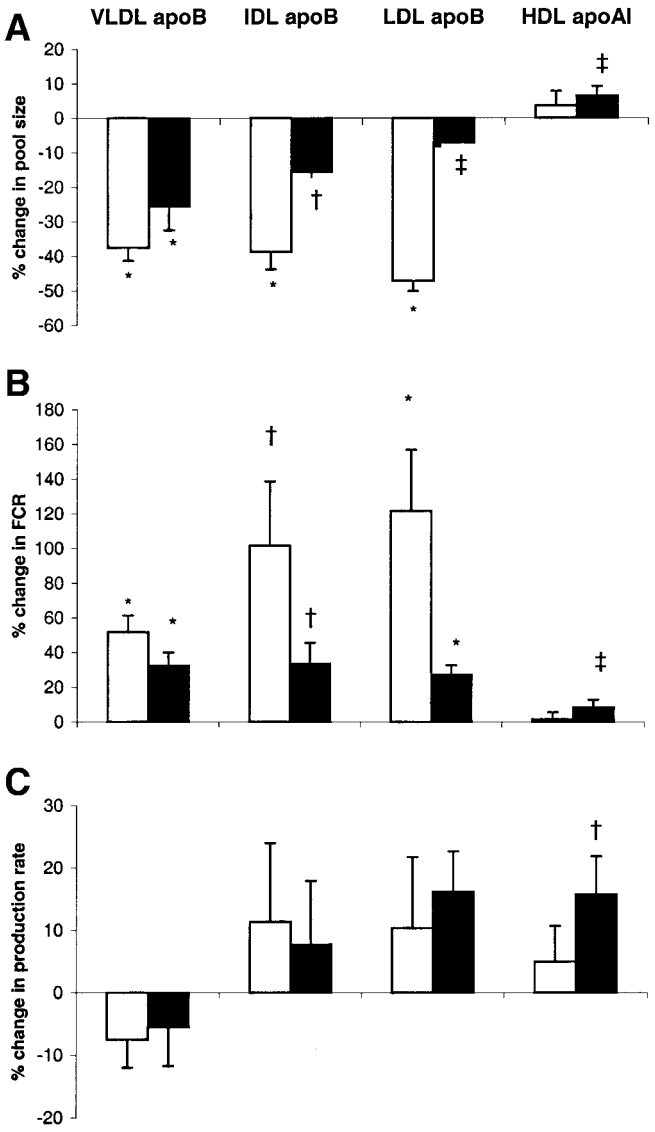


**FIG. 4. Percentage change (means ± SEM) in pool size (*A*), FCR (*B*), and production rate (*C*) of VLDL apoB, IDL apoB, LDL apoB, and HDL apoAI after treatment with atorvastatin or fenofibrate relative to placebo. **P* < 0.001, †*P* < 0.01, ‡*P* < 0.05 vs. placebo.**

persistently elevated FCR of apoAI, as well as by the lack of stimulation of apoAI synthesis and plasma LCAT activity. The persistent hypercatabolism of apoAI with atorvastatin could either be a direct consequence of hepatic insulin resistance (11,35,36) or increased uptake of apoE-containing HDL particles due to upregulation of LDL receptors (3).

The mechanism for increased catabolism of all apoB-containing lipoproteins with fenofibrate was probably chiefly due to hepatic activation of PPAR-α (19). Increased expression of lipoprotein lipase (23) and decreased hepatic apoCIII synthesis (27) could partly explain the increase in FCR of VLDL apoB and IDL apoB, respectively, as well as the increased production of LDL apoB (42,43). The fall in plasma lathosterol agrees with work showing that fibrates suppress cholesterol biosynthesis by inhibiting HMGCoA reductase (47). Hence, reciprocal stimulation of LDL receptor activity could account for the enhanced catabolism of both IDL and LDL apoB. Another PPAR-mediated effect of fibrates is reduction in hepatic triglyceride synthesis and secretion (19,21), consistent with our finding of decreased VLDL triglyceride–to–apoB ratio in the absence of reduction in hepatic apoB secretion. The lack of change in VLDL apoB secretion in the fenofibrate could again be due to persistent insulin resistance (1,6–8). The lack of effect of fenofibrate on CETP activity compared with atorvastatin could be related to increased transport rate of LDL apoB and the unchanged pool size of the corresponding cholesterol acceptor lipoprotein. Fibrates have not been shown consistently to decrease CETP activity (48,49). Hence, in our study the increase in plasma HDL cholesterol and apoAI concentrations with fenofibrate could be attributable chiefly to the increase in apoAI synthesis, given also the unaltered plasma LCAT activity and the significant increase in FCR of apoAI. The increase in apoAI synthesis reported here is consistent with the PPAR-mediated effect of fenofibrate that increases transcription of apoAI in hepatocytes and enterocytes (24). Increased expression of the ABCA1 transporter with fenofibrate could also contribute to the increase in plasma HDL cholesterol levels (26), but this needs confirmation in vivo. Enhancement of apoAI FCR with fenofibrate was seen despite an increase in plasma apoAII concentration that has been reported to impair the catabolism of AI (50). This suggests differential regulation of AI and AII kinetics by fenofibrate. The elevation in apoAI FCR may in part be related to increased activity of hepatic LDL receptors (3), as well as to an increase in hepatic lipase activity (16,21), but these possibilities require further investigation. The significant increase in plasma concentrations of both $HDL_2$ and $HDL_3$ cholesterol with fenofibrate are comparable with the effects on apoAI kinetics.

Clinical trials demonstrate that reduction of plasma apoB-containing lipoproteins with statins decreases cardiovascular events in dyslipidemic subjects (12,14). Similar effects have been reported with fibrates (20–22), but cardiovascular benefits are more closely associated with increase in plasma HDL concentrations (3,21). Our results provide kinetic bases for the changes in plasma lipids and lipoproteins that contributed to improved outcomes in these trials, specifically in subjects with the metabolic syndrome. In this setting, the increase in apoAI flux and concentrations with fenofibrate may be as anti-atherogenic as the greater increase in catabolic rate of apoB lipoproteins with atorvastatin. These different kinetic properties support the combined use of these agents to optimally regulate the dyslipoproteinemia of insulin resistance. The lack of therapeutic effect on hepatic oversecretion of apoB and/or hypercatabolism of apoAI in the present study also points to further investigation of the effects of other additional therapies (e.g., niacin and insulin sensitizers) on lipoprotein kinetics and cardiovascular disease in subjects with the metabolic syndrome.

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