Regulatory Effects of HMG CoA Reductase Inhibitor and Fish Oils on Apolipoprotein B-100 Kinetics in Insulin-Resistant Obese Male Subjects With Dyslipidemia

Dick C. Chan,[1] Gerald F. Watts,[1] P. Hugh R. Barrett,[1] Lawrence J. Beilin,[1] Trevor G. Redgrave,[2] and Trevor A. Mori[1]

Hepatic accumulation of lipid substrates perturbs apolipoproteinB-100 (apoB) metabolism in insulin-resistant, obese subjects and may account for increased risk of cardiovascular disease. In a placebo-controlled trial, we examined the independent and combined effects of decreasing cholesterol synthesis with atorvastatin (40 mg/day) and triglyceride synthesis with fish oils (4 g/day) on apoB kinetics. The subjects were 48 viscerally obese, insulin-resistant men with dyslipidemia who were studied in a fasted state. We found that atorvastatin significantly decreased plasma apoB-containing lipoproteins ($P < 0.001$, main effect) through increases in the fractional catabolic rate (FCR) of VLDL-, IDL-, and LDL-apoB ($P < 0.01$). Fish oils significantly decreased plasma levels of triglycerides and VLDL-apoB ($P < 0.001$), decreased the VLDL-apoB secretion rate ($P < 0.01$), but increased the conversion of VLDL to LDL ($P < 0.001$). Compared with placebo, combined treatment with atorvastatin and fish oils decreased VLDL-apoB secretion ($P < 0.03$) and increased the FCR of apoB in each lipoprotein fraction ($P < 0.03$) and the percent conversion of VLDL to LDL ($P < 0.05$). None of the treatments altered insulin resistance. In conclusion, in visceral obesity, atorvastatin increased hepatic clearance of all apoB-containing lipoproteins, whereas fish oils decreased hepatic secretion of VLDL-apoB. The differential effects of atorvastatin and fish oils on apoB kinetics support their combined use in correcting defective apoB metabolism in obese, insulin-resistant subjects. *Diabetes* 51:2377–2386, 2002

From the [1]Department of Medicine, University of Western Australia and the Western Australian Institute for Medical Research, Crawley, Western Australia; and [2]Department of Physiology, University of Western Australia, Crawley, Western Australia.

Address correspondence and reprint requests to Prof. G.F. Watts, Department of Medicine, University of Western Australia, GPO Box X2213, Perth, Western Australia 6847. E-mail: gfwatts@cyllene.uwa.edu.au.

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apo, apolipoprotein; apoB, apolipoprotein B-100; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FCR, fractional catabolic rate; GLM, general linear model; HOMA, homeostasis model assessment; IDL, intermediate-density lipoprotein; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator–activated receptor; RLP, remnant-like particle; SREBP-1c, sterol regulatory element binding protein-1c.

Visceral obesity is frequently associated with insulin resistance, which in turn is causally related to dyslipoproteinemia and increased risk of type 2 diabetes and cardiovascular disease (CVD) (1–3). Insulin resistance perturbs the metabolism of lipoproteins containing apolipoprotein B-100 (apoB), including VLDL, intermediate-density lipoprotein (IDL), and LDL. These abnormalities may account for the increased incidence of CVD in subjects with insulin resistance and type 2 diabetes (2,4).

Hepatic insulin resistance, involving both the diminished ability of insulin to suppress hepatic glucose production and increased lipogenesis, may ultimately be a consequence of adipose tissue insulin resistance that increases fatty acid flux to the liver (5). Increased fatty acid released from adipose tissue may also partly account for impaired uptake of glucose by skeletal muscle. Both hepatic and peripheral insulin resistance may result from impaired insulin receptor signaling pathways involving decreased phosphatidylinositide 3-kinase activity (6). In visceral obesity, insulin resistance increases hepatic apoB secretion by several mechanisms: increased fatty acid flux to the liver, resistance to a direct inhibitory effect of insulin on apoB secretion, increased expression of microsomal triglyceride transfer protein, and increased de novo lipogenesis (2). Increased lipogenesis may also be caused by overexpression of sterol regulatory element binding protein-1c (SREBP-1c) (1). Chronic hyperinsulinemia also channels hepatic fatty acids and storage triglyceride pools into a secretory pool that directly impacts VLDL secretion (7). These metabolic sequelae of insulin resistance support the notion that the availability of core lipids regulates apoB secretion by the liver (8,9), although the precise quantitative and homeostatic effects that individual lipids exert on this process remains unknown. Insulin resistance also downregulates LDL receptor expression and activity through a direct mechanism as well as through regulating cholesterol biosynthesis (10), thereby delaying the hepatic clearance of all apoB-containing lipoproteins from plasma.

Statins regulate lipoprotein metabolism (11) and decrease the incidence of clinical CVD in high-risk patients, including those with insulin resistance and diabetes (12). Statins competitively inhibit 3-hydroxy-3-methylglutaryl

coenzyme A, thereby decreasing cholesterol biosynthesis, reciprocally upregulating hepatic LDL receptors, and enhancing clearance of apoB-containing particles (13). Inhibition of cholesterogenesis by statins may also reduce the hepatic output of apoB (14,15). The kinetic effects of statins on apoB metabolism have not been elucidated in insulin-resistant subjects.

Marine oils, a rich source of two n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), also regulate lipid metabolism (16). Fish oils may diminish cardiovascular events in high-risk subjects (17). They have been shown to decrease hepatic triglyceride synthesis (18) via inhibition of diacylglycerol acyltransferase, fatty acid synthase, and acetyl-CoA carboxylase enzyme activities. They also enhance fatty acid β oxidation by stimulating peroxisome proliferator–activated receptors (PPAR-α). In addition, fish oils decrease the hepatic pool of triglycerides by suppressing transcription of the SREBP-1c gene, thereby inhibiting de novo synthesis of fatty acids and triglycerides (19). Accordingly, fish oils decrease hepatic VLDL-apoB secretion in both humans and experimental animals (18,20). The kinetic effects of fish oils on apoB metabolism in insulin-resistant, obese subjects have not been investigated.

In the present study, we examined the independent and combined effects of decreasing cholesterol synthesis with atorvastatin and decreasing triglyceride synthesis with fish oils on apoB kinetics in insulin-resistant men with visceral obesity. We hypothesized that by regulating both hepatic cholesterol and triglyceride availability, atorvastatin and fish oils exert independent and additive effects in improving apoB metabolism in these subjects.

RESEARCH DESIGN AND METHODS

Subjects. We recruited 48 obese men for this study. Obesity was defined by a waist circumference >100 cm, a waist-to-hip ratio >0.97, and a BMI >29 kg/m^2. Subjects were selected for having insulin resistance, defined as a homeostasis model assessment (HOMA) score (21) >5.1 (i.e., 1 SD above the mean for a reference population of 22 lean, normolipidemic men of similar age). All subjects had plasma triglyceride levels >1.2 mmol/l and cholesterol >5.2 mmol/l. None of the subjects had diabetes, apoE2/E2 genotype, macroproteinuria, creatinemia, hypothyroidism, or abnormal liver enzymes. Subjects consumed less than one fish meal per week and <30 g alcohol per day and took no fish oils supplements. None reported a history of CVD or was taking agents affecting lipid metabolism. For comparison purposes, 10 normolipidemic, insulin-sensitive, nonobese men (age 53 ± 9 years, BMI 24.8 ± 2.9 kg/m^2, total cholesterol 4.3 ± 0.3 mmol/l, and triglycerides 0.77 ± 0.25 mmol/l) also underwent apoB kinetic studies. All subjects provided written consent, and the study was approved by the local Ethics Committee.

Study design and clinical protocols. Subjects entered a randomized, double-blind, placebo-controlled intervention trial involving a 3-week run-in period with body weight variations of <2%. After the run-in period, subjects were randomized to one of four treatment groups for 6 weeks: atorvastatin (40 mg/day), fish oils (four Omacor [Pronova Biocare, Oslo, Norway] capsules, consisting of 45% EPA and 39% DHA, taken orally at night; 4 g/day), atorvastatin plus fish oils, or atorvastatin placebo plus 4 g/day placebo corn oil. Compliance was checked by tablet/capsule count. Adherence to fish oil regimen was also confirmed through measurement of plasma EPA and DHA levels.

Subjects were admitted to the metabolic ward after a 14-h fast. They were studied in a semi-recumbent position and allowed only water. Venous blood samples were collected for biochemical analyses. Plasma volume was determined by multiplying body weight by 0.045 (22), adjusting for the decrease in relative plasma volume associated with an increase in body weight (23). Dietary intake was assessed for energy and major nutrients throughout the study.

A single bolus of [$^2H_3$]leucine (5 mg/kg body wt) was administered intravenously into an antecubital vein via a 21-G butterfly needle. Blood samples were taken at baseline and at 5, 10, 20, 30, and 40 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 h after isotope injection. Additional fasting blood samples were collected in the morning on the following 4 days (24, 48, 72, and 96 h). All procedures were repeated after 6 weeks.

Isolation and measurement of isotopic enrichment of VLDL-, IDL-, and LDL-apoB. Laboratory methods for isolation and measurement of isotopic enrichment of apoB have been fully described (24). Briefly, apoB in VLDL, IDL, and LDL fractions was separated by sequential ultracentrifugation, precipitated by isopropanol, delipidated, hydrolyzed, and derivatized with acetonitrile/N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide. Isotopic enrichment was determined by ion monitoring of derivatized samples at mass-to-charge ratios of 305 and 302. Tracer/tracee ratios were derived for each sample.

Quantification of apoB and other analytes. Plasma samples were combined to yield five pooled VLDL, IDL, and LDL samples per patient study. ApoB in VLDL, IDL, and LDL fractions from the pooled plasma samples was isolated and determined by a modified Lowry method (24,25).

Laboratory methods for measurement of lipid and lipoprotein have been previously described (26). Plasma remnant-like particle (RLP) cholesterol was determined with a JIMRO-II assay kit (Japan Immunoresearch Laboratories, Takasaki, Japan). Plasma nonesterified fatty acids (NEFAs) were measured with a kit (Randox, Antrim, N. Ireland). Plasma insulin was measured by radioimmunoassay (DiaSorini, Saluggia, Italy). Plasma glucose concentration was measured by the hexokinase method. Insulin resistance was estimated using the HOMA formula (21). Plasma lathosterol concentration was assayed using gas chromatography–mass spectrometry (27). Plasma EPA and DHA levels were measured using gas chromatography (Hewlett-Packard) (28). ApoE genotype was determined as previously described (29).

Model of apoB metabolism and calculation of kinetic parameters. A multicompartmental model (Fig. 1), similar to that of Phair et al. (30), was used to describe VLDL-, IDL-, and LDL-apoB leucine tracer/tracee ratios. The SAAM II program (SAAM Institute, Seattle, WA) was used to fit the model to the tracer data. Kinetic estimates were derived from the model parameters giving the best fit. Part of the model consists of a four-compartment subsystem (compartments 1–4) that describes plasma leucine kinetics. The subsystem is connected to an intrahepatic delay in compartment 5 that accounts for the time required for the synthesis and secretion of apoB into plasma. This model provides for the direct secretion of apoB into VLDL, IDL, and LDL fractions. Compartments 6–10 are used to describe the kinetics of apoB in VLDL. Compartments 6–9 represent a delipidation cascade. The fraction of each compartment in the cascade is converted to the slowly turning over VLDL compartment (compartment 10). VLDL particles in compartment 9 can be converted to IDL or removed directly from plasma. Plasma IDL kinetics are described by compartments 11–12. Compartment 12 represents a slowly turning over pool of IDL. IDL in compartment 11 can be converted to LDL (compartment 13), or removed directly from plasma. The LDL section of the model consists of two compartments: 13 describes plasma LDL and 14 describes extravascular LDL exchange. VLDL, IDL, and LDL-apoB metabolic parameters, including secretion rate, fractional catabolic rate (FCR), and percent conversion of VLDL to IDL and LDL, were derived after fitting the model to the apoB tracer/tracee ratio data.

Statistical analysis. Group characteristics were compared by *t* tests. Adjustment for differences in baseline covariates and changes in variables during the study were performed using general linear models (GLMs). The factorial analysis of the GLM provides information on the main and additive effects of the interventions.

RESULTS

Table 1 shows the pretreatment clinical and biochemical characteristics of the obese subjects randomized to the four treatment groups. On average, subjects were middle-aged, centrally obese, normotensive, and insulin resistant. There were no significant group differences in any of the variables in Table 1. Of the 48 obese subjects, 31 were E3/E3 homozygotes, 2 were E2/E3 heterozygotes, 13 were E3/E4 heterozygotes, and 2 were E4/E4 homozygotes. There were no statistically significant differences in the frequency distribution of E alleles among the groups.

Tablet/capsule counts confirmed subjects' compliance with randomization to active intervention or placebo was >95%. Plasma EPA and DHA concentrations increased from 1.0 ± 0.4 to 3.5 ± 1.3% and from 1.6 ± 0.5 to 3.1 ±

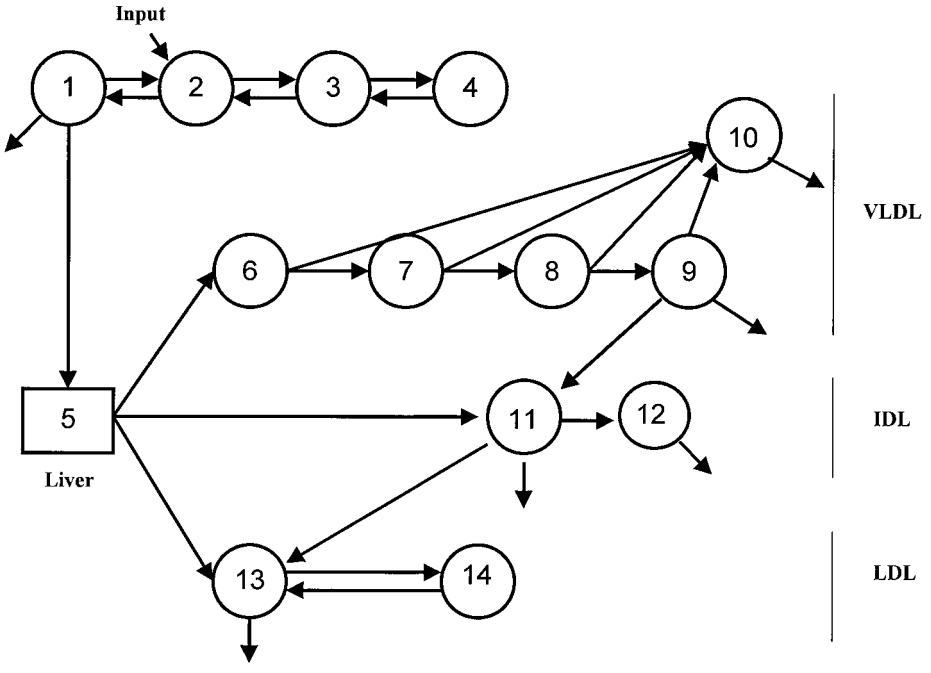

FIG. 1. Multicompartmental model for apoB metabolism. Compartments 1–4 represent plasma leucine; compartment 5 is an intrahepatic delay compartment for apoB synthesis and secretion; compartments 6–10 represent a delipidation cascade for VLDL-apoB; compartment 11 represents plasma IDL converting to LDL or being removed directly from plasma; compartment 12 represents a slowly turning over pool of IDL apoB; compartment 13 represents plasma LDL; and compartment 14 represents an extravascular LDL exchange compartment.

0.8% ($P < 0.01$), respectively, confirming good compliance with fish oil capsules.

Table 2 shows macronutrient and alcohol intake and exercise expenditure in the four groups at baseline and after intervention. These measurements did not differ significantly among groups at baseline, nor were they altered significantly during the study. Hence, these factors were unlikely to confound treatment effects on the study outcome variables.

Table 3 shows the effects of the interventions on plasma lipid, lipoprotein, apo, and lathosterol concentrations. There were no significant interactions between atorvastatin and fish oil treatment for any of the variables. In a factorial analysis carried out with GLM, there was a significant ($P < 0.001$) main effect of atorvastatin in decreasing plasma triglycerides, apoB, lathosterol, and total, non-HDL, LDL, and RLP cholesterol and in increasing HDL cholesterol ($P < 0.01$). There was also a significant ($P < 0.05$) main effect of fish oils in lowering plasma triglycerides and raising HDL cholesterol. Fish oils had no significant effect on other lipid-related variables. There were no significant within-group differences in body weight, waist circumference, waist-to-hip ratio, blood pressure, plasma NEFAs, glucose, or HOMA scores (data not shown).

Figure 2 shows isotopic enrichment curves for VLDL-, IDL-, and LDL-apoB after the administration of [D_3]leucine in a representative subject from each group before and after treatment. All enrichment curves were of similar contour and demonstrated a precursor-product relation among VLDL-, IDL-, and LDL-apoB. Enrichment curves with placebo were not altered between weeks 0 and 6 (Fig. 2*A*). With atorvastatin, enrichment curves on treatment displayed more rapid appearance and disappearance com-

TABLE 1.
Pretreatment clinical and biochemical characteristics of 48 obese subjects

	Placebo	Atorvastatin	Fish oils	Atorvastatin + fish oils
n	12	13	12	11
Age (years)	51 ± 9.3	52 ± 9.7	58 ± 7.9	54 ± 8.1
Body weight (kg)	100 ± 8.3	107 ± 17.6	106 ± 18.0	101 ± 14.7
BMI (kg/m^2)	32.2 ± 2.7	34.5 ± 5.2	35.2 ± 4.4	32.5 ± 3.05
Waist circumference (cm)	110 ± 6.2	118 ± 13.2	115 ± 9.3	110 ± 7.8
Waist-to-hip ratio	1.00 ± 0.05	1.04 ± 0.07	1.00 ± 0.03	1.01 ± 0.03
Systolic blood pressure (mmHg)	128 ± 11.1	132 ± 19.2	132 ± 12.6	138 ± 17.1
Diastolic blood pressure (mmHg)	78 ± 9.7	79 ± 11.9	74 ± 9.7	83 ± 8.1
Fasting NEFAs (mmol/l)	0.26 ± 0.10	0.29 ± 0.13	0.36 ± 0.19	0.27 ± 0.06
Fasting glucose (mmol/l)	5.4 ± 0.54	5.6 ± 0.57	5.7 ± 0.97	5.3 ± 0.72
Fasting insulin (mU/l)	32.1 ± 8.6	32.5 ± 12.7	40.6 ± 12.4	32.7 ± 6.9
Insulin resistance (HOMA)	7.7 ± 2.6	8.1 ± 3.6	10.4 ± 4.7	7.8 ± 2.4

Data are means ± SD.

TABLE 2
Daily energy and macronutrient intake and exercise energy expenditure of the subjects at baseline and postintervention

	Placebo	Atorvastatin	Fish oils	Atorvastatin + fish oils
Energy (kJ)				
Baseline	10110 ± 469	9952 ± 341	9291 ± 362	10299 ± 376
Postintervention	9611 ± 274	10087 ± 378	9260 ± 301	9912 ± 352
Protein (% energy)				
Baseline	20.3 ± 1.6	18.1 ± 0.7	20.3 ± 1.7	17.5 ± 1.3
Postintervention	19.9 ± 1.3	18.8 ± 1.3	21.9 ± 1.2	20.0 ± 1.8
Carbohydrate (% energy)				
Baseline	38.3 ± 2.2	44.8 ± 2.8	43.3 ± 1.6	43.7 ± 3.3
Postintervention	38.4 ± 2.7	45.4 ± 2.6	39.5 ± 3.0	42.5 ± 3.4
Fat (% energy)				
Baseline	33.3 ± 1.2	30.8 ± 2.3	33.1 ± 1.7	31.6 ± 1.2
Postintervention	35.7 ± 1.4	29.9 ± 2.3	33.8 ± 3.1	30.1 ± 1.9
Alcohol (% energy)				
Baseline	7.8 ± 1.8	6.5 ± 1.2	3.3 ± 1.6	7.1 ± 2.4
Postintervention	5.8 ± 1.8	5.8 ± 1.2	2.8 ± 1.0	5.5 ± 1.6
Exercise energy expenditure (kJ)				
Baseline	6029 ± 762	7339 ± 894	8121 ± 615	6558 ± 722
Postintervention	6416 ± 901	7211 ± 933	7942 ± 581	6354 ± 716

Data are means ± SE.

pared with during the off-treatment period. Peak enrichment values were higher, reflecting reductions in VLDL-, IDL-, and LDL-apoB pool sizes with atorvastatin (Fig. 2*B*). With fish oils, the peak apoB enrichment in VLDL fraction was also higher, reflecting reductions in VLDL-apoB pool size (Fig. 2*C*). However, there were no differences in the IDL and LDL fractions on the apoB enrichment curves before and after fish oil supplementation. The pattern of change of all tracer enrichment curves with combination treatment was similar to those with atorvastatin treatment (Fig. 2*D*).

Compared with nonobese control subjects, the 48 obese subjects had higher VLDL-apoB production (14.7 ± 1.0 vs. 9.7 ± 1.2 mg · kg^{-1} · day^{-1}; $P < 0.05$), lower conversion of VLDL- to LDL-apoB (28 ± 3 vs. 50 ± 8%; $P < 0.01$), and a lower FCR of LDL-apoB (0.27 ± 0.02 vs. 0.43 ± 0.07 pools/day; $P < 0.001$). Table 4 shows the effects of the interventions on VLDL-, IDL-, and LDL-apoB metabolism. There were no significant interactions between atorvastatin and fish oil treatments on any of the variables shown in Table 4. There was a significant ($P < 0.01$) main effect of atorvastatin on decreasing VLDL-, IDL-, LDL-apoB and total apoB pool size. This was coupled with a significant increase ($P < 0.01$) in the FCR of each apoB fraction; posttreatment values of LDL-apoB FCR did not differ significantly from those of nonobese control subjects. Atorvastatin did not significantly alter apoB production or the percent VLDL-IDL-LDL conversion rates. With fish oils, there was a significant ($P < 0.001$) main effect in lowering plasma VLDL-apoB pool size and decreasing VLDL-apoB secretion ($P < 0.01$); posttreatment values of VLDL-apoB secretion did not differ significantly from those of nonobese control subjects. Fish oils did not significantly alter apoB pool sizes or secretion rates of IDL- and LDL-apoB. Furthermore, fish oils did not alter the FCRs of VLDL-, IDL-, and LDL-apoB. However, fish oils did increase the percent conversion of VLDL to IDL and IDL to LDL ($P < 0.05$). The results in Table 4 still held after adjusting for baseline group differences in insulin, HOMA, alcohol intake, and other variables (Table 1) using GLM. We also found that atorvastatin increased the total apoB FCR ($P < 0.01$) without changing total apoB secretion, whereas fish oils decreased ($P < 0.05$) both total apoB production and the FCR (data not shown).

Figure 3 shows the percent change in plasma apoB pool sizes and metabolic variables in VLDL-, IDL-, and LDL-apoB on treatments. Consistent with the above analysis, the reduction in VLDL-apoB pool size (−62%; −255 ± 43 mg/l; $P < 0.01$) was significantly greater with combination treatment versus treatment with either atorvastatin or fish oils alone. This difference reflected both a significant decrease in VLDL-apoB secretion (−33%; −5.9 ± 2.1 mg · kg^{-1} · day^{-1}; $P < 0.03$) and an increase in VLDL-apoB FCR (+68%; 2.5 ± 0.7 pools/day; $P < 0.03$). Compared with the placebo group, there was also a significant increase in the FCR of IDL-apoB (+49%; 2.1 ± 0.6 pools/day; $P < 0.02$) and LDL-apoB (+107%; 0.22 ± 0.04 pools/day; $P < 0.01$), as well as in the percent conversion of VLDL- to IDL-apoB (+39%; 13 ± 5%; $P < 0.05$), VLDL- to LDL-apoB (+51%; 17 ± 6%; $P < 0.03$), and IDL- to LDL-apoB (+20%; 10 ± 5%; $P < 0.05$). However, the reduction in IDL- and LDL-apoB or the increase in the FCRs of each apoB fraction with combination treatment did not differ significantly from treatment with atorvastatin alone. Similarly, there were no significant differences in the reduction in VLDL-apoB secretion or increase in apoB conversion with fish oil alone or combination treatment. Posttreatment values of VLDL-apoB secretion (8.8 ± 1.1 vs. 9.7 ± 1.2 mg · kg^{-1} · day^{-1}), FCR of LDL-apoB (0.50 ± 0.05 vs. 0.43 ± 0.07 pools/day), and percent conversion of VLDL- to LDL-apoB (55 ± 6 vs. 50 ± 8%) with combination treatment were not significantly different from those in the nonobese control subjects.

DISCUSSION

This is the first study to examine the independent and combined effects of a statin and fish oils on apoB kinetics in insulin-resistant subjects. We showed that dysregulation of apoB metabolism in these subjects responds differen-

TABLE 3
Plasma lipid, lipoprotein, apolipoprotein and lathosterol concentrations, and HOMA score in the subjects at baseline and postintervention

	Placebo	Atorvastatin	Fish oils	Atorvastatin + fish oils	Main effects (*P*)	
					Atorvastatin	Fish oils
Triglycerides (mmol/l)						
Baseline	1.7 ± 0.18	1.9 ± 0.13	2.0 ± 0.34	2.0 ± 0.21	−0.32 ± 0.08	−0.28 ± 0.08
Week 6	1.6 ± 0.15	1.4 ± 0.12*	1.5 ± 0.21*	1.2 ± 0.17*	(0.001)	(0.001)
Total cholesterol (mmol/l)						
Baseline	5.8 ± 0.14	5.8 ± 0.17	5.9 ± 0.22	6.3 ± 0.32	−1.82 ± 0.17	−0.04 ± 0.17
Week 6	5.6 ± 0.13	3.6 ± 0.12*	5.5 ± 0.22	3.9 ± 0.27*	(0.001)	(NS)
HDL cholesterol (mmol/l)						
Baseline	1.05 ± 0.06	1.00 ± 0.05	0.99 ± 0.06	1.10 ± 0.09	0.10 ± 0.04	0.07 ± 0.04
Week 6	1.03 ± 0.06	1.04 ± 0.05	1.00 ± 0.04	1.25 ± 0.09*	(0.007)	(0.041)
Non-HDL cholesterol (mmol/l)						
Baseline	4.77 ± 0.12	4.81 ± 0.18	4.94 ± 0.21	5.15 ± 0.26	−1.93 ± 0.16	−0.11 ± 0.16
Week 6	4.58 ± 0.11	2.58 ± 0.11*	4.46 ± 0.24	2.72 ± 0.22*	(0.001)	(NS)
LDL cholesterol (mmol/l)						
Baseline	3.80 ± 0.13	3.81 ± 0.16	3.92 ± 0.22	4.04 ± 0.28	−1.78 ± 0.14	0.02 ± 0.14
Week 6	3.83 ± 0.11	1.84 ± 0.12*	3.68 ± 0.17	2.15 ± 0.19*	(0.001)	(NS)
Remnant-like particle-cholesterol (mmol/l)						
Baseline	0.35 ± 0.04	0.43 ± 0.07	0.45 ± 0.08	0.41 ± 0.04	−0.08 ± 0.04	−0.07 ± 0.04
Week 6	0.32 ± 0.04	0.29 ± 0.08*	0.31 ± 0.04	0.20 ± 0.02*	(0.035)	(NS)
Apolipoprotein A-I (mg/dl)						
Baseline	128 ± 5	119 ± 5	118 ± 4	128 ± 6	5 ± 4	5 ± 4
Week 6	126 ± 4	123 ± 4	121 ± 4	135 ± 9	(NS)	(NS)
Apolipoprotein B (mg/dl)						
Baseline	129 ± 4	122 ± 6	128 ± 6	134 ± 6	−49 ± 4	−2 ± 4
Week 6	123 ± 3	69 ± 3*	118 ± 6	73 ± 5*	(0.001)	(NS)
Lathosterol (μmol/l)						
Baseline	9.3 ± 1.0	10.8 ± 1.2	11.2 ± 1.5	13.0 ± 1.0	−7.80 ± 0.88	1.50 ± 0.89
Week 6	9.6 ± 0.7	1.9 ± 0.3*	11.2 ± 1.1	3.8 ± 1.1*	(0.001)	(NS)
Insulin resistance (HOMA score)						
Baseline	7.8 ± 0.7	8.1 ± 1.0	10.4 ± 1.4	7.8 ± 0.7	−0.3 ± 0.7	0.9 ± 0.8
Week 6	7.4 ± 0.6	8.0 ± 1.2	12.1 ± 2.4	8.0 ± 0.7	(NS)	(NS)

Data are means ± SE. General linear modeling was used to assess the main and interactive effects of atorvastatin and fish oil treatments. There were no significant interactive effects between the treatments. NS, no significant. *$P < 0.05$ for t test comparison with placebo group.

tially to treatment with atorvastatin and fish oils. Inhibition of cholesterol synthesis, and hence the availability of both free and esterified cholesterol, with atorvastatin enhanced clearance of all apoB-containing lipoproteins, whereas inhibition of triglyceride synthesis, and hence the amount of newly synthesized triglycerides, with fish oils decreased the hepatic secretion of VLDL-apoB and increased the conversion of VLDL- to LDL-apoB. Although both atorvastatin and fish oils had independent beneficial effects on apoB kinetics, combination treatment attained the optimal improvement in apoB metabolism. These effects were achieved with no significant alteration in insulin resistance, body weight, or dietary intake.

Previous kinetic studies. Dyslipidemia in insulin-resistant subjects, including patients with obesity and type 2 diabetes, results from overproduction of VLDL and decreased clearance of apoB-containing lipoproteins (2,24,31–33). Our pretreatment data were consistent with those from previous kinetics studies. The independent effects of statins (34,35) or fish oils (18,20,36,37) on apoB kinetics have been examined only in nonobese, insulin-sensitive subjects. The present study extends previous studies to insulin-resistant subjects.

Effect of fish oils on apoB metabolism. Animal and human studies have shown that fish oils lower plasma triglycerides by inhibiting hepatic VLDL-apoB secretion (18,20). This finding agrees with our observation that the VLDL-apoB pool size and secretion rate decreased with fish oils. These effects were achieved without a change in cholesterol synthesis, as reflected by the plasma lathosterol concentration, suggesting that hepatic triglyceride synthesis may play a significant role in regulating VLDL-apoB secretion. Our data also demonstrated a significant increase in the conversion of VLDL to IDL and LDL. Because fish oils reduced hepatic VLDL-apoB secretion, the absolute flux of VLDL-apoB into the circulation would be expected to likewise fall. Decreased VLDL particle number would conceivably lead to a greater proportion of VLDL being converted into LDL. Moreover, these effects may entail enhanced lipolysis via the stimulation of lipoprotein lipase activity, as reported elsewhere (38). Other studies have also shown that enrichment of VLDL particles with n-3 fatty acids favors conversion of VLDL to LDL (39). Moreover, fish oils could downregulate hepatic receptor activity and hinder removal of apoB (40). Reduction in VLDL particle size may also enhance apoB conversion (41). Despite increased apoB conversion, we did not demonstrate a significant effect of fish oil on IDL- and LDL-apoB pool sizes, nor on the FCRs of apoB in all lipoprotein fractions. Our data showing that total apoB pool size did not change with fish oil supplementation are consistent with a mechanism of action of fish oils that

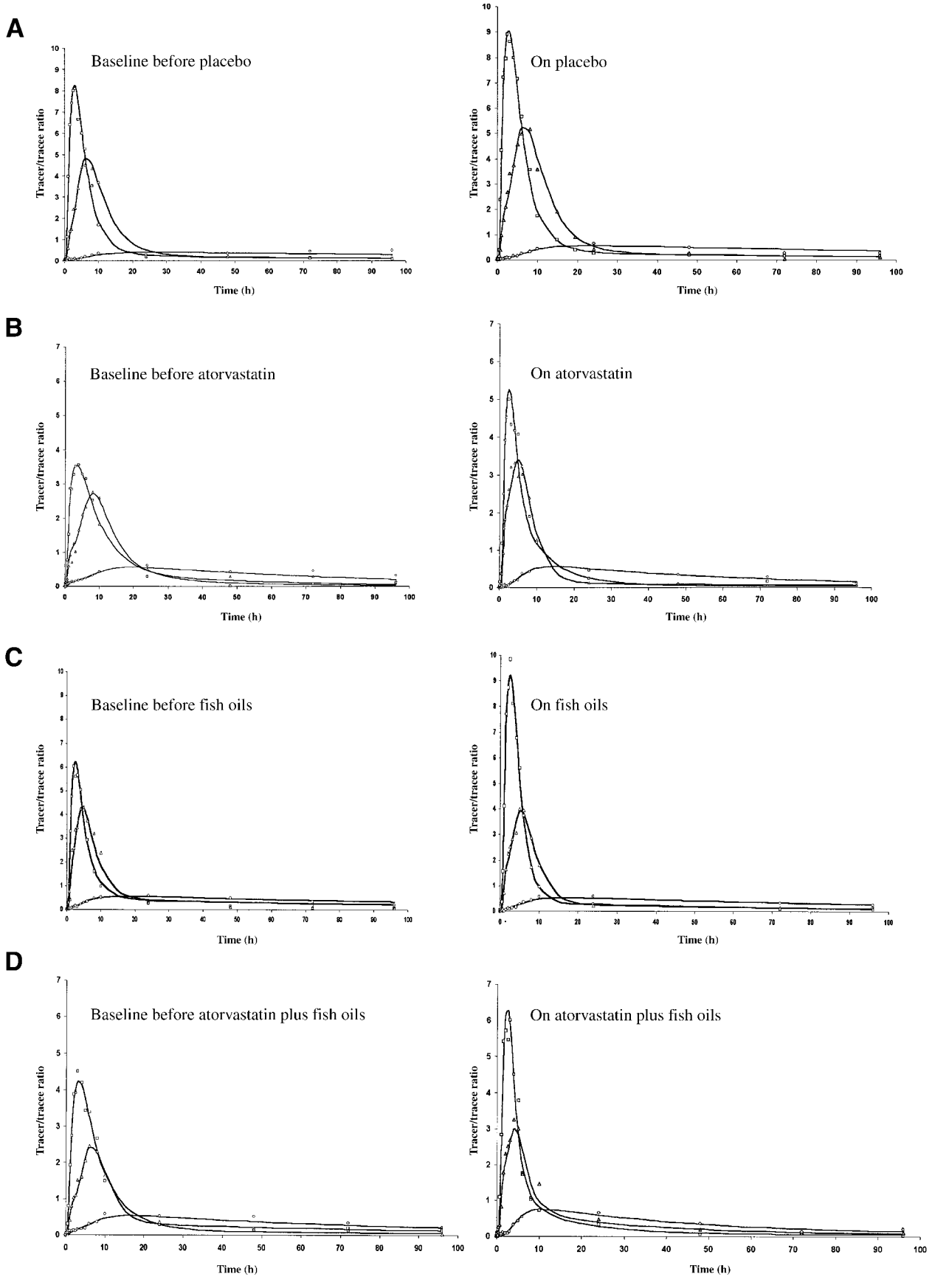

FIG. 2. Plasma isotopic tracer curves for VLDL- (□), IDL- (△) and LDL-apoB (○) after the administration of [D_3]leucine in a representative subject from placebo (*A*) , atorvastatin (*B*), fish oils (*C*), and atorvastatin plus fish oils (*D*) groups before and after treatment. Observed values (symbols) and model-predicted values (lines) are shown.

TABLE 4
Effect of interventions on VLDL-, IDL-, and LDL apoB-100 metabolism

	Placebo	Atorvastatin	Fish oil	Atorvastatin + fish oil	Main effects (*P*)	
					Atorvastatin	Fish oil
Apo B-100 pool size (mg)						
Total						
Baseline	2798 ± 167	2780 ± 163	2641 ± 151	2850 ± 196	−1107 ± 86	18 ± 86
Week 6	2681 ± 145	1606 ± 90	2638 ± 185	1627 ± 134	(0.001)	(NS)
VLDL						
Baseline	390 ± 46	367 ± 39	338 ± 39	404 ± 53	112 ± 20	−92 ± 20
Week 6	346 ± 39	228 ± 28*	235 ± 27*	148 ± 18*	(0.001)	(0.001)
IDL						
Baseline	145 ± 11	161 ± 11	159 ± 14	162 ± 12	−40.3 ± 7.5	−2.3 ± 7.5
Week 6	135 ± 10	115 ± 11*	152 ± 16	106 ± 10*	(0.001)	(NS)
LDL						
Baseline	2263 ± 140	2253 ± 139	2144 ± 134	2285 ± 186	−949 ± 76	109 ± 76
Week 6	2199 ± 118	1263 ± 77*	2252 ± 155	1372 ± 126*	(0.001)	(NS)
Production rate (mg · Kg^{-1} · day^{-1})						
VLDL						
Baseline	16.2 ± 2.0	12.8 ± 1.7	14.8 ± 2.3	14.7 ± 2.1	−0.86 ± 1.66	−3.3 ± 1.6
Week 6	15.7 ± 2.5	13.8 ± 2.0	10.1 ± 1.2*	8.8 ± 1.1*	(NS)	(0.002)
IDL						
Baseline	8.2 ± 1.2	5.5 ± 0.7	5.6 ± 0.7	6.8 ± 0.6	−0.29 ± 0.58	−0.28 ± 0.57
Week 6	7.2 ± 0.9	5.8 ± 0.8	5.8 ± 0.7	5.8 ± 0.5	(NS)	(NS)
LDL						
Baseline	6.6 ± 0.5	4.8 ± 0.5	5.4 ± 0.8	5.8 ± 0.6	−0.15 ± 0.70	0.66 ± 0.69
Week 6	6.1 ± 0.5	5.8 ± 0.7	6.8 ± 0.8	6.4 ± 0.6	(NS)	(NS)
FC Rate (pools/day)						
VLDL						
Baseline	4.3 ± 0.4	3.8 ± 0.3	4.5 ± 0.5	3.7 ± 0.3	2.2 ± 0.6	−0.18 ± 0.56
Week 6	4.5 ± 0.4	6.3 ± 0.8*	4.3 ± 0.3	6.2 ± 0.8*	(0.001)	(NS)
IDL						
Baseline	5.3 ± 0.6	3.7 ± 0.3	3.9 ± 0.5	4.2 ± 0.2	1.5 ± 0.4	0.57 ± 0.44
Week 6	5.0 ± 0.7	5.0 ± 0.3*	4.3 ± 0.4	6.2 ± 0.7*	(0.002)	(NS)
LDL						
Baseline	0.29 ± 0.02	0.25 ± 0.03	0.27 ± 0.04	0.28 ± 0.04	0.22 ± 0.04	0.02 ± 0.04
Week 6	0.27 ± 0.02	0.50 ± 0.06*	0.32 ± 0.03	0.50 ± 0.05*	(0.001)	(NS)
Lipoprotein conversion (%)						
VLDL to IDL						
Baseline	44 ± 7	37 ± 6	29 ± 4	42 ± 5	1 ± 5	16 ± 5
Week 6	38 ± 6	32 ± 3	44 ± 5*	55 ± 6*	(NS)	(0.002)
VLDL to LDL						
Baseline	34 ± 7	27 ± 4	23 ± 4	30 ± 5	4 ± 4	16 ± 4
Week 6	27 ± 4	28 ± 3	39 ± 5*	47 ± 6*	(NS)	(0.001)
IDL to LDL						
Baseline	75 ± 7	78 ± 7	82 ± 8	77 ± 10	3 ± 4	6 ± 4
Week 6	78 ± 6	88 ± 4	93 ± 3*	87 ± 5*	(NS)	(0.043)

Data are means ± SE. General linear modeling was used to assess the main and interactive effects of atorvastatin and fish oil treatments. NS, not significant. There were no significant interactive effects among the treatments; *$P < 0.01$ for *t* test comparison with placebo group.

decreases VLDL-apoB secretion and increases the proportion of VLDL particles converted to LDL. Because fish oil treatment did not alter insulin sensitivity in the present study, it is possible that insulin resistance downregulated the expression and activity of LDL receptors, leading to a persistent suppression of the FCRs of apoB. Hence our findings suggest that the shift in apoB trafficking could be related to a reduction in the VLDL particle number with fish oil supplementation.

Effect of atorvastatin on apoB metabolism. Increased de novo cholesterol synthesis is associated with increased hepatic VLDL-apoB secretion (9). We have previously reported in normolipidemic and familial hypercholesterolemic subjects that statins decrease cholesterogenesis and hepatic secretion of VLDL-apoB (14,15). In the present study, our findings demonstrated that atorvastatin substantially inhibits cholesterol synthesis, as reflected by plasma lathosterol concentration. However, reduction in hepatic cholesterol availability by atorvastatin did not result in decreased VLDL-apoB secretion, nor in the direct secretion of IDL or LDL from the liver (data not shown). Given that atorvastatin did not alter insulin sensitivity, one could argue that insulin resistance was still driving the overproduction of apoB (2). In the absence of a significant change in plasma fatty acids, insulin resistance may increase the influx of free fatty acids to the liver, thereby driving triglyceride synthesis and hepatic secretion of VLDL. Hence, in the setting of insulin resistance, our results suggest that the availability of hepatic triglycerides, rather than cholesterol, plays a significant role in regulat-

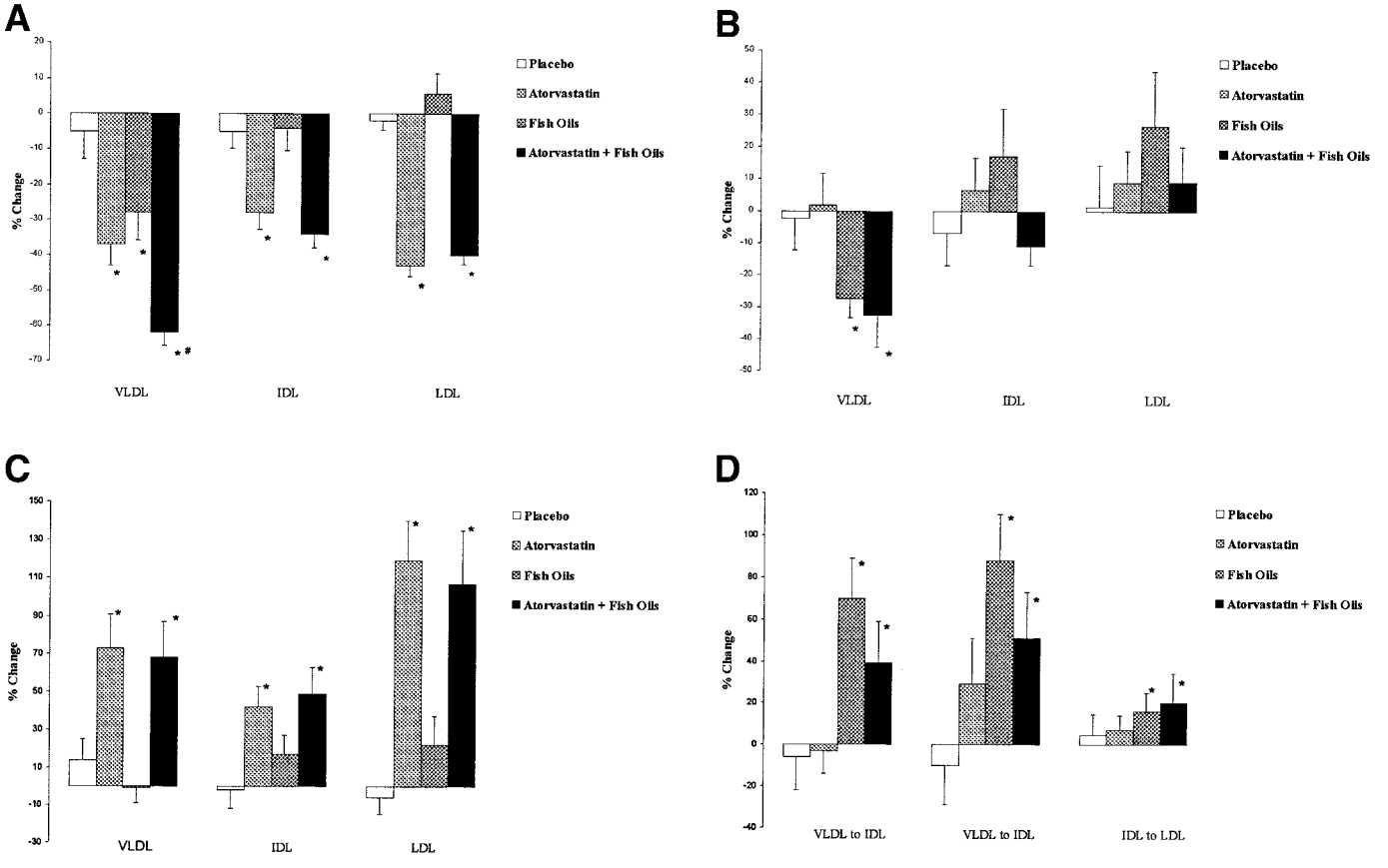

FIG. 3. Percentage change in apoB pool sizes (*A*), secretion rate (*B*), FCR (*C*), and conversion (*D*) in VLDL, IDL, and LDL on placebo, atorvastatin, fish oils, and atorvastatin plus fish oils, respectively. *$P < 0.01$ compared with the placebo group; **$P < 0.05$ compared with the atorvastatin or fish oil groups.

ing VLDL-apoB secretion**.** The unaltered HOMA scores suggest that these regulatory processes occur in the absence of a change in hepatic glucose metabolism. LDL-receptor independent uptake of cholesterol may influence apoB secretion (9) and could potentially contribute to a decrease in apoB secretion. However, our results suggest that this mechanism may also be overridden by an effect of hepatic insulin resistance on lipid metabolism.

Given the central role of the LDL receptor in the removal of all apoB-containing lipoproteins by the liver (13), one might expect that clearance of all apoB-containing lipoproteins would be enhanced with statin treatment. This concept is supported by our observations that the plasma pool sizes of all major apoB-containing lipoproteins decreased after atorvastatin, in association with increases in their FCRs. Our finding of increased VLDL- and IDL-apoB FCRs with atorvastatin is consistent with effects of statins in upregulating LDL receptor and LPL activities and inhibiting expression of apoC-III (42). Statins may also inhibit inflammatory cytokines and stimulate PPAR-α activity (43,44), which could contribute to improvement in apoB metabolism, but these mechanisms require further investigation.

Effect of atorvastatin plus fish oils on apoB metabolism. Our findings demonstrated that the combination of atorvastatin and fish oils effectively normalized the kinetic abnormalities in apoB metabolism. Our statistical analyses showed that the improvements resulted from the additive effects of combination treatment on VLDL-, IDL-, and LDL-apoB kinetics. Consistent with this finding, our results demonstrated further reductions in plasma triglycerides and VLDL-apoB pool size with combination treatment than with either of the monotherapies. Despite increased conversion of VLDL to IDL or LDL with combination treatment, the pool sizes of IDL- and LDL-apoB fell significantly by –35 and –40%, respectively. This suggests that the increased flux of apoB from VLDL to IDL or LDL was overcome by enhanced hepatic clearance of these lipoproteins. This mechanism may be related to activation of LDL receptor-mediated pathways by atorvastatin.

Limitations. A major assumption of the present study was that atorvastatin and fish oils exclusively decrease de novo synthesis of cholesterol and triglycerides, respectively. Statins may also inhibit cholesterol esterification rate and transcription of apoB cDNA and gene products required for VLDL-apoB assembly and secretion (35). Fish oils may also directly enhance intracellular degradation of apoB before secretion. In hamster hepatocytes, fish oils can perturb initial lipidation of VLDL precursor particles in the rough endoplasmic reticulum and thus target apoB for degradation (45). Therefore, the inhibitory mechanisms of atorvastatin and fish oils may operate at different stages in VLDL assembly and secretion, but this requires further investigation. However, we cannot exclude the possibility that in this study, atorvastatin and fish oils regulated apoB metabolism by these mechanisms.

Statins and fish oils have been shown to affect insulin sensitivity (46,47). In this study, changes in insulin sensi-

tivity could have been measured with a euglycemic clamp. HOMA scores, however, are well correlated with this technique and reflect hepatic insulin resistance with respect to glucose metabolism (48). Our isolation of apoB fractions could include a small portion of apoB-48 particles. However, because our subjects fasted for at least 14 h, it is unlikely that this would significantly confound our findings. Despite our using a factorial design to increase the power of our study, we cannot strictly exclude the possibility that our sample size was not sufficient to demonstrate that atorvastatin might have had a small but significant impact in decreasing hepatic apoB secretion in our patients. Examination of the effect of statins and fish oils on free fatty acid and triglyceride kinetics may further help to clarify the precise contribution of triglyceride and cholesterol availability on apoB metabolism. Because insulin sensitivity was not altered, further studies should examine the incremental effect of weight reduction or insulin sensitizers together with a statin or fish oil regimen on apoB metabolism in dyslipidemic, insulin-resistant subjects.

Implications and conclusions. The increased cardiovascular morbidity and mortality observed in human obesity may be caused by insulin resistance and dyslipidemia. Considering the key role of dyslipidemia in the development of CVD, we suggest that the improvement in apoB metabolism by either atorvastatin or fish oils may have clinical significance, consistent with evidence that these agents decrease the risk of coronary disease. Statins have been shown to reduce cardiovascular events in subjects with diabetes and insulin resistance (12). Our study provides the kinetic bases for this event by mechanisms involving regulation of lipid metabolism. Fish oils also reduced cardiovascular events in subjects with coronary disease (17), but the effects on events have not been studied in insulin-resistant or diabetic subjects. More importantly, our data suggest that the combination of statin and fish oils, but not treatment with one of these alone, normalizes all metabolic parameters of apoB without any significant change in insulin sensitivity or body weight. This suggests that this combination treatment may have achieved an optimal effect in treating insulin-resistant subjects with dyslipidemia; whether this treatment improves clinical outcomes remains to be fully demonstrated in clinical trials. The change in plasma HDL cholesterol levels also suggests that the benefits of this treatment may extend to reversing cholesterol transport. Two previous studies (43,49) have found that statin plus fish oil treatment may improve cardiovascular risk by nonlipid pleitropic mechanisms. Those results, taken together with the finding of the present study, provide a rationale for a clinical trial to assess the efficacy of statin plus fish oils in the prevention of CVD in subjects with insulin resistance and type 2 diabetes.

In conclusion, the results of the present study indicated that in obese subjects with insulin resistance and dyslipidemia, atorvastatin increased hepatic clearance of all apoB-containing lipoproteins, whereas fish oils decreased hepatic secretion of VLDL-apoB. Although these agents individually improved apoB metabolism, when used in combination they attained the best overall improvement in apoB metabolism in these subjects.

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