Effect of atorvastatin on chylomicron remnant metabolism in visceral obesity: a study employing a new stable isotope breath test

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Abstract Elevated plasma concentration of chylomicron remnants may be causally related to atherosclerosis in obesity. We examined the effect of atorvastatin on chylomicron remnant metabolism in 25 obese men with dyslipidaemia. A remnant-like emulsion labeled with cholesteryl [^{13}C]oleate was injected intravenously into patients; the fractional catabolic rate (FCR) of the remnant-like emulsion was determined by measurement of $^{13}CO_2$ in the breath and analyzed using compartmental modelling. Compared with placebo, atorvastatin significantly decreased the plasma concentrations of total cholesterol, triglycerides, LDL cholesterol, apolipoprotein B (apoB), and lathosterol ($P < 0.001$). ApoB-48 and remnant-like particle-cholesterol (RLP-C) both decreased significantly by 23% ($P = 0.002$) and 33% ($P = 0.045$), respectively. The FCR of the remnant-like emulsion increased significantly from 0.054 ± 0.008 to 0.090 ± 0.010 pools/h ($P = 0.002$). The decrease in RLP-C was associated with the decrease in plasma triglycerides ($r = 0.750$, $P = 0.003$). Furthermore, the change in FCR of remnant-like emulsions was inversely associated with the change in LDL-C ($r = -0.575$, $P = 0.040$), suggesting removal of LDL and chylomicron remnants by similar hepatic receptor pathways. We conclude that in obese subjects, inhibition of cholesterol synthesis with atorvastatin decreases the plasma concentrations of both LDL-C and triglyceride-rich remnants and that this may be partially due to an enhancement in hepatic clearance of these lipoproteins.—Chan, D. C., G. F. Watts, P. H. R. Barrett, I. J. Martins, A. P. James, J. C. L. Mamo, T. A. Mori, and T. G. Redgrave. **Effect of atorvastatin on chylomicron remnant metabolism in visceral obesity: a study employing a new stable isotope breath test.** ***J. Lipid Res.*** **2002.** 43: **706–712.**

Supplementary key words HMG-CoA reductase inhibitor • dyslipidaemia • triglyceride-rich lipoproteins

The precise reason for increased risk of cardiovascular disease in visceral obesity remains unclear, but may relate to dyslipoproteinaemia (1). Hypertriglyceridaemia is the most consistent lipid disorder in visceral obesity and may reflect elevation in the plasma concentrations of triglyceride-rich lipoproteins (TRLs) and chylomicron remnants. Increasing evidence suggests the postprandial accumulation of chylomicron remnants in plasma is atherogenic and may contribute to the development of atherosclerosis (2–4). This defect may be due to oversecretion of intestinally derived apolipoprotein B-48 (apoB-48) containing particles and/or to reduction in the clearance of these lipoprotein remnants, but this remains to be further investigated.

While a hypocaloric diet and other lifestyle modifications in obese subjects may achieve significant weight loss and correct lipoprotein metabolism (5), including postprandial dyslipidaemia, adherence to such programs is generally unsuccessful (6). Effective management of dyslipidaemia in obesity may therefore often require the use of lipid-regulating pharmacotherapy (7, 8). HMG-CoA reductase inhibitors have been shown to be effective in treating dyslipidaemia (9), but their effect and mechanism of action on chylomicron remnant metabolism in subjects with visceral obesity and insulin resistance is not fully established. Because de novo cholesterol synthesis is co-regulated with the expression of the LDL receptor (10), which may in turn clear chylomicron remnants, statins, by inhibiting cholesterol synthesis, may decrease plasma chylomicron remnant concentrations by increasing their fractional catabolism.

Standard methods for assessing the kinetics of chylomicron remnant metabolism involve measurement of the response of plasma triglycerides, retinyl esters, or apoB-48 to an oral fat load (2, 11). ApoB-48 and remnant-like particle (RLP) cholesterol are static markers for the metabolism of chylomicron and their remnants (12, 13). A single fasting plasma level of apoB-48 may also predict the kinetic re-

Abbreviations: apo, apolipoprotein; FCR, fractional catabolic rate; HOMA, homeostasis model assessment; RLP, remnant-like particle.

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sponse of retinyl esters and apoB-48 to a fat load (14). However, none of these tests provides specific information relating to chylomicron remnant kinetics or metabolism. We have previously described and validated a novel breath test based on the injection of a remnant-like emulsion labeled with cholesteryl [^{13}C]oleate and subsequent measurement of $^{13}CO_2$ enrichment in breath (15) that provides a specific functional assessment of chylomicron remnant metabolism in human (16, 17) and animal studies (18, 19).

In this study, we undertook a controlled trial to examine the effect of an HMG-CoA reductase inhibitor (atorvastatin) on chylomicron remnant metabolism in viscerally obese men with dyslipidaemia based on the the kinetics of chylomicron remnant-like emulsions measured with the breath test, and measurements of fasting apoB-48 and RLP cholesterol.

MATERIALS AND METHODS

Subjects

Twenty-five obese men (body mass index >29 kg/m^2, waist circumference >100 cm, waist-hip ratio >0.97) with dyslipidaemia at screening (plasma triglycerides >1.2 mmol/l and cholesterol >5.2 mmol/l) while consuming ad libitum, weight-maintenance diets were recruited from the community. None of the subjects had diabetes mellitus (excluded by oral glucose tolerance test), apolipoprotein E2/E2 genotype, macroproteinuria, creatinaemia (>120 μmol/l), hypothyroidism, or abnormal liver and muscle enzymes, or consumed more than 30 g alcohol/day. None reported a history of cardiovascular disease, FH, or were taking medication or other agents known to affect lipid metabolism. All subjects provided written consent and the study was approved by the Ethics Committee of the Royal Perth Hospital.

Study design

The study reported herein represents a substudy of a larger randomized, doubled-blind, placebo-controlled intervention trial examining the effects of atorvastatin and other interventions on aspects of lipoprotein metabolism in obesity, which will be reported separately. Eligible patients entered a 3 week run-in diet-stabilizing period, at the end of which they were randomized to a 6 week treatment period of either atorvastatin (40 mg orally at night) or matching placebo. All subjects were advised to continue their habitual isocaloric diet, and to keep physical exercise constant. Compliance with atorvastatin and placebo was checked by tablet count at week 3 and 6 treatment periods.

Clinical protocol

All subjects were admitted to the metabolic ward in the morning after a 14 h fast. They were studied in a semi-recumbent position and allowed water only. Venous blood was collected for measurements of biochemical analyses. Arterial blood pressure was recorded after 3 min in the supine position using a Dinamap 1846 SX/P monitor (Critikon Inc, Tampa, FL).

The sterile isotopically labeled chylomicron remnant-like emulsion (14 ml) was injected intravenously into an antecubital vein via a 21G butterfly needle. End-expiratory breath samples were collected into a vacutainer (Labco Ltd) at baseline and post-injection every 10 min for the first hour, every 20 min for the second hour, every 30 min for the next 5 h and hourly for another 3 h. Subjects were then given a snack and allowed to go home. Two additional breath samples were collected on the following days (24 h). During the first 10 h of collection of breath samples subjects sat quietly in a chair and were allowed to drink only water. After leaving the metabolic ward, they were requested to refrain from vigorous activity and to eat a light supper before retiring.

Dietary analysis

Dietary intake was assessed for energy and major nutrients using at least two 24 h dietary diaries. Diets were subsequently analyzed using DIET 4 Nutrient Calculation Software (Xyris Software, Queensland, Australia). Dietary intake, alcohol, and exercise diaries were also completed at baseline and at the end of the study.

Preparation of stable isotope-labeled remnant-like emulsions

This was carried out as described previously (16). Briefly, pure lipid mixtures containing triolein (135 mg), phosphatidylcholine (75 mg), cholesteryl [^{13}C]oleate (70 mg), and cholesterol (24 mg) were emulsified by sonication for 1 h in 2.2% glycerol in water. After sonication the mixture was centrifuged for 10 min to remove titanium fragments and then filtered into sterile vessels. All emulsion preparations were confirmed to be sterile and pyrogen-free (Pharmacy Dept., Royal Perth Hospital). Uniformly labeled [^{13}C]oleate was purchased from Novachem Pty. Ltd., Victoria, Australia, and cholesteryl [^{13}C]oleate was synthesized from cholesterol and [^{13}C]oleic acids as described previously (15).

Biochemical analyses

Plasma triglyceride and cholesterol concentrations were determined by standard enzymatic methods using a Hitachi 917 Biochemical Analyser (Hitachi Ltd, Tokyo, Japan). HDL cholesterol (HDL-C) was measured by an enzymatic colorimetric method using a commercial kit (Boehringer Mannheim, Mannheim, Germany). LDL-C was calculated using the Friedewald equation. ApoB and apoA-I were determined by immuno-nephelometry. ApoB-48 was measured as described previously (14) with the exception that apoB-48 was assayed directly from plasma rather than 1.063 g/ml lipoprotein fraction. Values obtained using either method are identical (J. C. L. Mamo and A. P. James, unpublished observation). Plasma RLP-C was determined with a JIMRO-II (Japan Immunoresearch Laboratories, Takasaki, Japan) assay kit using an immunoseparation method described by Nakajima et al. (20). Plasma non-esterified fatty acids (NEFAs) were measured by an enzymatic, colorimetric method using a commercial kit (Randox, Co., Antrim, UK). Plasma insulin was measured by radioimmunoassay (DiaSorini s.r.l., Saluggia, Italy) and glucose concentration by a hexokinase method on a Hitachi 917 analyser. Insulin resistance was estimated by the homeostasis model assessment (HOMA) that employs the formula: fasting insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5, as described by Matthews et al. (21). Plasma lathosterol concentration and apoE genotype were determined as described elsewhere (22). CETP activity was determined using a CETP Activity Kit (Roar Biomedical Inc.): briefly, 10 μl of plasma was diluted (1:10) in 90 μl of sample buffer; 20 μl of the diluted plasma was combined with 4 μl of donor (synthetic phospholipid and cholesteryl ester) and 4 μl of VLDL acceptor particle to a total volume of 200 μl and incubated for 3 h at 37°C in a fluorescence-compatible microtiter plate; the fluorescence intensity was then read at excitation wavelength of 465 nm and emission wavelength of 535 nm in a fluorescence spectrometer. Plasma liver (alanine transferase, asparate transferase, alkaline phosphatase) and muscle (creatinine kinase) enzymes were measured at the beginning and end of the study. Interassay CV of all measurements were less than 6%. The expired CO_2 in the exhaled breath samples was analyzed at each time point by isotope ratio mass

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spectroscopy using a Finnigan BreathPlus machine (Thermoquest Systems Pty. Ltd, Sydney, Australia). The ratio of $^{13}CO_2$/$^{12}CO_2$ was referenced to Peedeebelimnite standard values and the delta unit value was calculated using the Breathmat software. The delta units reference a sample of limestone, a standard in the 13-carbon isotope ratio field, and basal (non-enriched) values correspond approximately to 1% 13 carbon (16).

Kinetic analysis

A compartment model describing the appearance of labeled CO_2 in breath was developed using the SAAM II program (SAAM Institute, Seattle, WA, USA). The model was developed assuming that the fractional rate constants [k(i,j)] were time invariant and first order. The model used to fit the tracer data is shown in **Fig. 1**. The differential equations describing this model are shown below:

$$\frac{dq1}{dt} = -k(2,1)q1 - k(3,1)q1 + ex1$$

$$\frac{dq2}{dt} = -k(0,2)q2 + k(2,1)q1 + k(2,3)q3s$$

$$\frac{dq3s}{dt} = -k(2,3)q3s + k(3,1)q1$$

$$q3(t) = \Sigma q3s(t)$$

$$FCR = k(3,1) + k(2,1)$$

where, q_i is the tracer mass in each compartment; k(i,j) is the fractional transfer rate to compartment i from compartment j; $ex1$ is the input of tracer into compartment 1; $q3(t)$ is the mass of tracer in compartment 3 (the delay compartment) at time t and is the sum of the subcompartments $q3s(t)$ that provide the delay function. The average delay time provided by compartment 3 is equivalent to the product of the number of delay subcompartments and the inverse of k(2,3). As shown above, the fractional catabolic rate (FCR) of the labeled emulsion from compartment 1 was determined as the sum of the two rate constants out of this compartment, k(3,1) and k(2,1).

Compartment 1 of the model represents the plasma compartment into which the labeled remnant-like emulsion is injected. Material in compartment 1 can be lost from plasma to compartments 2 or 3. Compartment 2 represents labeled CO_2 in breath and is sampled during the course of the study. The primary pathway for clearance of emulsion and appearance of labeled CO_2 is via compartment 3. This compartment may include the hepatic and extrahepatic processes associated with uptake, hydrolysis, and oxidation of the labeled oleate.

The compartment model was fitted to the observed $^{13}CO_2$ breath data to provide estimates of the model rate constants and FCR. By using measures of model order, such as the Akaike information criterion (23), the model structure shown in Fig. 1 was the simplest model that was consistent with the tracer data. In the absence of additional data, a more complex model, including hepatic compartmentalization, could not be included in the present model structure.

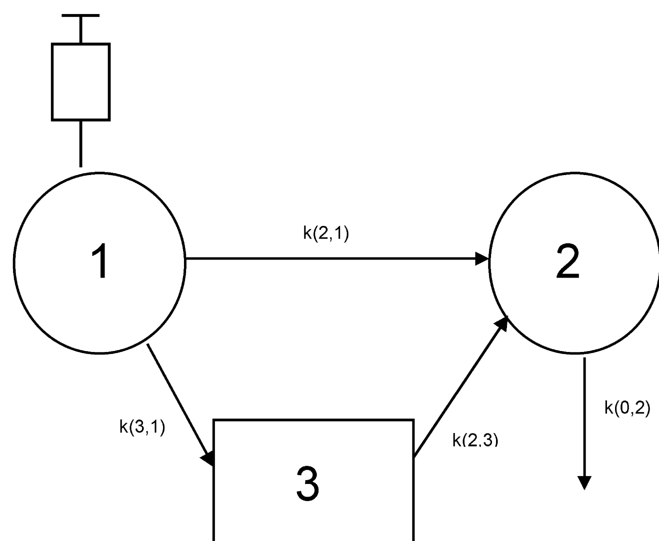

Fig. 1. Three-compartment model employed to fit the breath appearance of $^{13}CO_2$ following injection of cholesteyl [^{13}C]oleate labeled chylomicron remnant-like emulsion.

Statistical analysis

All analyses were carried out using SPSS 10.1 (SPSS, Inc., Chicago, IL). Skewed variables were examined after logarithmic transformation. Differences were tested using one-sample and two-sample t-tests. Associations were examined by linear regression methods. Statistical significance was defined at the 5% level using a two-tailed test.

RESULTS

There were no significant differences between the two groups for any of the clinical characteristics of the 25 patients studied at baseline (**Table 1**). On average, the men were middled-aged, obese, and normotensive. Although no patients had diabetes, the HOMA score indicated that they were as a whole, insulin resistant compared with lean controls (22). Eighteen of the men were E3/E3 homozygotes, one was E2/E3 heterozygote, five were E3/E4 heterozygotes, and one was E4/E4 homozygote. There were no statistically significant differences in the frequency distribution of E-alleles between the groups. Compliance with atorvastatin and placebo tablets was >95%, and none of the subjects reported untoward clinical side effects or showed significant increase in liver or muscle enzymes.

Table 2 shows the plasma lipid, lipoprotein, apolipoprotein, and lathosterol concentrations before and after treatment in the atorvastatin and placebo groups. There were no significant differences between the two groups for any of the biochemical variables at baseline. Compared with placebo, there was a significant decrease with atorvastatin in the plasma concentrations of total cholesterol (−38%), triglycerides (−26%), LDL-C (−52%), RLP-C (−33%), apoB (−43%), apoB-48 (−24%), and lathosterol (−82%);

TABLE 1. Clinical and biochemical characteristics of subjects at baseline

	Atorvastatin (n = 13)	Placebo (n = 12)
Age, years	51.7 ± 9.7	50.6 ± 9.3
Body weight, kg	107 ± 18	100 ± 8
Body mass index, kg/m^2	34.5 ± 5.2	32.2 ± 2.7
Waist:hip ratio	1.04 ± 0.07	1.00 ± 0.04
Systolic blood pressure, mmHg	132 ± 19	128 ± 11
Diastolic blood pressure, mmHg	79.2 ± 11.9	77.8 ± 9.7
Fasting NEFAs, mmol/l	0.29 ± 0.13	0.26 ± 0.10
Fasting glucose, mmol/l	5.57 ± 0.57	5.35 ± 0.54
Fasting insulin, mU/l	32.5 ± 12.7	32.1 ± 8.6
Insulin resistance, HOMA score	8.12 ± 3.65	7.71 ± 2.56

Values are mean ± SD.

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TABLE 2. Changes in plasma lipids, lipoproteins, apolipoproteins, lathosterol, and CETP activity in obese patients after 6 week treatment with atorvastatin and placebo

	Atorvastatin		Placebo		Treatment Effect	
	Baseline	Week 6	Baseline	Week 6	Mean (95% CI)	P*
Total cholesterol, mmol/l	5.81 ± 0.17	3.62 ± 0.13	5.82 ± 0.14	5.62 ± 0.13	−1.99 (−2.32, −1.67)	0.001
Triglyceride, mmol/l	1.88 ± 0.13	1.40 ± 0.12	1.69 ± 0.18	1.57 ± 0.15	−0.37 (−0.56, −0.18)	0.001
HDL-cholesterol, mmol/l	1.01 ± 0.05	1.04 ± 0.05	1.05 ± 0.06	1.03 ± 0.06	0.05 (−0.04, 0.13)	0.262
LDL-cholesterol, mmol/l	3.81 ± 0.16	1.84 ± 0.12	3.80 ± 0.13	3.83 ± 0.11	−1.99 (−2.30, −1.69)	0.001
Non-HDL- cholesterol, mmol/l	4.81 ± 0.18	2.58 ± 0.11	4.77 ± 0.12	4.58 ± 0.11	−2.04 (−2.34, −1.74)	0.001
RLP-cholesterol, mmol/l	0.43 ± 0.07	0.29 ± 0.08	0.35 ± 0.04	0.32 ± 0.04	−0.12 (−0.23, −0.03)	0.045
Apolipoprotein A-I, mg/dl	119 ± 5	123 ± 4	129 ± 5	126 ± 4	6.23 (−2.53, 15)	0.155
Apolipoprotein B, mg/dl	123 ± 6	68.7 ± 0.3	129 ± 4	123 ± 3	−47 (−58, −37)	0.001
Apolipoprotein B-48, mg/l	23.2 ± 1.9	17.7 ± 2.1	22.5 ± 2.0	21.9 ± 2.4	−4.94 (−7.83, −2.05)	0.002
Lathosterol, μmol/l	10.8 ± 1.2	1.86 ± 0.26	9.33 ± 1.03	9.65 ± 0.72	−9.29 (−12, −6.47)	0.001
CETP activity, nmol/l/h	94.2 ± 10.6	83.8 ± 8.9	117.8 ± 7.3	102.7 ± 7.3	5.124 (−25.2, 14.9)	0.602

Values are mean ± SEM; CI, confidence interval.
* *P* value for comparison of change in atorvastatin group versus change in placebo group.

HDL-C and apoA-I increased by 4% and 3%, respectively, but these changes failed to reach statistical significance. There was no significant change in plasma CETP activity with atorvastatin compared with the placebo group. The post-intervention levels of plasma apoB-48 and RLP-C remained elevated compared with a reference, normolipidaemic population (24).

Body weight, waist-hip ratio, blood pressure, plasma NEFAs, glucose, insulin, and HOMA score did not alter significantly compared with baseline levels in the atorvastatin and placebo groups (data not shown). Dietary intake of carbohydrate, fat, protein, alcohol, and total energy, as well as level of physical activity, did not alter significantly in either of the treatment groups during the study.

Figure 2 shows the enrichment of $^{13}CO_2$ in the breath before and during treatment with atorvastatin over time following injection of the emulsion. In both groups there was a rapid increase in enrichment of $^{13}CO_2$ with a peak at approximately 6 h, reflecting the catabolism of remnant-like particles via lipolysis and hepatic removal, followed by a slow decline period over the following 19 h. Although enrichment curves were of similar contour, peak enrichment in the atorvastatin group was higher and maximum enrichment was attained earlier. **Table 3** shows the model rate constants and FCR for the labeled chylomicron remnants in the two groups. Relative to placebo, there were no significant changes in $k(0,2)$ and $k(2,3)$, but $k(2,1)$, $k(3,1)$, and FCR increased significantly after atorvastatin treatment. The FCR during treatment with atorvastatin was also lower compared with a reference, normolipidaemic population (16, 18).

In the atorvastatin-treated group, there was a statistically significant inverse association between the change in FCR of the remnant-like emulsions and the change in plasma LDL-C concentration ($P = -0.575$, $P = 0.040$), but no significant associations were found with the other variables. The decrease in plasma RLP-C was also significantly associated with the decrease in plasma triglycerides ($r = 0.750$, $P = 0.003$), but not with other variables. There was no significant association between the change in

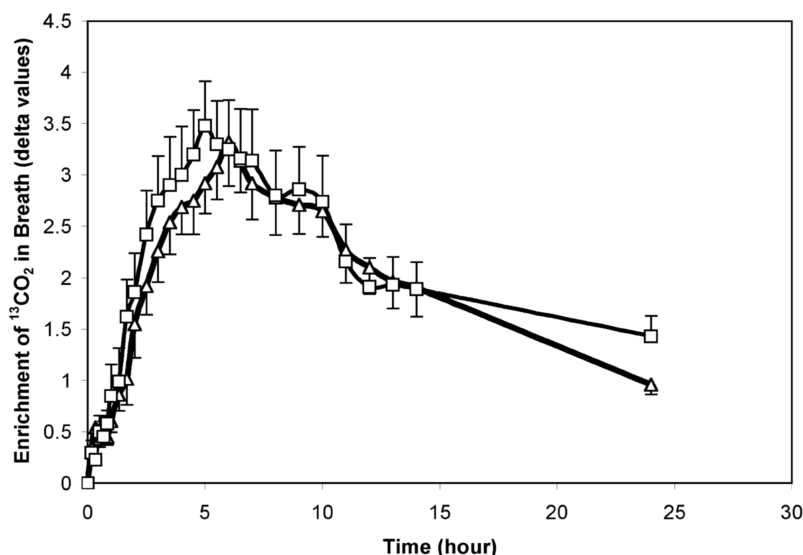

Fig. 2. Appearance of $^{13}CO_2$ in the breath (mean ± SEM) over time following injection of the chylomicron remnant emulsion before (open triangle) and after (open square) 6 week atorvastatin treatment.

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TABLE 3. Changes in model rate constants and fractional catabolic rate (FCR) for the injected chylomicron remnant-like emulsion in obese patients after 6 weeks treatment atorvastatin and placebo

	Atorvastatin		Placebo		Treatment Effect	
	Baseline	Week 6	Baseline	Week 6	Mean (95% CI)	P*
k(2,1), pools/h	0.015 ± 0.003	0.025 ± 0.005	0.015 ± 0.004	0.012 ± 0.003	0.014 (0.001, 0.028)	0.047
k(3,1), pools/h	0.039 ± 0.006	0.064 ± 0.008	0.059 ± 0.007	0.048 ± 0.009	0.030 (0.007, 0.054)	0.013
k(2,3), pools/h	6.52 ± 1.00	5.92 ± 1.16	7.20 ± 1.19	7.85 ± 1.11	−1.17 (−5.45, 3.11)	0.578
k(0,2), pools/h	0.95 ± 0.19	1.20 ± 0.19	1.49 ± 0.30	1.14 ± 0.17	0.59 (−0.24, 1.42)	0.153
FCR, pools/h	0.054 ± 0.008	0.090 ± 0.011	0.074 ± 0.007	0.067 ± 0.009	0.045 (0.018, 0.072)	0.002

Values are mean ± SEM; CI, confidence interval.
* P value for comparison of change in atorvastatin group versus change in placebo group.

plasma apoB-48 and changes in RLP-C, FCR, or other variables.

DISCUSSION

In this double-blind, randomized, placebo controlled trial we provide new information about the effect of atorvastatin on chylomicron remnant metabolism employing a stable isotope breath test in subjects with visceral obesity and dyslipidaemia. Our results demonstrate that inhibition of cholesterol synthesis with the HMG-CoA reductase inhibitor atorvastatin decreases the concentration of TRLs, as reflected by a decrease in plasma concentrations of apoB-48 and RLP-C. This was also associated with increased catabolism of chylomicron remnants as reflected by the increase in FCR of remnant-like emulsions. These effects of atorvastatin were achieved with no significant alterations in plasma NEFAs, insulin resistance, CETP activity, or body weight.

The effects of HMG-CoA reductase inhibitors on intestinally-derived triglyceride-rich lipoprotein metabolism have been examined previously (25–29). In two uncontrolled trials, retinyl palmitate was used as a postprandial marker. Cabezas et al. (25) reported in patients with familial combined hyperlipidaemia that simvastatin improved postprandial chylomicron remnant clearance. Cianflone et al. (26) also reported increased remnant clearance with lovastatin in normotriglyceridaemic subjects. However, the reliability of retinyl esters as a postprandial marker is questionable (2, 30). The rationale for its use as a postprandial marker is based on the assumption that the retinyl esters remain associated with the chylomicron remnant. However, Krasinski et al. (30) found that about one third of the postprandial retinyl esters associated with HDL and LDL. In another two uncontrolled trials using apoB-48 as a postprandial maker, Parhofer et al. (27) found that chylomicron remnant-apoB-48 clearance was increased with atorvastatin in normolipidaemic subjects. Battula et al. (28) found in Type 2 diabetes that cerivastatin improved postprandial response of chylomicron apoB-48. However, in these two trials they failed to detect a decrease in fasting plasma apoB-48 level with statin treatment. Using a radioactive labeled chylomicron-like emulsion, Santos et al. (29) recently reported in patients with coronary artery diseases pravastatin increased chylomicron remnant clearance. Not all studies suggest that statins universally improve postprandial lipidaemia (31), although the clearance of chylomicron remnants has not been specifically examined in studies reporting no treatment effects. We have extended the aforementioned studies by investigating the effect of atorvastatin on chylomicron remnant metabolism in obesity employing a stable isotope remnant-like emulsion and measurements of fasting plasma apoB-48 and RLP-C. Our data are consistent with the findings that atorvastatin increases catabolism of chylomicron remnants in obese patients as reflected by the increase in FCR of remnant-like emulsions. Although we did not examine postprandial response of apoB-48, we would expect atorvastatin would have attenuated postprandial apoB-48 response based on our previous findings that the fasting apoB-48 predicts the postprandial response (14).

That visceral obesity is associated with disturbances in lipoprotein metabolism is well recognized (1, 32, 33). As seen in our patients, the principal abnormalities include elevation in plasma TRLs and to a lesser extent LDL-C, with a reciprocal decrease in HDL-C. Elevated fasting levels and postprandial responses of apoB-48 and apoB-100 containing TRLs have been shown in viscerally obese subjects, and these may be partially related to insulin resistance (34). Insulin resistance has two potential effects on chylomicron remnant metabolism. First, it down-regulates LDL receptor expression and activity (35). Second, it increases hepatic de novo cholesterol synthesis and subsequently VLDL apoB-100 secretion (5, 36). These effects would result in increased competition for hepatic receptors between chylomicron and VLDL remnants, thereby disturbing or delaying the uptake of chylomicron remnants by this pathway. As recently emphasized, the binding of apoE on the remnant particle to the hepatic LDL/E receptor is central to the disposal of chylomicron remnants (37). Compared with a reference normolipidaemic population (17, 24), plasma apoB-48 and RLP-C levels were markedly elevated and the FCR of remnant-like emulsion was lower in our obese patients. This reflects delayed chylomicron remnant clearance in these obese subjects with dyslipidaemia and insulin resistance.

HMG-CoA reductase inhibitors have been shown to decrease de novo cholesterol synthesis, thereby upregulating LDL receptor activity and decreasing hepatic secretion of apoB containing lipoproteins. If there was an increased LDL receptor activity and decreased competition with apoB-100 containing lipoproteins for receptor-mediated

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clearance pathway, the clearance of chylomicron remnants following atorvastatin would be subsequently enhanced. Our findings show that in the setting of insulin resistance, inhibition of cholesterol synthesis with atorvastatin, as reflected by a significant decrease in plasma lathosterol, does improve these abnormalities.

Our findings demonstrated there was a greater reduction on atorvastatin in plasma RLP-C (−33%) than in apoB-48 (−23%) levels, and the decrease in plasma triglyceride in our study was associated with RLP-C but not with apoB-48. This may be due to several reasons. The decrease in plasma apoB-48 and RLP-C levels may reflect a reduction in chylomicron remnant number and size. Plasma apoB-48 level exclusively reflects intestinal-derived chylomicrons and their remnants, whereas RLP-C constitutes a spectrum of intestinal and hepatic derived triglyceride-rich lipoprotein remnants (20). The action of atorvastatin may be to primarily decrease triglyceride-rich lipoproteins of hepatic origin. Given these factors, we might expect that RLP-C would be more sensitive to the change in TRL metabolism on atorvastatin treatment than apoB-48. However, apoB-48 and RLP-C do not specifically reflect chylomicron remnants. These plasma markers of chylomicron remnant metabolism have considerable kinetic heterogeneity. Hence, the lack of correlation between apoB-48, RLP-C, and the FCR of the emulsion might have been anticipated. The decrease in apoB-48 and RLP-C might also possibly have been due to a decrease in apoB-48 and hepatic VLDL apoB-100 secretions (38). That plasma apoB-48 and RLP-C levels and FCR of the remnant-like particle emulsion did not return to normal following treatment reflects the effects of continuing insulin resistance of TRLs, including chylomicron remnants, as was reviewed previously (34).

The significant inverse association between changes in FCR- and LDL-C also supported the notion that there was a decreased competition for the hepatic LDL/E receptor. The importance of the LDL/E receptor in regulating chylomicron remnant metabolism has been shown in previous studies (18, 37). However, we recently reported that in patients with homozygous or heterozygous FH the FCR of the emulsion was not different from that in normolipidaemic controls (17) and was also not altered by simvastatin treatment (39). The discrepancies with the present report might be due to differences between FH and obese subjects in the expression and functionality of LDL and other receptors that clear chylomicron remnants, as well as to group differences in the response of these receptors to statin therapy. In our study, the significant increase in the rate constants $k(3,1)$ and $k(2,1)$ values reflect a parallel increase in both hepatic and extrahepatic removal of remnant-like emulsions on atorvastatin treatment. Total FCR of remnant-like emulsions, however, predominantly involved an increase in their removal by the liver, as reflected by the relative magnitude of these two rate constants.

Direct measurement of chylomicron remnant metabolism is technically difficult. The traditional methods for accessing chylomicron metabolism lack specificity for distinguishing defects in lipolysis from defects in remnant clearance. The breath test is relatively simple to administer in clinical studies. It provides a specific functional assessment of chylomicron remnant metabolism and has been previously validated in patients with familial dyslipidaemias (16, 17). We standardized conditions for carrying out the breath test by studying volunteers in the postabsorptive state, restricting physical activity, and maintaining habitual dietary intake constant.

The breath test depends not only on the plasma clearance of remnant-like emulsions, but also on variations in respiratory quotient, fatty acid uptake, and oxidation. This might explain why the FCR of the emulsion using this test was of smaller magnitude than that reported by Santos et al., who used direct measurements in plasma to estimate the FCR of chylomicron-like emulsion (29). We cannot exclude the possibility of increased insulin sensitivity and decreased cytosolic triglyceride stores in the liver by atorvastatin would enhance the FCR of remnant-like emulsions. However, there were no significant changes in body weight, HOMA score, or plasma NEFAs on atorvastatin treatment. The confounding influence of CETP also needs to be acknowledged. The absence of significant effect of atorvastatin on plasma CETP activity in this study suggests that the effect of atorvastatin on the FCR of the emulsion is not likely to be mediated by CETP. Also, CETP activity was not correlated with the FCR of the emulsion at baseline (data not shown). However, further in vivo studies of the effect of CETP activity on the breath test are required.

In conclusion, we suggest that in obese subjects inhibition of cholesterol synthesis with atorvastatin improves chylomicron remnant metabolism by increasing chylomicron remnant catabolism. Since elevation of triglyceride-rich remnants is a risk factor for atherosclerosis (40), our results suggest that statins may decrease coronary heart disease risk in obesity not only by improving the kinetics of apoB-100 containing lipoproteins but also by improving chylomicron remnant kinetics. Further studies should examine the additive effect of weight reduction or insulin sensitizers together with a statin in dyslipidaemic obese subjects. Finally, the kinetic bases for other lipoprotein changes also require investigation.

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