Inhibition of ACAT by avasimibe decreases both VLDL and LDL apolipoprotein B production in miniature pigs

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Abstract **An orally bioavailable acyl coenzyme A:cholesterol acyltransferase (ACAT) inhibitor, avasimibe (CI-1011), was used to test the hypothesis that inhibition of cholesterol esterification, in vivo, would reduce hepatic very low density (VLDL) apolipoprotein (apo) B secretion into plasma. ApoB kinetic studies were carried out in 10 control miniature pigs, and in 10 animals treated with avasimibe (10 mg/kg/d, n = 6; 25 mg/kg/d, n = 4). Pigs were fed a diet containing fat (34% of calories) and cholesterol (400 mg/d; 0.1%). Avasimibe decreased the plasma concentrations of total triglyceride, VLDL triglyceride, and VLDL cholesterol by 31–40% 39–48%, and 31–35%, respectively. Significant reductions in plasma total cholesterol (35%) and low density lipoprotein (LDL) cholesterol (51%) concentrations were observed only with high dose avasimibe. Autologous ^{131}I-labeled VLDL, ^{125}I-labeled LDL, and [^{3}H]leucine were injected simultaneously into each pig and apoB kinetic data were analyzed using multicompartmental analysis (SAAM II). Avasimibe decreased the VLDL apoB pool size by 40–43% and the hepatic secretion rate of VLDL apoB by 38–41%, but did not alter its fractional catabolism. Avasimibe decreased the LDL apoB pool size by 13–57%, largely due to a dose-dependent 25–63% in the LDL apoB production rate. Hepatic LDL receptor mRNA abundances were unchanged, consistent with a marginal decrease in LDL apoB FCRs. Hepatic ACAT activity was decreased by 51% ($P = 0.050$) and 68% ($P = 0.087$) by low and high dose avasimibe, respectively. The decrease in total apoB secretion correlated with the decrease in hepatic ACAT activity ($r = 0.495$; $P = 0.026$). We conclude that inhibition of hepatic ACAT by avasimibe reduces both plasma VLDL and LDL apoB concentrations, primarily by decreasing apoB secretion.**—Burnett, J. R., L. J. Wilcox, D. E. Telford, S. J. Kleinstiver, P. H. R. Barrett, R. S. Newton, and M. W. Huff. **Inhibition of ACAT by avasimibe decreases both VLDL and LDL apolipoprotein B production in miniature pigs.** ***J. Lipid Res.*** **1999.** 40: **1317–1327.**

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Acyl coenzyme A:cholesterol acyltransferase (ACAT; EC 2.3.1.26) is an intracellular enzyme that catalyzes the formation of cholesteryl esters from cholesterol and long chain fatty acids (1, 2). ACAT is an integral membrane protein located in the rough endoplasmic reticulum (ER) and ACAT activity is present in a variety of cells and tissues, including the adrenal, aorta, intestine, and liver (2). The regulation of ACAT is a key step in cholesterol homeostasis. ACAT protects cells from an excess of membrane free cholesterol as ACAT-derived cholesteryl esters are stored in cytoplasmic droplets within the cell. ACAT is postulated to play a physiological role in hepatic and intestinal lipoprotein assembly and secretion, steroid hormone production, and dietary cholesterol absorption (1). In early atherogenesis, ACAT-derived cholesteryl esters accumulate within macrophages and smooth muscle cells resulting in foam cell formation (3). The atherogenic lipoproteins, chylomicron remnants, very low density lipoprotein (VLDL) remnants, and low density lipoproteins (LDL) contain intestinal and hepatic ACAT-derived cholesteryl esters and are the supply of cholesterol to the arterial intima. Therefore, an understanding of the regulation of ACAT should allow insights into the physiological functions of this enzyme that impact on the atherogenic process.

Hepatic apolipoprotein (apoB)-containing lipoprotein secretion into plasma requires the synthesis and assembly of apoB and lipids. Microsomal triglyceride transfer protein (MTP) facilitates the association of these lipids, in-

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; ALT, alanine aminotransferase; apo, apolipoprotein; AST, aspartate aminotransferase; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; FCR, fractional catabolic rate; GGT, gamma glutamyltransferase; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPGC, high performance gel chromatography; IDL, intermediate density lipoprotein; IV, intravenous; LDL, low density lipoprotein; mRNA, messenger ribonucleic acid; MTP, microsomal triglyceride transfer protein; PR, production rate; VLDL, very low density lipoprotein.

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cluding cholesteryl ester, triglyceride, and phospholipid with apoB (4, 5). If apoB fails to associate with sufficient lipid, apoB is targeted for degradation mediated by cellular proteases. There is accumulating evidence from both in vitro and in vivo studies that cholesterol and/or cholesteryl ester availability can regulate hepatic VLDL apoB secretion (5–8). The concentration of free cholesterol substrate in the cell regulates enzymatic activity of ACAT (9). Furthermore, inhibition of newly synthesized cholesteryl ester catalyzed by ACAT has been shown to decrease apoB secretion in HepG2 cells (6, 10–16), primary hepatocytes (11, 17, 18), perfused monkey livers (19) as well as in several small animal models (8). ACAT inhibitors decrease plasma cholesterol and apoB concentrations in rats and rabbits fed cholesterol-supplemented diets (20–23). However, most ACAT inhibitors show poor systemic bioavailability, and thus, the primary mechanism of action of these compounds has been ascribed to the inhibition of cholesterol absorption (1, 2).

ApoB kinetic studies from this laboratory provided the first in vivo evidence, in a large animal model, that inhibition of ACAT decreases hepatic apoB secretion (24). The intravenous (IV) administration of DuP 128 (2.2 mg/kg/d) to miniature pigs fed a low fat, cholesterol-free diet decreased the hepatic secretion of VLDL apoB into plasma by 65% (24). Hepatic microsomal ACAT activity was decreased by 68% with DuP 128 treatment. ACAT inhibition by DuP 128 did not affect LDL apoB kinetic parameters. Consistent with these findings, Carr, Hamilton, and Rudel (19) using perfused monkey livers demonstrated that three different ACAT inhibitors, when added to the liver perfusate, decreased hepatic cholesteryl ester concentrations and apoB secretion (19). In further studies (25), we have demonstrated that in pigs fed a diet higher in fat, IV DuP 128 treatment resulted in a more modest reduction in VLDL apoB secretion. Plasma VLDL apoB concentrations were decreased by 30% due to a 28% reduction in VLDL apoB production. Hepatic microsomal ACAT activity was decreased by 51%. As observed with the low fat diet, LDL apoB concentrations and production rates (PR) were unaffected by DuP 128 treatment.

Avasimibe (previously known as CI-1011) is a potent, new, orally bioavailable inhibitor of ACAT (26). This compound was effective in decreasing plasma total cholesterol concentrations in cholesterol-fed rats, cholesterol-fed hamsters, casein-fed rabbits, normal chow-fed rats, and chow-fed monkeys (26, 27). Krause and Auerbach (28) demonstrated plasma triglyceride reductions in normal chow-fed and in sucrose-fed rats with avasimibe treatment. Using Triton WR-1339 to block VLDL catabolism, they found significant decreases in plasma cholesterol and VLDL cholesteryl ester output, but no significant effect of avasimibe on triglyceride production. In the hypercholesterolemic hamster model, Nicolosi, Wilson, and Krause (29) observed that in addition to decreasing plasma lipids, avasimibe treatment reduced early atherosclerotic lesion development. In the same model, the addition of avasimibe (30 mg/kg/d) to the diet of the hamsters resulted in a 90% regression in lesion area over an 8-week period compared to untreated animals (29). Moreover, in monkeys, Ramharack et al. (27) showed that avasimibe (30 mg/kg/d) decreased lipoprotein[a] concentrations independent of apoB reductions. In a preliminary report, Koren et al. (30) recently demonstrated in hypertriglyceridemic human subjects, that avasimibe treatment (50–500 mg/d) significantly decreased plasma triglyceride and VLDL cholesterol concentrations. The mechanism for these significant lipid-lowering effects with avasimibe treatment is unknown.

The coordinated synthesis and assembly of apoB and lipids is necessary for apoB-containing lipoprotein secretion into plasma. Under normal circumstances, sufficient cholesteryl esters are produced for apoB-containing lipoprotein synthesis and assembly. The inhibition of ACAT by a potent ACAT inhibitor should significantly limit the availability of cholesteryl esters for incorporation into hepatic VLDL. In the present studies, an orally bioavailable ACAT inhibitor, avasimibe, was used to test the hypothesis that inhibition of cholesterol esterification, in vivo, would reduce hepatic VLDL apoB secretion into plasma.

MATERIALS AND METHODS

Animals and diets

Miniature pigs weighing 26.53 ± 0.43 kg were obtained from a local supplier (Premier Quality Genetics Inc., West Lorne, Ontario). After being acclimatized for 1 week, animals were maintained on the experimental diet for 21 days before and during the lipoprotein turnover studies. One week prior to the turnover study, an indwelling silicone elastomer (Silastic) catheter (1.96 mm ID) was surgically implanted in an external jugular vein (31). Isoflurane USP (Abbott Laboratories Ltd.) was used as the anesthetic and ketamine USP (Vetrepharm Canada Inc.) as the preanesthetic. Catheters, that were kept patent by filling with 7% EDTA-Na_2, allowed for ease of sample injection as well as blood sampling throughout each turnover study in unrestrained, unanesthetized animals. The experimental protocol was approved by the Animal Care Committee of the University of Western Ontario.

Pigs were studied in pairs, with each pair being same-sex litter mates. Six animals received the ACAT inhibitor, avasimibe (Parke-Davis) at a dose of 10 mg/kg per d and four animals, avasimibe at a dose of 25 mg/kg per d for 21 days prior to the turnover studies. Avasimibe, sulfamic acid [[2,4,6-tris(1-methylethyl)phenyl]acetyl]-2-6-bis(1-methylethyl)phenyl ester, is a synthetic inhibitor of ACAT. In vitro IC_{50} values and chemical structure of avasimibe have been reported previously (26). We have determined that the in vitro IC_{50} of avasimibe for pig microsomal ACAT activity is 4 μmol/L. Avasimibe was placed in gelatin capsules and, to ensure ingestion, was administered by hand before the daily feeding. The 10 control animals received a placebo capsule. The avasimibe was given at 9 am each day. Each animal received a 590 g ration of diet (B.W.S. Hog Grower, B-W Feed and Seed Ltd, New Hamburg, Ontario) supplemented with lard, unsalted butter, and safflower oil (1:0.6:0.2) generating a final polyunsaturated: monounsaturated: saturated fatty acid ratio of 1:1:1. Cholesterol (Fisher Scientific) was added to the diet to a final concentration of 0.1% (0.2 mg/kcal). This diet provided 34% of calories as fat, 49% as carbohydrate, and 17% as protein.

Lipoprotein turnover studies

Lipoprotein turnover studies were performed essentially as described previously (24, 31, 32) with minor modifications (33).

VLDL (S_f 20–400) and LDL (S_f 0–12) were isolated by sequential ultracentrifugation, from plasma (100–150 mL) obtained after a 24-h fast and subsequently radiolabeled with ^{131}I and ^{125}I, respectively (31). All labeled lipoproteins were autologous. Radiolabeling was performed using the iodine monochloride technique (32). Lipoproteins were sterilized by the addition of gentamicin sulfate (100 mg/mL) and checked for pyrogenicity and sterility. Of the total VLDL radioactivity, <2% was free iodine, 14–36% was bound to lipid, and 34–55% of the protein-bound label was bound to apoB. Of the total LDL radioactivity, <1% was free iodine, 8–30% was bound to lipid, and 78–91% of the protein-bound label was bound to apoB. After a 24-h fast, each animal received 20 μCi ^{131}I-labeled VLDL apoB, 15 μCi ^{125}I-labeled LDL apoB, and 2.5 mCi l-[4,5-^{3H}]leucine (Amersham Canada Ltd.; specific activity 155 Ci/mmol) given as a bolus by the indwelling catheter. After injection, blood samples (20 mL) were collected into tubes containing EDTA-Na_2 at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 12, 24, 30, 36, 48, 72, 96, and 120 h. The pigs received no food (but had free access to water) until the 12-h sample on day 1 and 36 h sample on day 2, at which time they received half their daily ration and either avasimibe or placebo. Animals were given their full daily ration and either avasimibe or placebo after the 48, 72, 96, and 120 h samples.

As described previously (24, 31–33), VLDL (d < 1.006 g/mL), IDL (d 1.006–1.019 g/mL), and LDL (d 1.019–1.063 g/mL) were separated from plasma by sequential ultracentrifugation. ApoB was isolated from each lipoprotein fraction by isopropanol precipitation and apoB specific activities were calculated after counting the washed pellet (31, 33) and assaying for protein by a modified Lowry procedure (34). The plasma concentrations of apoB in each plasma and lipoprotein fraction were measured by enzyme-linked immunosorbent assay (33). VLDL, IDL, and LDL apoB concentrations were constant over the sampling time period (31). As described previously (33), leucine concentrations were determined in deproteinized plasma isolated by a modification of the method of Hamilton (35) and plasma leucine specific activities were determined.

Kinetic analysis

The turnover data were analyzed by using the multicompartmental modeling program SAAM II (SAAM Institute, Seattle, WA) running on a Pentium-based PC. The model structure, the assumptions made in developing the model, and the constraints applied to the model were essentially those as previously reported (33). The model allows for direct apoB input into VLDL, IDL, and LDL. Although the majority of the IDL was derived from VLDL, direct IDL apoB input was required for optimal model fit. The percentage of IDL apoB derived directly was quantitatively small (<2%) and no significant differences were observed between treatment groups. This model was simultaneously fit to the three sets of tracer data for all lipoprotein fractions. This approach permitted the integration of all tracer data into a single model. Because of the different methods by which the two iodinated tracers and [^{3}H]leucine were introduced into the system, the information contained in each data set helped support different aspects of the model structure.

As described previously (33), aliquots of plasma (taken at time t = 0) were spiked with a small aliquot of the ^{131}I-labeled VLDL injected dose. Each spiked sample was then processed with the other plasma samples to determine the amount of radioactivity in apoB in the VLDL, IDL, and LDL fractions. For these studies, the mean distribution of ^{131}I apoB radioactivity in the spiked samples was 93.9 ± 0.6%, 4.6 ± 0.5%, and 1.5 ± 0.2%, in the VLDL, IDL, and LDL fractions, respectively. Based on the distribution of radioactivity in the spiked samples, the initial conditions (the initial amount of radioactivity in each fraction) were incorporated into the compartmental model.

Total and microsomal liver lipids

As described previously (33), total liver lipids were extracted using the method of Folch, Lees, and Sloane Stanley (36) from 1.0 g sections of liver obtained at killing that had been stored at −80°C. As previously described (24), microsomes were isolated from liver homogenates. Lipids were extracted from microsomes (1 mg protein) using the method of Folch et al. (36). Total cholesterol, free cholesterol, and triglyceride concentrations were quantitated in liver lipid extracts and isolated microsomes using a modification (33) of the method of Carr, Andresen, and Rudel (37) by enzymatic, colorimetric assays with reagents obtained from Boehringer Mannheim GmbH, Germany.

Oleate incorporation into hepatic cholesteryl ester

Liver samples obtained at killing were immediately frozen in liquid N_2 and stored at −80°C until analyzed. The activity of hepatic ACAT in crude homogenates was determined by the rate of incorporation of [1-^{14}C]oleic acid (Amersham) into cholesteryl ester, essentially as described by Gallo, Wadsworth, and Vahouny (38). ACAT activity was determined in liver homogenates, rather than microsomes, due to the loss of avasimibe during microsome preparation.

RNase protection assay for liver and intestine apoB, LDL receptor, and HMG-CoA reductase mRNA abundances

As described previously (33), liver and small intestine samples obtained at killing were immediately frozen in liquid N_2 and stored at −80°C until analysis. Total RNA was isolated, its integrity was verified, and content was determined. Pig specific cDNAs for apoB and LDL receptor, cloned into Bluescript plasmid (kindly provided by Dr. Alan D. Attie, University of Wisconsin-Madison, Madison, WI), and a Hind III/PST I fragment of human HMG-CoA reductase (ATCC), subcloned into Bluescript plasmid served as templates to synthesize antisense RNA probes. As described previously, (33), these riboprobes were then used to measure hepatic and intestinal apoB, LDL receptor, and HMG-CoA reductase mRNA abundances in a modification of the RNase protection solution hybridization assay of Azrolan and Breslow (39).

Analyses

Fasting blood samples (20 mL) were taken on days 1, 4, and 6 of the turnover study and plasma concentrations of total cholesterol and triglyceride, VLDL cholesterol and triglyceride, and high density lipoprotein (HDL) cholesterol were measured (31). VLDL was obtained after ultracentrifugation at d < 1.006 g/mL, and HDL was obtained after precipitation of other lipoproteins by dextran sulfate–magnesium chloride (31). LDL cholesterol was calculated by difference. The concentrations of total cholesterol, triglyceride, free cholesterol, and phospholipid were measured in the plasma and various lipoprotein fractions by enzymatic, colorimetric assays using reagents obtained from Boehringer Mannheim GmbH, Germany. Esterified cholesterol was calculated by the difference between total and free cholesterol determinations. Lipoprotein protein was determined by a modification of the method of Lowry (34). To determine whether avasimibe altered the size distribution of the lipoproteins and/or whether the distribution of cholesterol among the lipoprotein classes was affected by avasimibe, plasma was separated by the high performance gel chromatographic (HPGC) method of Kieft, Bocan, and Krause (40). On-line cholesterol content of each fraction was determined, and peak retention times were used to compare the relative sizes of the lipoproteins. Dietary fatty acid composition was determined by gas chromatography. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), or gamma glutamyltransferase (GGT) concentrations were determined by enzymatic, colorimetric assays using reagents obtained from Boehringer Mannheim, GmbH Ger-

TABLE 1. Plasma lipid and lipoprotein concentrations in control and avasimibe-treated miniature pigs

	Triglyceride				Cholesterol								ApoB			
	Total		VLDL		Total		VLDL[a]		LDL[b]		HDL[c]		VLDL[d]		LDL[d]	
Dose of Avasimibe	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A
	mmol/L				*mmol/L*								*mg/L*			
10 mg/kg/d (n = 6)																
Mean	0.222	0.153	0.141	0.086	2.781	2.607	0.046	0.031	1.436	1.253	1.295	1.322	16.71	10.10	191.4	166.4
SEM	0.043	0.033	0.033	0.018	0.151	0.079	0.008	0.004	0.155	0.059	0.050	0.075	1.34	1.10	13.6	14.2
P	0.004		0.024		NS		0.029		NS		NS		0.0002		NS	
25 mg/kg/d (n = 4)																
Mean	0.203	0.122	0.127	0.067	2.887	1.875	0.042	0.023	1.371	0.667	1.475	1.185	18.08	10.28	198.2	85.8
SEM	0.012	0.017	0.010	0.009	0.154	0.178	0.002	0.002	0.139	0.141	0.024	0.058	0.70	0.77	17.7	21.6
P	0.002		0.008		0.019		0.002		0.043		0.013		0.005		0.028	

Each lipid value is a mean of three determinations from each animal. ApoB, apolipoprotein B; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; C, control pigs; A, avasimibe-treated pigs; NS, not significant.

[a] VLDL cholesterol was determined after ultracentrifugation at d < 1.006 g/mL.

[b] LDL cholesterol was calculated as total cholesterol minus the sum of VLDL cholesterol and HDL cholesterol.

[c] HDL cholesterol was determined after precipitation of the apoB-containing lipoproteins from plasma.

[d] VLDL (d < 1.006 g/mL) and LDL (d 1.019–1.063 g/mL) apoB are the mean of all samples obtained during the kinetic study in the respective lipoprotein fractions separated by ultracentrifugation.

many. Tests for statistical significance of differences were compared by paired *t*-test (41). A P value <0.05 was considered significant.

RESULTS

The effects of avasimibe on plasma and lipoprotein lipid concentrations are shown in **Table 1**. At the lower avasimibe dose of 10 mg/kg per d, total plasma and VLDL triglyceride concentrations were significantly reduced by 31% ($P = 0.004$) and 39% ($P = 0.024$), respectively. VLDL cholesterol and VLDL apoB concentrations were significantly decreased by 31% ($P = 0.029$) and 40% ($P = 0.0002$), respectively. Total plasma cholesterol, HDL cholesterol, LDL cholesterol, and LDL apoB concentrations

TABLE 2. Metabolic parameters of VLDL apoB metabolism in control and avasimibe-treated miniature pigs

	VLDL Pool Size[a]		VLDL FCR[b]		Total VLDL Production		VLDL Conversion to LDL[c]		VLDL Direct Removal[d]	
Dose of Avasimibe	C	A	C	A	C	A	C	A	C	A
	mg/kg		h^{-1}		*mg/kg/h*		*mg/kg/h (%)*		*mg/kg h (%)*	
10 mg/kg/d										
1	0.882	0.600	2.926	2.619	2.581	1.571	0.240 (9)	0.092 (6)	2.394 (93)	1.508 (96)
2	0.668	0.478	3.313	3.880	2.214	1.856	0.053 (2)	0.049 (3)	2.171 (98)	1.821 (98)
3	0.598	0.343	3.414	4.399	2.042	1.509	0.054 (3)	0.114 (8)	1.930 (95)	1.395 (92)
4	0.598	0.347	4.365	4.353	2.609	1.508	0.094 (4)	0.053 (4)	2.513 (96)	1.473 (98)
5	0.870	0.475	3.197	3.346	2.782	1.589	0.075 (3)	0.034 (2)	2.619 (94)	1.571 (99)
6	0.594	0.301	4.109	3.760	2.442	1.132	0.088 (4)	0.062 (6)	2.282 (93)	1.083 (96)
Mean	0.702	0.424	3.554	3.726	2.445	1.528	0.101 (4)	0.067 (5)	2.318 (95)	1.475 (96)
SEM	0.056	0.046	0.228	0.273	0.112	0.095	0.029 (1)	0.012 (1)	0.101 (1)	0.098 (1)
P	0.0002		NS		0.002		NS (NS)		0.002 (NS)	
25 mg/kg/d										
1	0.832	0.439	3.629	4.162	3.018	1.828	0.215 (7)	0.058 (3)	2.867 (95)	1.793 (98)
2	0.769	0.482	3.977	3.516	3.060	1.694	0.064 (2)	0.022 (1)	2.845 (93)	1.624 (96)
3	0.689	0.468	3.641	3.809	2.508	1.782	0.138 (6)	0.026 (1)	2.444 (98)	1.760 (99)
4	0.747	0.338	3.511	3.903	2.623	1.318	0.123 (5)	0.075 (6)	2.393 (91)	1.262 (96)
Mean	0.759	0.432	3.690	3.847	2.802	1.655	0.135 (5)	0.045 (3)	2.637 (94)	1.610 (97)
SEM	0.030	0.003	0.100	0.133	0.139	0.116	0.031 (1)	0.013 (1)	0.127 (1)	0.122 (1)
P	0.005		NS		0.004		0.047 (NS)		0.003 (0.020)	

VLDL, very low density lipoprotein; apoB, apolipoprotein B; FCR, fractional catabolic rate; LDL, low density lipoprotein; C, control pigs; A, avasimibe-treated pigs; NS, not significant.

[a] Pool size refers to the plasma VLDL apoB concentration multiplied by 0.042, making the assumption that in the pig there are 42 mL plasma per kilogram body weight.

[b] FCR is determined by [FLUX(0,1) + FLUX(2,1) + FLUX(6,1)]/VLDL apoB pool size, where [FLUX(0,1) + FLUX(2,1) + FLUX(6,1)] is the production rate of VLDL apoB, where the numbers in brackets refer to compartments of the model (33).

[c] Values in parentheses represent VLDL conversion to LDL as a percent of total VLDL production.

[d] Values in parentheses represent VLDL direct removal as a percent of total VLDL production.

were unaltered by the ACAT inhibitor. At the higher avasimibe dose of 25 mg/kg per d, total plasma and VLDL triglyceride concentrations were significantly reduced by 40% ($P = 0.002$) and 48% ($P = 0.008$), respectively. Total plasma, VLDL, LDL, and HDL cholesterol concentrations were significantly reduced by 35% ($P = 0.019$), 45% ($P = 0.002$), 51% ($P = 0.043$), and 20% ($P = 0.013$), respectively. VLDL and LDL apoB concentrations were significantly decreased by 43% ($P = 0.005$) and 57% ($P = 0.028$), respectively.

Autologous ^{131}I-labeled VLDL, ^{125}I-labeled LDL, and [^{3}H]leucine were simultaneously injected into each control and avasimibe-treated pig. The kinetic parameters of apoB were determined from the simultaneous analysis of all the specific activity data by using the model described previously (33). The kinetic parameters are summarized in **Table 2** and **Table 3**. A fit of the model to the apoB specific radioactivity curves for ^{131}I-labeled VLDL, ^{131}I-labeled IDL, and ^{131}I-labeled LDL for one representative animal is shown in **Fig. 1**. A fit of the model to the apoB specific radioactivity curves for [^{3}H]VLDL, [^{3}H]IDL, and [^{3}H]LDL for the same animal is shown in **Fig. 2**. In this model, approximately 90% of apoB enters the circulation as VLDL, 5% of VLDL apoB flux is converted to LDL (either through the IDL pool or directly without passing through plasma IDL), and 10% of apoB enters the circulation directly as LDL.

At the lower avasimibe dose, the VLDL apoB pool size was decreased by 40% ($P = 0.0002$) (Table 2). This was primarily due to a significant 38% ($P = 0.002$) reduction in the VLDL apoB PR, as the FCR was unchanged. The amount of VLDL apoB converted to LDL apoB was decreased by 33%. Although not statistically significant, conversion was reduced in four of six animals studied. The flux of apoB cleared directly, without conversion to IDL or LDL, was significantly reduced by 36% ($P = 0.002$). Neither the percentage of VLDL apoB flux converted to LDL nor the percent cleared directly were affected by avasimibe treatment. Similarly, at the higher avasimibe dose, the VLDL apoB pool size decreased by 43% ($P = 0.005$). This was primarily due to a significant 41% ($P = 0.004$) reduction in the VLDL apoB PR, as the FCR was unchanged. The amount of VLDL apoB converted to LDL apoB was significantly decreased by 66% ($P = 0.047$). The percentage of VLDL apoB flux converted to LDL was not affected by avasimibe treatment. The flux of apoB cleared without conversion to IDL or LDL was significantly reduced by 39% ($P = 0.003$); however, when expressed as a percent of the total VLDL apoB flux, a small but significant increase in the percentage of apoB cleared directly was observed with avasimibe treatment. The FCRs for VLDL apoB direct catabolism [k(0,1), k(0,2) and k(0,3)] and for IDL direct catabolism [k(0,4) and k(0,5)] were not significantly affected by either dose of avasimibe treatment (data not shown). The model allowed us to determine the production or flux of VLDL apoB that was converted to LDL without being transported through the plasma IDL pool, as well as the amount of VLDL apoB converted to LDL via the IDL fraction. VLDL apoB converted to LDL via IDL were decreased by 36% and 65%

TABLE 3. Metabolic parameters of LDL apoB metabolism in control and avasimibe-treated miniature pigs

	LDL Pool Size[a]		LDL FCR[b]		Total LDL Production		LDL Direct Production[c]		Total ApoB Production[d]	
Dose of Avasimibe	C	A	C	A	C	A	C	A	C	A
	mg/kg		*h^{-1}*		*mg/kg/h*		*mg/kg/h (%)[e]*		*mg/kg h*	
10 mg/kg/d										
1	6.55	7.28	0.054	0.046	0.351	0.334	0.111 (32)	0.243 (73)	2.692	1.813
2	8.75	5.40	0.052	0.043	0.459	0.234	0.406 (88)	0.185 (79)	2.620	2.041
3	10.10	9.60	0.045	0.044	0.453	0.418	0.399 (88)	0.304 (73)	2.441	1.813
4	8.77	7.14	0.046	0.038	0.408	0.275	0.313 (77)	0.222 (81)	2.922	1.730
5	7.39	6.48	0.049	0.033	0.361	0.214	0.286 (79)	0.180 (84)	3.067	1.769
6	6.68	6.01	0.045	0.047	0.299	0.281	0.211 (70)	0.218 (78)	2.653	1.351
Mean	8.04	6.99	0.049	0.042	0.389	0.293	0.288 (72)	0.225 (78)	2.733	1.753
SEM	0.57	0.60	0.002	0.002	0.026	0.030	0.046 (9)	0.018 (2)	0.092	0.092
P	NS		0.047		0.040		NS (NS)		0.0008	
25 mg/kg/d										
1	9.34	5.57	0.047	0.049	0.435	0.272	0.230 (53)	0.214 (79)	3.248	2.042
2	6.93	2.27	0.044	0.033	0.302	0.075	0.244 (81)	0.054 (71)	3.305	1.747
3	9.84	1.87	0.052	0.041	0.511	0.076	0.373 (73)	0.050 (66)	2.881	1.832
4	7.18	4.71	0.048	0.035	0.345	0.166	0.222 (64)	0.090 (55)	2.846	1.408
Mean	8.32	3.60	0.048	0.039	0.398	0.147	0.268 (68)	0.102 (68)	3.070	1.758
SEM	0.84	0.91	0.002	0.004	0.047	0.047	0.036 (6)	0.038 (5)	0.120	0.132
P	0.028		NS		0.028		NS (NS)		0.001	

LDL, low density lipoprotein; apoB, apolipoprotein B; FCR, fractional catabolic rate; C, control pigs; A, avasimibe-treated pigs; NS, not significant.

[a] Pool size refers to the plasma LDL apoB concentration multiplied by 0.042, making the assumption that in the pig there are 42 mL plasma per kilogram body weight.

[b] FCR is the rate constant k(0,6) determined from the model (33).

[c] Direct production (direct synthesis) is the production of apoB directly into compartment 6.

[d] Production of apoB into the plasma compartment calculated as VLDL apoB production plus LDL apoB direct production.

[e] Values in parentheses represent LDL direct production as a percent of total LDL production.

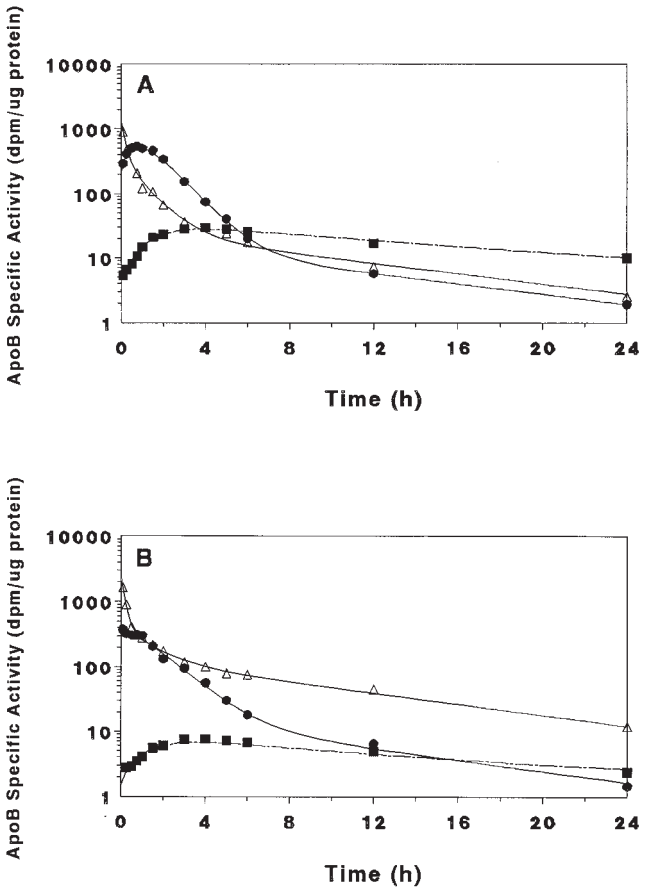

Fig. 1. Line graph showing apoB specific activity time curves for very low density lipoprotein (VLDL) (△), intermediate density lipoprotein (●), and low density lipoprotein (■) after injection of radiolabeled ^{131}I-labeled VLDL. Data points represent the observed data, and the lines are the best fit generated by the kinetic model. Panel A represents a control pig; panel B, an avasimibe-treated pig.

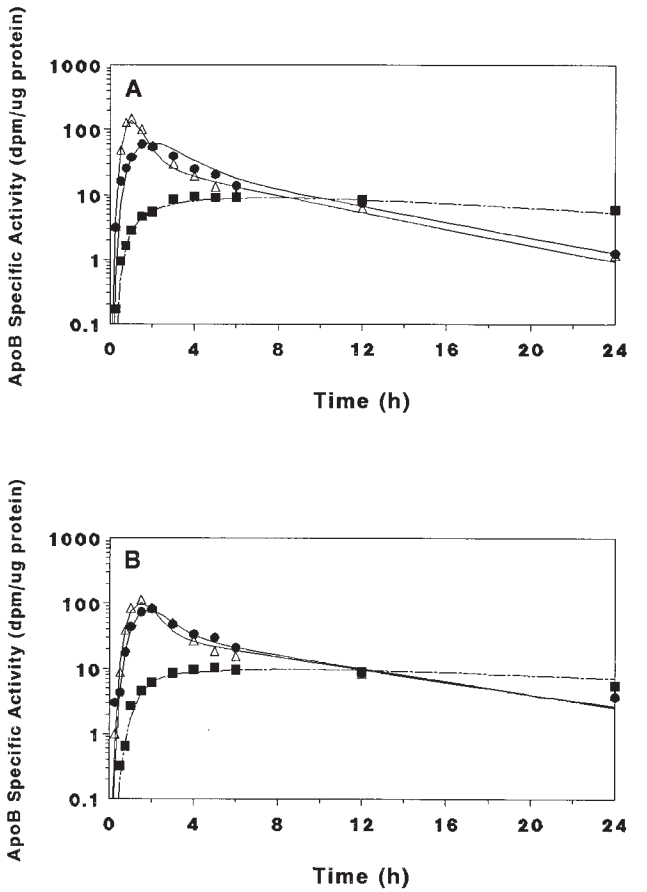

Fig. 2. Line graph showing apoB specific activity time curves for very low density lipoprotein (△), intermediate density lipoprotein (●), and low density lipoprotein (■) after injection of radiolabeled [^{3}H]leucine. Data points represent the observed data and the lines are the best fit generated by the kinetic model. Panel A represents a control pig; panel B, an avasimibe-treated pig.

with low and high dose avasimibe treatment, respectively (P = NS; data not shown).

At the lower avasimibe dose, the LDL apoB pool size appears to be decreased, primarily, due to a significant 25% ($P = 0.040$) reduction in the LDL apoB PR (Table 3). The observed change would appear to be the result of a decrease both in LDL apoB derived from VLDL apoB catabolism and LDL apoB direct synthesis. Total apoB production into plasma, calculated as the sum of VLDL apoB production plus LDL apoB direct production decreased significantly by 36% ($P = 0.0008$). Avasimibe treatment resulted in a small, but significant 14% decrease in the LDL apoB FCR ($P = 0.047$). At the higher avasimibe dose, the LDL apoB pool size was reduced to a greater extent (57%; $P = 0.028$) (Table 3). This was due to a significant 63% ($P = 0.028$) reduction in the LDL apoB PR, which was accounted for by the combination of a 66% decrease in LDL apoB derived from VLDL apoB catabolism ($P = 0.047$) and an apparent decrease (62%) in LDL apoB direct synthesis. Total apoB production into plasma decreased significantly by 43% ($P = 0.001$). The LDL apoB FCR at the higher avasimibe dose was unchanged.

VLDL and LDL were analyzed for lipid and protein composition (**Table 4**). The percent free cholesterol in VLDL increased significantly at both avasimibe doses. In addition, at the high dose, the percent esterified cholesterol in VLDL decreased and the triglyceride/esterified cholesterol ratio increased. The percent composition of LDL at the low avasimibe dose was unchanged. However, at the high avasimibe dose, the percent free cholesterol increased and the percent esterified cholesterol in LDL decreased. The surface/core lipid ratio increased. Plasma cholesterol distribution among plasma lipoprotein classes, as assessed by HPGC, however, showed no major changes at either avasimibe dose. The elution peak retention times for VLDL, LDL and HDL were unchanged (data not shown) suggesting no significant effect of avasimibe treatment on particle size.

Approximately 24 h after the last dose of avasimibe was administered, the pigs were killed, and sections of liver and small intestine were removed and stored at −80°C prior to analyses. Hepatic total or microsomal free cholesterol concentrations were not significantly altered by avasimibe treatment (**Table 5**). However, at the high avasimibe

TABLE 4. Percent composition of VLDL and LDL isolated from control and avasimibe-treated miniature pigs

Dose of Avasimibe	VLDL			LDL		
	Control	Avasimibe	*P*	Control	Avasimibe	*P*
10 mg/kg/d (n = 6)						
Triglyceride	62.8	62.3	NS	3.1	2.8	NS
Free cholesterol	3.3	4.4	0.003	11.4	14.9	NS
Esterified cholesterol	5.3	5.6	NS	36.6	36.5	NS
Phospholipid	10.9	10.0	NS	25.1	22.0	NS
Protein	17.7	17.7	NS	23.9	23.8	NS
TG/EC[a]	12.1	11.2	NS	0.09	0.08	NS
Surface/core[b]	0.21	0.22	NS	0.92	0.95	NS
25 mg/kg/d (n = 4)						
Triglyceride	60.3	59.0	NS	2.9	2.7	NS
Free cholesterol	3.1	5.0	0.048	12.4	15.9	0.008
Esterified cholesterol	5.5	4.2	0.014	36.1	32.0	0.010
Phospholipid	11.5	11.6	NS	24.6	25.8	NS
Protein	19.6	20.2	NS	24.0	23.6	NS
TG/EC	10.9	14.2	0.017	0.08	0.08	NS
Surface/core	0.22	0.26	NS	0.96	1.21	0.009

Values are percent of total lipoprotein mass and are means of determinations on VLDL and LDL from control and avasimibe-treated animals. VLDL, very low density lipoprotein; LDL, low density lipoprotein; EC, esterified cholesterol; TG, triglyceride; NS, not significant.

[a] Ratios are weight ratios.

[b] Surface/core is the ratio of (phospholipid + free cholesterol)/(triglyceride + esterified cholesterol).

dose, mean increases in hepatic total and microsomal esterified cholesterol concentrations of 5.7- and 1.5-fold, respectively, were observed over control animals. A dose-dependent effect on hepatic total triglyceride concentrations was observed, with a mean increase of 2.4-fold at the low avasimibe dose and 7-fold at the high dose. Increases in hepatic triglyceride concentrations were observed in all four animals treated with the high avasimibe dose; however, due to between animal variation, this failed to achieve statistical significance ($P = 0.065$). Hepatic microsomal triglyceride concentrations were significantly increased by 2.4-fold at the high dose ($P = 0.037$). Despite the markedly elevated hepatic triglyceride concentrations, no increases in plasma ALT, AST, or GGT concentrations were observed with either avasimibe dose (data not shown). Hepatic ACAT activities measured in crude liver homogenates were inhibited by 51% and 68% by low and high dose avasimibe, respectively (Table 5). Hepatic ACAT activity was significantly correlated with total apoB production rate ($r = 0.495$; $P = 0.026$) suggesting a coordinate regulation of cholesterol esterification and apoB secretion.

Hepatic apoB mRNA abundance was significantly decreased by 13% in the low dose avasimibe-treated animals ($P = 0.004$) (**Table 6**), however, no significant effect (mean decrease of 9%) was observed at the high avasimibe dose. Intestinal apoB mRNA abundances were unchanged. Hepatic and intestinal LDL receptor and HMG-CoA mRNA abundances were unaltered by avasimibe treatment.

DISCUSSION

Hepatic apoB-containing lipoprotein secretion can be regulated by lipid availability. However, the relative importance of the individual neutral lipids in this process remains unclear (5–7, 42, 43). In vivo apoB kinetic studies

TABLE 5. Hepatic total and microsomal lipid concentrations and ACAT activity in control and avasimibe-treated miniature pigs

Dose of Avasimibe	Total Lipids			Microsomal Lipids			ACAT Activity
	FC	EC	TG	FC	EC	TG	
	mg/g wet wt			*μg/mg protein*			*nmol/min/mg*
10 mg/kg/d (n = 6)							
Control	2.15 ± 0.07	0.31 ± 0.05	2.75 ± 0.41	79 ± 18	5 ± 1	90 ± 22	2.42 ± 0.48
Avasimibe	1.90 ± 0.13	0.53 ± 0.12	6.66 ± 2.34	68 ± 8	8 ± 2	123 ± 29	1.20 ± 0.17
P	NS	NS	NS	NS	NS	NS	0.050
25 mg/kg/d (n = 4)							
Control	1.70 ± 0.10	0.23 ± 0.02	2.41 ± 0.23	129 ± 13	11 ± 1	131 ± 12	2.04 ± 0.54
Avasimibe	1.87 ± 0.10	1.32 ± 0.52	16.80 ± 5.20	126 ± 17	17 ± 4	311 ± 42	0.65 ± 0.11
P	NS	NS	NS	NS	NS	0.037	NS

Values are the mean ± SEM of determinations from control and avasimibe treated pigs. Livers were removed approximately 24 h after the last dose of avasimibe. Sections from several lobes of liver were excised and frozen at −80°C until analysis. Liver ACAT activity and cholesterol and triglyceride concentrations were determined as described in Methods. FC, free cholesterol; EC, esterified cholesterol; TG, triglyceride; NS, not significant.

TABLE 6. ApoB, LDL receptor and HMG-CoA reductase mRNA content in control and avasimibe-treated miniature pigs

Dose of Avasimibe	ApoB mRNA			LDL Receptor mRNA			HMG-CoA Reductase mRNA		
	Control	Avasimibe	*P*	Control	Avasimibe	*P*	Control	Avasimibe	*P*
	pg mRNA/μg total RNA			*pg mRNA/μg total RNA*			*pg mRNA/μg total RNA*		
10 mg/kg/d (n = 6)									
Liver	137 ± 17	119 ± 16	0.004	2.38 ± 0.42	2.29 ± 0.36	NS	0.38 ± 0.07	0.39 ± 0.12	NS
Intestine	56 ± 9	60 ± 9	NS	2.60 ± 0.55	3.65 ± 0.41	NS	0.52 ± 0.11	0.57 ± 0.12	NS
25 mg/kg/d (n = 4)									
Liver	122 ± 10	112 ± 9	NS	4.73 ± 0.66	4.71 ± 0.74	NS	0.81 ± 0.25	0.79 ± 0.33	NS
Intestine	58 ± 10	70 ± 13	NS	3.76 ± 0.24	3.36 ± 0.26	NS	0.58 ± 0.07	0.48 ± 0.11	NS

Values are the mean ± SEM of determinations from control and avasimibe-treated pigs. Liver and intestine were removed approximately 24 h after the last dose of avasimibe. Sections were excised and frozen at −80°C until analysis. Liver and intestine apoB, LDL receptor, and HMG-CoA reductase mRNA abundances were determined as described in Methods. ApoB, apolipoprotein B; LDL, low density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; NS, not significant.

using orally administered inhibitors of HMG-CoA reductase (8, 33) and IV administered inhibitors of ACAT (24, 25) have demonstrated a fundamental regulatory role for cholesterol and/or cholesteryl esters in hepatic apoB secretion into plasma. It is evident that the inhibition of ACAT may play an important role in the treatment of hyperlipidemia and atherosclerosis. However, a lack of efficacy and/or toxicity (44) after oral administration of ACAT inhibitors to humans (45) or to animals fed physiologically relevant amounts of fat and cholesterol have limited their potential usefulness.

The results of the apoB kinetic experiments in the present study clearly demonstrate, for the first time, that oral administration of the ACAT inhibitor avasimibe significantly reduces the secretion of apoB-containing lipoproteins into plasma. Avasimibe treatment of miniature pigs fed normal amounts of fat and cholesterol resulted in the following major findings. *1*) VLDL apoB secretion rates decreased by 38–41%, resulting in a 40–43% reduction in VLDL apoB pool sizes; *2*) LDL apoB PRs were significantly decreased by 25–63%, resulting in a 13–57% reduction in the LDL apoB pool sizes; *3*) the conversion of VLDL apoB to LDL apoB and direct LDL apoB synthesis were decreased by 33–66% and 22–62%, respectively; *4*) total apoB secretion decreased by 36–43%; *5*) hepatic and intestinal LDL receptor mRNA abundances were unchanged, consistent with a marginal decrease in LDL apoB FCRs; and *6*) hepatic ACAT activities were reduced by 51–68%. This study supports the concept that hepatic assembly and secretion into plasma of apoB-containing lipoproteins is dependent on the ACAT-catalyzed synthesis of cholesteryl esters. Furthermore, the results are consistent with our previous apoB kinetic studies using an IV administered ACAT inhibitor (DuP 128) (24, 25) and studies with monkey livers perfused with ACAT inhibitors (19).

How ACAT inhibition regulates the assembly and secretion of apoB-containing lipoproteins within the hepatocyte has not been clearly established. This conundrum has been compounded by inconsistent in vitro findings in cultured hepatocytes (12, 14, 46–50). We hypothesize that reduced cholesterol esterification may compromise the ability of apoB to fold appropriately and/or reduce full lipidation and subsequent secretion. In turn, the rates of apoB degradation and/or translocation of apoB across the ER membrane may be affected. Moreover, the rates of apoB degradation and translocation can determine the fate of newly synthesized apoB (8). Recently completed work by Wilcox et al. (15), in HepG2 cells, has demonstrated a significant dose-dependent reduction in apoB secretion in the media with avasimibe treatment (10 nm to 10 μm), an effect associated with increased intracellular degradation of apoB. These findings are consistent with a preliminary report by Kulinski et al. (16) who demonstrated a decreased translocation efficiency and increased cotranslational degradation of apoB in avasimibe-treated HepG2 cells. However, a reduction in apoB secretion is not a universal finding of ACAT inhibitors in HepG2 cells, as DuP 128 had no effect, even though cellular cholesterol esterification was inhibited to an extent similar to that with avasimibe (15). Neither inhibitor affected triglyceride or phospholipid synthesis, both of which have been shown to affect apoB secretion in cultured hepatocytes (51, 52). Also, other investigators using HepG2 cells, have not observed any effect of ACAT inhibitors (58-035, CL2777,082, 447C88) on apoB secretion (48, 50). Thus it is possible that in HepG2 cells, avasimibe blocks apoB secretion by mechanisms unrelated to inhibition of cholesterol esterification. Although the same may occur in porcine liver after avasmibe treatment, we observed that oral avasimibe and IV DuP 128 (24, 25) both inhibited hepatic ACAT and both decreased apoB secretion into plasma. This suggests that hepatic ACAT activity in the pig may be more tightly linked to apoB secretion than in HepG2 cells.

ACAT activity is present in a variety of cells and tissues (2) and after the cloning by Chang et al. (53) of the human ACAT gene (ACAT1), evidence has accumulated that more than one ACAT enzyme is present in mammals. The cloning and expression of a second ACAT isoform (ACAT2) has recently been reported in mice by Cases et al. (54), in nonhuman primates by Anderson et al. (55), and in humans by Oelkers et al. (56). In contrast to ACAT1, whose expression is more or less ubiquitous, ACAT2 mRNA was expressed in the liver and intestine of both mice (54) and primates (55) and also detected in

HepG2 cells (54). Interestingly, although ACAT1 does not appear to play a major catalytic role in mouse liver (54), immunodepletion experiments using primary human hepatocytes suggest that it does play a role adult human liver (57).

ACAT2, like ACAT1, is predicted to be an ER integral membrane protein (54, 55). The active site of ACAT1 by topology is predicted to be cytoplasmic, whereas ACAT2 is predicted to be on the lumenal side of the ER membrane (55). Furthermore, ACAT1 and ACAT2 responded in a different manner to ACAT inhibitors of differing structures and classes (54). Therefore, one possibility that would be consistent with the conflicting in vitro and in vivo findings using ACAT inhibitors is that ACAT1 represents the ER enzyme responsible for generating cholesteryl esters for cytoplasmic storage, whereas ACAT2 represents the enzyme responsible for generating cholesteryl esters destined for lipoprotein assembly and secretion (55). If avasimibe proves to be a potent inhibitor of ACAT2, this could explain the significant reduction we observed in VLDL apoB secretion and direct LDL production.

A significant decrease in LDL apoB production with avasimibe treatment was observed that was due to decreases in both direct LDL apoB production and conversion of VLDL apoB to LDL apoB. The latter was due to a marked decrease in VLDL apoB production by avasimibe. In contrast, our previous in vivo studies using IV DuP 128 showed no effect on LDL apoB kinetic parameters (24, 25). The reasons for the difference are not readily apparent. As discussed above, it is possible that compared to avasimibe, DuP 128 did not achieve a sufficient inhibition of ACAT2 to decrease the regulatory cholesteryl ester pool involved in the assembly and secretion of apoB-containing lipoproteins, measured kinetically as direct LDL production.

The decreased conversion of VLDL apoB to LDL apoB, in the present study, would appear to be related to the reduced total VLDL apoB production that results in both a decreased conversion to LDL apoB and a decreased direct removal of VLDL apoB. Increasing the avasimibe dose did not produce a significant further decrease in VLDL apoB PR, however, the conversion of VLDL apoB to LDL apoB was reduced to a greater extent. As discussed below, this was not due to an enhanced LDL receptor expression. Rather, the changes in VLDL composition, i.e., an increase in percent free cholesterol (both avasimibe doses) and decrease in percent esterified cholesterol (high dose), may, in part, account for the decreased VLDL to LDL conversion. However, these biochemical changes did not result in any significant effect on the percent of plasma cholesterol carried in VLDL or VLDL particle size (as assessed by peak retention time) using HPGC. At the high avasimibe dose, the percent free cholesterol was increased and the percent esterified cholesterol was decreased in LDL. The surface/core lipid ratio was significantly increased consistent with the production of a smaller particle. Nevertheless, this did not result in any significant effect on LDL apoB catabolism.

Cholesterol biosynthesis, LDL receptor expression, and cholesterol esterification respond rapidly to changes in cellular concentrations of free cholesterol (58). The inhibition of ACAT by avasimibe would be expected to increase the hepatic free cholesterol concentration of the ER resulting in down-regulation of LDL receptor expression. However, total hepatic and microsomal free cholesterol concentrations were unaffected by avasimibe treatment, consistent with our inability to demonstrate any change in hepatic or intestinal LDL receptor abundances. Although not measured in the present study, it is possible that an accumulation of hepatic free cholesterol was prevented by a decrease in HMG-CoA reductase activity (59) and increases in 7α-hydroxylase activity and bile secretion (60).

Total hepatic triglyceride and esterified cholesterol concentrations were increased 7-fold and 5.7-fold with high dose avasimibe treatment, respectively, and these increases were positively correlated with microsomal triglyceride and esterified cholesterol concentrations, respectively ($r = 0.859$, $P < 0.001$; $r = 0.732$, $P = 0.002$). The histological appearances were those of hepatic steatosis (fatty liver). However, plasma ALT, AST, and GGT concentrations were not elevated (data not shown). Hepatic steatosis has been described in subjects with hypobetalipoproteinemia and abetalipoproteinemia (61), conditions associated with an inability to secrete apoB-containing lipoproteins into plasma. Our apoB kinetic studies demonstrate that avasimibe significantly decreases VLDL secretion into plasma, suggesting that the dose-dependent hepatic triglyceride accumulation results from reduced export of hepatic triglyceride as VLDL. However, a 28% decrease in VLDL apoB secretion in DuP 128-treated pigs or a 34% decrease in atorvastatin-treated animals was not associated with any hepatic triglyceride elevations (25, 33).

MTP is capable of facilitating apoB translocation, in addition to its role in mediating delivery of core lipid to nascent apoB (62–64). The absence of apoB-containing lipoproteins in subjects with abetalipoproteinemia (65, 66) indicates the critical role of MTP in apoB secretion. The marked reduction in VLDL apoB secretion by avasimibe associated with an increase in hepatic esterified cholesterol and triglyceride concentrations would be consistent with an inhibitory effect on MTP. Hepatic triglyceride accumulation has been observed in hamsters treated with an MTP inhibitor (67). It is possible that avasimibe, in addition to inhibiting ACAT, may directly or indirectly affect MTP activity. In preliminary long term studies in HepG2 cells, we observed that avasimibe decreased both MTP protein and MTP mRNA abundance (68).

For ACAT inhibitors to have beneficial antiatherogenic effects, ideally they should be absorbed and inhibit cholesterol esterification linked to both apoB secretion as well as arterial wall foam cell formation. The avasimibe-induced decrease in hepatic apoB-containing lipoprotein secretion may significantly impact on the atherogenic process, a concept supported by the decreased atherosclerotic lesion development and complexity observed in avasimibe-treated hamsters and rabbits (29, 69). However, as stated above, the potential benefits of ACAT inhibition must be weighed against possible hepatic lipid accumulation.

In conclusion, the inhibition of ACAT by avasimibe de-

creases both VLDL and LDL apoB concentrations, primarily by decreasing apoB secretion into the plasma. Hepatic ACAT activity was significantly correlated with total apoB production rate suggesting a coordinate regulation of cholesterol esterification and apoB secretion. We postulate that avasimibe reduces cholesteryl ester synthesis below a critical level required for apoB translocation and lipoprotein assembly and secretion, thereby stimulating the rate of apoB degradation. Our results are consistent with the concept that modulation of apoB synthesis and secretion is an important mechanism whereby ACAT inhibitors lower the plasma concentration of apoB-containing lipoproteins.

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