Endogenous cholesterol synthesis is associated with VLDL-2 apoB-100 production in healthy humans

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Abstract Subjects with high plasma cholesterol levels exhibit a high production of VLDL apolipoprotein B-100 (apoB-100), suggesting that cholesterol is a mediator for VLDL production. The objective of the study was to examine whether endogenous cholesterol synthesis, reflected by the lathosterol-cholesterol ratio (L-C ratio), affects the secretory rates of different VLDL subfractions. Ten healthy subjects were studied after overnight fasting. During a 10 h primed, constant infusion of ^{13}C-valine (15 μmol/kg/h), enrichment was determined in apoB-100 from ultracentrifugally isolated VLDL-1 and VLDL-2 by gas chromatography mass spectrometry. The synthesis rates of VLDL-1 apoB-100 and VLDL-2 apoB-100, catabolism, and transfer were estimated by compartmental analysis. Mean VLDL-1 apoB-100 pool size was 90 ± 15 mg, and mean VLDL-2 apoB-100 pool size was 111 ± 14 mg. Absolute synthesis rate of VLDL-1 apoB-100 was 649 ± 127 mg/day and 353 ± 59 mg/day for VLDL-2 apoB-100. There was a strong association between the absolute synthesis rate of VLDL-2 apoB-100 and L-C ratio ($r^2 = 0.61$, $P < 0.01$). In contrast, no correlation was observed between L-C ratio and absolute synthesis rate of VLDL-1 apoB-100 ($r^2 = 0.302$, $P = 0.09$). In conclusion, these data provide additional support for an independent regulation of VLDL-1 apoB-100 and VLDL-2 apoB-100 production. Endogenous cholesterol synthesis is correlated only with the VLDL-2 apoB-100 production.—Prinsen, B. H. C. M. T., J. A. Romijn, P. H. Bisschop, M. M. J. de Barse, P. H. R. Barrett, M. Ackermans, R. Berger, T. J. Rabelink, and M. G. M. de Sain-van der Velden. **Endogenous cholesterol synthesis is associated with VLDL-2 apoB-100 production in healthy humans. *J. Lipid Res.* 2003.** 44: **1341–1348.**

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Cardiovascular diseases are the most common cause of mortality in the Western population. In addition to the traditional factors known from studies like the Framingham study and the PROCAM study, evidence is accumulating that VLDL is an important determinant for the development of atherosclerosis (1–6).

The assembly of VLDL particles is a complex process and consists of two steps. In the first step, two VLDL precursors are formed simultaneously and independently. These particles consist of apolipoprotein B (apoB)-containing VLDL precursor and a VLDL-sized lipid droplet lacking apoB. In the second step, both particles fuse to produce a mature VLDL particle. However, the respective roles of cholesterol and triglycerides in the regulation of assembly and production of VLDL are poorly understood. Some studies showed that intracellular cholesterol plays a key role in apoB-100 production (7–9), whereas other studies showed that triglycerides or free fatty acids are the main determinants for apoB production (10–14). However, most studies have been performed in vitro (HepG2 cells), and the discrepancies might be explained by differences in experimental models and study design.

In vivo studies in healthy normolipidemic subjects using stable isotopes suggest that the availability of cholesterol may be more important than triglyceride substrate in determining apoB-100 production (8, 9). The models used in these studies to determine hepatic production of VLDL apoB-100 have limitations. Since a one-compartmental model or a monoexponential function was used to calculate the kinetics of total VLDL, it was not possible to make inferences regarding VLDL subfractions. The assumption that VLDL apoB-100 is a homogenous pool has been shown to be invalid. There is now clear evidence that production of VLDL-1 apoB-100 and VLDL-2 apoB-100 are independently regulated (15). Malmstrom et al. (15) showed that insulin suppresses VLDL-1 apoB-100 production but has no

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effect on VLDL-2 apoB-100 production. Furthermore, a primary effect of ethanol in humans on the stimulation of production of VLDL-1 particles has been suggested (16). Since it has been demonstrated that the increased VLDL-2 apoB-100 pool in moderate hypercholesterolemia (17) and in familial hypercholesterolemia patients (18) is predominantly explained by increased production of VLDL-2 apoB-100, one can speculate that the availability of intracellular cholesterol or endogenous cholesterol synthesis is an important regulator for VLDL-2 apoB-100 production.

Endogenous hepatic cholesterol synthesis can be assessed by determination of the activity of the rate-limiting enzyme for cholesterol synthesis HMG-CoA reductase. Since this information is hampered by the requirement of a liver biopsy, several sterol precursors in plasma [mevalonic acid (MVA), squalene, lanosterol, and lathosterol] have been proposed as alternate indicators for endogenous cholesterol synthesis. MVA is not preferred, since plasma MVA exhibits diurnal variation (19) and might reflect changes in renal function (20). Although cholesterol precursors exhibit diurnal variations (21), the lathosterol-cholesterol (L-C) ratio is less marked than the plasma MVA, probably due to the differences in half-life (40 min for MVA vs. 24 h for lathosterol) and is now accepted as a good indicator for cholesterol synthesis (9).

In this study, we tested the hypothesis that production rates of different VLDL apoB-100 subfractions and cholesterol synthesis (determined by the L-C ratio) are closely coupled in healthy control subjects. VLDL-1 apoB-100 and VLDL-2 apoB-100 production, as well as the L-C ratio (which reflects endogenous cholesterol synthesis), in healthy control subjects were measured.

MATERIALS AND METHODS

Subjects

After an overnight fast, 10 healthy control subjects (8 male/2 female) were admitted to the hospital. Mean age of the control subjects was 46 ± 3 years (mean ± SEM, range 32–55 years), and the subjects had a normal body mass index of 24.2 ± 0.6 kg/m^2 (range 20.4–27.0 kg/m^2). One intravenous cannula was placed in the brachial vein for infusion of the labeled valine, while blood samples were taken from the contralateral brachial vein. No food was given during the tracer infusion, and the subjects were only allowed to drink water. Clinical details are shown in **Table 1**. No subjects had apoE2/E2 phenotype. Before the start of the study, all volunteers agreed to participate after signing an informed consent form, in accordance with the Helsinki Declaration of Human Rights. The Institutional Ethical Committee for studies in humans approved this study.

Experimental protocol

The infusion protocol has been described in detail elsewhere (22). Briefly, at t = 0, a priming dose of 15 μmol/kg L-[1-^{13}C]valine was administered intravenously in 2 min, followed by a continuous infusion of 15 μmol/kg/h L-[1-^{13}C]valine for 10 h. EDTA and heparin samples were taken from the contralateral arm at t = 0, 30, 60, 180, 300, 360, 420, 480, 540, and 600 min. Samples were kept on ice (maximum 1 h) until plasma was separated by centrifugation (20 min, 3,000 rpm, 4°C). Plasma samples were immediately stored at −80°C.

Isotope and chemicals

L-[1-^{13}C]valine (isotope mole fraction >0.99) (MassTrace, Woburn, MA) was dissolved in sterile 0.9% saline and sterilized through a 0.22 μm filter. High-purity solvents (hexane and ethanol) were purchased from Merck (Darmstadt, Germany). Bis-trimethyl-silyl-trifluoro-acetamide (BSTFA) was obtained from Sigma-Aldrich Chemie (Steinheim, Germany), and pyridine was from Pierce (Rockford, IL). Lathosterol (5β-cholest-7en-3β-ol) was purchased from Sigma-Aldrich, cholesterol (5δ-3β-cholestenol) was from IGN Biomedicals Inc. (Aurora, OH) and stigmasterol (internal standard) was from Acros Organics. All other chemicals were obtained from Riedel de Haën (Seelze, Germany). Density solutions were made with KBr in 0.9% NaCl.

Preinfusion measurements

Plasma triglycerides were measured with standard laboratory methods on a Vitros 950 (Johnson and Johnson, Clinical Diagnostics). Plasma cholesterol was measured using gas chromatography mass spectrometric (GC-MS) techniques (23). ApoE genotyping was isolated from blood according to the method described by Slooter et al. (24).

Isolation of free amino acids, apoB-100 from lipoproteins

The isolation of free amino acids from plasma was performed as described in detail elsewhere (22). The apoB-100-containing lipoproteins (VLDL-1, VLDL-2, IDL, and LDL) were isolated

TABLE 1. Clinical data in 10 normal control subjects

Number	Age	Gender	BMI	TC	TG	ApoB	Lathosterol	L-C Ratio	ApoE Genotyping
	years		*kg/m^2*	*mmol/l*		*g/l*	*μmol/l*		
1	43	M	25.5	3.5	1.0	0.80	2.31	0.65	E3/E4
2	55	M	22.6	6.5	1.1	1.19	2.28	0.35	E3/E4
3	40	M	22.0	3.1	0.5	0.65	1.92	0.61	E3/E3
4	32	M	23.1	4.9	1.5	1.14	3.58	0.73	E3/E4
5	32	M	20.4	3.5	0.3	0.65	1.44	0.41	E3/E3
6	55	M	26.2	4.2	1.0	0.75	5.30	1.26	E3/E3
7	55	M	25.5	5.2	1.4	1.20	3.36	0.65	E3/E4
8	55	M	25.3	5.1	0.9	0.97	3.01	0.59	E3/E3
9	54	F	27.0	8.1	1.3	1.14	8.30	1.02	E3/E4
10	48	F	24.0	6.3	1.0	0.90	4.69	0.75	E3/E3
Mean ± SEM	46 ± 3	8M/2F	24.2 ± 0.6	5.0 ± 0.5	1.0 ± 0.1	0.93 ± 0.06	3.61 ± 0.64	0.70 ± 0.08	

BMI, body mass index; L-C ratio: lathosterol-cholesterol ratio; TC, total plasma cholesterol measured by mass spectrometry; TG, total plasma triglycerides.

from 2 ml EDTA plasma using a discontinuous salt gradient by cumulative ultracentrifugation. The method for isolation of VLDL-1 and VLDL-2 is described in detail elsewhere (25). After a third spin, the IDL fraction was isolated (2.35 h, 39,000 rpm, 15°C). The IDL fraction was removed in the top 0.5 ml of solution, and after a fourth spin (17.30 h, 33,000 rpm, 15°C), the LDL fraction was isolated in the top 0.5 ml. ApoB concentrations were determined using a nephelometric assay (Dade Behring, Marburg, Germany). The detection limit of the apoB assay was 12 mg/l. In subjects who had detectable lipoprotein [a]concentrations in the IDL and LDL fractions, the samples were pretreated with lysine-sepharose 4B as described in (26). ApoB-100 from the isolated lipoproteins was then precipitated using isopropanol and isolated as described before (27).

Determination of [^{13}C]valine enrichment

Derivatization of the isolated amino acids and of the isolated apoB-100 was done according to the method of Husek (28). The *N*(*O, S*)-methoxycarbonyl methyl ester derivatives of plasma-free amino acids and of apoB-100 from VLDL-1, VLDL-2, and IDL were analyzed by GC-MS, and the derivatives of LDL apoB-100 were analyzed by GC combustion isotope ratio MS as described previously (22).

Lathosterol and cholesterol measurements

For lathosterol determination, 500 μl of plasma was mixed with 100 μl of 0.01 mg/ml stigmasterol, while for cholesterol determination, 50 μl plasma was mixed with 100 μl of 1 mg/ml stigmasterol. Mixtures were saponified for 60 min at 60°C in 1 ml 4% (w/v) KOH in 90% ethanol. After saponification, the samples were mixed with 1 ml water and extracted twice with 2 ml hexane. The pooled hexane extracts were dried under nitrogen and derivatized with 50 μl BSTFA-pyridine (v/v 5:1) for 60 min at 60°C.

For selected ion monitory-GC-MS analysis, 2 μl of the derivative mixture was delivered by automatic injection to a HP-5890 gas chromatograph split injection port (1:20) leading to a 0.2 mm × 25 m Chrompack CP-sil 19 CB (WCOT Fused Silica) capillary column. The injection port contained a glass wool liner. The carrier gas was helium at a linear rate of 1 ml/min. The oven temperature started at 120°C and was raised to 260°C at 20°C/min, then to 280°C at 2°C/min, and finally to 300°C at 40°C/min and held for 5 min. A HP-5989B mass spectrometer was used as detector. Measurements were done in the electron impact mode at 70 eV with an ion source temperature of 250°C. The quadrupole temperature was 150°C. Mass spectrometric data were collected in the selected ion mode at m/z = 255 and 213 for lathosterol, m/z = 329 and 368 for cholesterol, and m/z = 255 and 394 for stigmasterol. Calibration curves for lathosterol were constructed by mixing 100 μl of 0.01 mg/ml stigmasterol with a standard range of 0–10 μmol/l lathosterol. Calibration curves for cholesterol were constructed by mixing 100 μl of 0.1 mg/ml stigmasterol with a standard range of 0–10 mmol/l cholesterol.

ApoB-100 kinetics

ApoB-100 production was measured as the rate of incorporation of ^{13}C-enriched valine into circulating VLDL-1,VLDL-2, IDL, and LDL apoB-100. The best fit to the data was determined using the SAAM II software (Simulation Analysis and Modelling, version 1.1.1., SAAM Institute, Seattle, WA) as described in **Fig. 1**. Compartment 1 represents plasma valine into which the valine tracer was injected. Compartment 2 represents delay compartment, and valine is incorporated in VLDL-1 apoB-100, VLDL-2 apoB-100, IDL apoB-100, and LDL apoB-100 via compartments 3, 4, 5, and 6. The absolute synthesis rate, which is the amount of protein synthesized per day, was calculated as the product of the FSR and the plasma pool (plasma volume × plasma concentration). Plasma volumes in all subjects were calculated from body surface area using the formulas described by Hurley et al. (29).

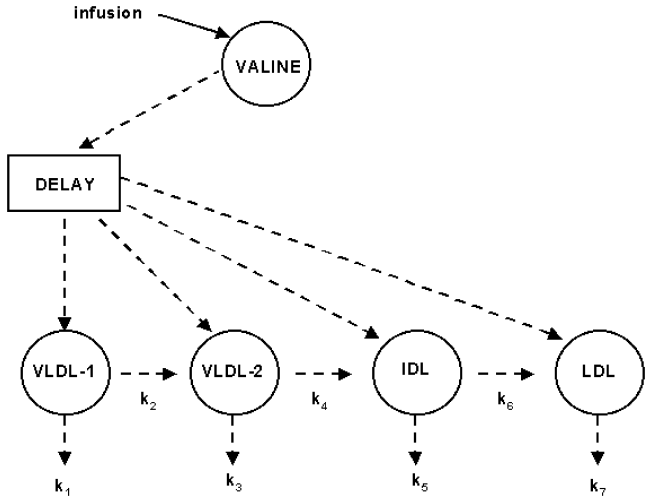

Fig. 1. Multicompartmental model for apolipoprotein B-100 (apoB-100) metabolism. Compartment 1 represents plasma valine into which the valine tracer was injected. Compartment 2 represents a delay compartment, and valine is incorporated in VLDL-1 apoB-100, VLDL-2 apoB-100, IDL apoB-100, and LDL apoB-100 via compartment 3, 4, 5, and 6. The *k* values represent the rate constants.

Statistics

Linear regression analysis was performed using Sigmastat software (Sigmastat for Windows, version 1.0). Data are expressed as mean ± SEM.

RESULTS

Baseline measurements

Baseline measurements of the control subjects are shown in Table 1. Mean plasma cholesterol concentration was 5.0 ± 0.5 mmol/l. Mean plasma triglycerides concentrations were 1.0 ± 0.1 mmol/l (range 0.3–1.5 mmol/l) and 0.93 ± 0.12 (range 0.65–1.20 g/l) for apoB-100. Mean plasma lathosterol concentration was 3.61 ± 0.64 μmol/l (range 1.44–8.30). The L-C-ratio, as defined by ratio of plasma L-C, was 0.70 ± 0.08 (range 0.35–1.26). Five subjects had the apoE3/E3 genotype, and five patients had the apoE3/E4 genotype.

ApoB-100 measurements

The enrichment of [^{13}C]valine in apoB-100 of VLDL-1, VLDL-2 IDL, and LDL increased in a linear fashion (**Table 2**), and in some cases, a plateau for VLDL-1 and VLDL-2 was reached (**Fig. 2**). The results of the kinetic analysis for VLDL-1 apoB-100, VLDL-2 apoB-100, IDL apoB-100, and LDL apoB-100 of the control subjects are presented in **Tables 3–6**.

Mean plasma VLDL-1 apoB-100 pool size was 90 ± 15 mg (range 29–202 mg), and mean VLDL-1 apoB-100 synthesis rate was 649 ± 127 mg/day (range 251–1363 mg/

TABLE 2. Mean [^{13}C]valine enrichment data for VLDL-1 apoB-100, VLDL-2 apoB-100, IDL apoB-100, and LDL apoB-100 in 10 normal control subjects

Time	VLDL-1 ApoB-100	VLDL-2 ApoB-100	IDL ApoB-100	LDL ApoB-100
min		%		
0	0.09 ± 0.06	0.05 ± 0.04	0.04 ± 0.03	0.01 ± 0.01
30	0.25 ± 0.12	0.09 ± 0.05	0.04 ± 0.02	0.02 ± 0.01
60	1.67 ± 0.34	0.69 ± 0.20	0.13 ± 0.03	0.07 ± 0.05
180	5.59 ± 0.28	3.46 ± 0.47	0.89 ± 0.13	0.11 ± 0.02
300	7.36 ± 0.40	6.27 ± 0.55	1.98 ± 0.22	0.32 ± 0.06
360	8.25 ± 0.37	7.54 ± 0.60	2.71 ± 0.33	0.36 ± 0.05
420	9.17 ± 0.35	8.09 ± 0.60	3.42 ± 0.46	0.48 ± 0.06
480	9.65 ± 0.32	9.07 ± 0.57	4.17 ± 0.54	0.66 ± 0.09
540	10.06 ± 0.29	9.65 ± 0.56	4.94 ± 0.53	0.84 ± 0.09
600	10.64 ± 0.19	10.07 ± 0.49	5.78 ± 0.55	1.00 ± 0.10

Data are expressed as mean ± SEM.

TABLE 3. VLDL-1 apoB-100 (S_f 60–400) metabolism in 10 control subjects

Number	VLDL-1 ApoB-100	Synthesis	Transfer	Direct Catabolism	Total FCR
	mg	*mg/day*		*pools/day*	
1	122	894	4.29	3.05	7.34
2	105	1,363	8.78	4.23	13.02
3	63	674	2.78	7.86	10.64
4	202	1,222	3.89	2.18	6.07
5	29	561	19.32	0.00	19.32
6	79	286	3.61	0.00	3.61
7	122	666	5.47	0.00	5.47
8	87	314	3.62	0.00	3.62
9	38	251	6.52	0.00	6.52
10	57	260	4.56	0.00	4.56
Mean ± SEM	90 ± 15	649 ± 127	6.28 ± 1.54	1.73 ± 0.84	8.01 ± 1.57

FCR, fractional catabolic rate.

day). Total VLDL-1 apoB-100 fractional catabolic rate (FCR) was the sum of the transfer of VLDL-1 apoB-100 into VLDL-2 apoB-100 and direct catabolism, and was an estimated 8.01 ± 1.57 pools/day (range 3.61–19.32 pools/day). The number of pools/day transferred to VLDL-2 apoB-100 was 6.28 ± 1.54 pools/day (range 2.78–19.32), and 1.73 ± 0.84 pools/day of VLDL-1 apoB-100 (range 0.00–7.86 pools/day) were direct cleared from plasma.

Mean VLDL-2 apoB-100 pool size was 111 ± 14 mg (range 45–197 mg), and mean VLDL-2 apoB-100 synthesis rate was 353 ± 59 mg/day (range 30–538 mg/day). Mean flux, which is calculated as the product of VLDL-1 transfer (pools/day) rate and the VLDL-1 apoB-100 pool size, was 474 ± 81 mg/day (range 30–592 mg/day). The number of pools/day transferred to IDL apoB-100 was 6.62 ± 1.35 pools/day (range 1.25–15.39 pools/day) and 1.73 ± 0.84 pools/day of VLDL-2 apoB-100 (range 0.00–7.86 pools/day) were direct cleared from plasma.

Mean IDL apoB-100 pool size was 236 ± 24 mg (range 160–360 mg), and mean IDL apoB-100 production rate was 90 ± 29 mg/day (range 0–211 mg/day). Mean flux, which is calculated as the product of VLDL-2 transfer (pools/day) rate and the VLDL-2 apoB-100 pool size was 651 ± 81 mg/day (range 83–940 mg/day). The number of pools/day transferred to LDL apoB-100 was 3.2 ± 0.5 pools/day (range 1.4–5.4 pools/day), and 0.3 ± 0.2 pools/day of IDL apoB-100 (range 0.00–1.5 pools/day) were direct cleared from plasma.

Mean LDL apoB-100 pool size was 1375 ± 105 mg (range 891–1916 mg) and mean LDL apoB-100 synthesis rate was 185 ± 34 mg/day (range 22–330 mg/day). Mean flux, which is calculated as the product of IDL transfer (pools/day) rate and the IDL apoB-100 pool size, was 684 ± 58 mg/day (range 294–929 mg/day). Total LDL apoB-100 FCR was 0.6 ± 0.0 pools/day (range 0.4–1.1 pools/day). Total apoB-100 production was 1,279 ± 102 mg/day (range 969–1,807 mg/day).

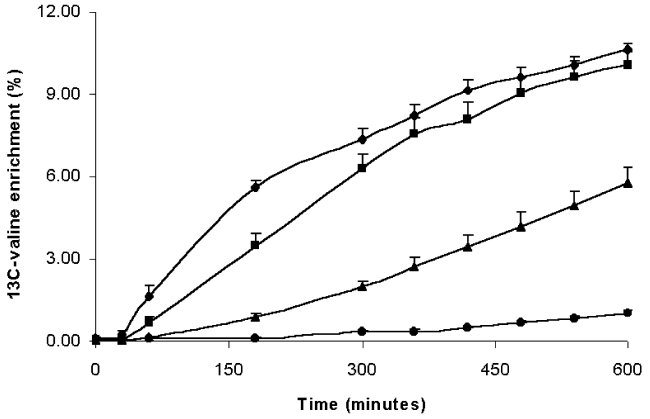

Fig. 2. [^{13}C]valine enrichment curves for VLDL-1 apoB-100 (diamond), VLDL-2 apoB-100 (square), IDL apoB-100 (triangle) and LDL apoB-100 (circle) in 10 healthy humans. Data are expressed as mean ± SEM.

Correlations

In the control group, VLDL-2 apoB-100 synthesis rate was strongly correlated with L-C ratio ($r^2 = 0.61$, $P < 0.01$, **Fig. 3A**), while no such correlation was found for L-C ratio and VLDL-1 apoB-100 production rate ($r^2 = 0.30$, $P = 0.09$, Fig. 3B), IDL apoB-100 production ($r^2 = 0.30$, $P = 0.10$), LDL apoB-100 production ($r^2 = 0.00$, $P = 0.96$), and total apoB-100 production ($r^2 = 0.15$, $P = 0.26$). Furthermore, VLDL-1 apoB-100 pool size correlated with VLDL-1 apoB-100 synthesis rate ($r^2 = 0.471$, $P = 0.02$), VLDL-2 apoB-100 pool size was strongly associated with total VLDL-2 apoB-100 FCR ($r^2 = 0.66$, $P = 0.004$), and VLDL-2 apoB-100 synthesis rate was strongly associated with the flux of apoB-100 from VLDL-1 ($r^2 = 0.62$, $P = 0.007$). No significant correlation was found for VLDL-1 apoB-100 production and lathosterol ($r^2 = 0.26$, $P = 0.12$) or VLDL-2 apoB-100 production and lathosterol ($r^2 = 0.29$, $P = 0.10$). Furthermore, no correlation was observed for plasma cholesterol and VLDL-1 apoB-100 ($r^2 = 0.01$, $P = 0.76$), the VLDL-2 apoB-100 synthesis rate ($r^2 = 0.001$, $P = 0.92$), the plasma triglycerides and VLDL-1 apoB-100 ($r^2 = 0.06$, $P = 0.49$), or the VLDL-2 apoB-100 synthesis rate ($r^2 = 0.002$, $P = 0.88$).

TABLE 4. VLDL-2 apoB-100 (S_f 20–60) metabolism in 10 control subjects

Number	VLDL-2 ApoB-100	Synthesis	Flux	Transfer	Direct Catabolism	Total FCR
	mg	*mg/day*			*pools/day*	
1	164	518	522	3.28	3.05	6.34
2	126	30	919	3.30	4.23	7.53
3	66	426	176	1.25	7.86	9.12
4	197	228	783	2.95	2.18	5.13
5	45	132	561	15.39	0.00	15.39
6	124	592	286	7.08	0.00	7.08
7	98	274	666	9.59	0.00	9.59
8	92	439	314	8.19	0.00	8.19
9	80	538	251	9.86	0.00	9.86
10	116	363	261	5.38	0.00	5.38
Mean ± SEM	111 ± 14	353 ± 59	474 ± 81	6.62 ± 1.35	1.73 ± 0.84	8.36 ± 0.93

Flux is VLDL-1 apoB-100 transfer (pools/day) × VLDL-1 apoB-100 pool size.

DISCUSSION

This study shows for the first time that endogenous cholesterol synthesis reflected by the L-C ratio is strongly associated with the VLDL-2 apoB-100 production rate. In contrast, no correlation was found for L-C ratio and the production rate of other apoB-100-containing lipoproteins (VLDL-1, IDL, and LDL) or total apoB-100 production rate in healthy controls. This suggests that only VLDL-2 apoB-100 production is driven by endogenous cholesterol synthesis.

In agreement with our data, an association between endogenous cholesterol synthesis and total VLDL apoB-100 synthesis was observed in healthy normolipidemic subjects (8, 9, 30), obese subjects (31, 32), and subjects with Type 2 diabetes (33). However, these associations were not as strong as observed in our controls. This discrepancy could be explained by the lack of separation of VLDL-1 apoB-100 and VLDL-2 apoB-100 production rates, since recent evidence strongly suggests that synthesis of both proteins is independently regulated (15).

Inhibition of endogenous cholesterol synthesis by HMG-CoA-reductase inhibitors (statin therapy) reduced VLDL apoB-100 production in healthy normolipidemic subjects (9), in miniature pigs (34), and in patients with combined hyperlipidemia (13, 35). In these studies, there were no significant changes in VLDL apoB-100 catabolism. However, other groups found no effect of statin therapy on VLDL apoB-100 synthesis (36–39). In most of these studies, patients had clinical signs of insulin resistance. Several studies demonstrated that statin therapy reduces endogenous cholesterol synthesis (9, 34), but no data are available concerning improvement of insulin sensitivity by statin therapy. Therefore, we speculate that VLDL apoB-100 production rate in insulin-resistant subjects could be explained predominantly by an overproduction of VLDL-1 apoB-100 and a reduced production of VLDL-2 apoB-100, resulting in a net normal rate of VLDL apoB-100 production. Additional indirect evidence for this explanation was provided by Riches et al. (40), who showed that improvement in insulin sensitivity following weight loss in viscerally obese men resulted in a reduction of hepatic VLDL apoB-100 production. In summary, reduced VLDL apoB-100 production due to statin intervention in subjects with normal insulin sensitivity is in agreement with the hypothesis that endogenous cholesterol synthesis is critical for VLDL apoB-100 assembly.

A model for the pools of free and esterified cholesterol and the main metabolic pathways involved in synthesis of bile acids and secretion of apoB-100-containing particles by the liver has been proposed by Thompson et al. (30). They hypothesized that the supply of cholesteryl ester for incorporation into VLDL originates from esterification by acyl coenzymeA:cholesterol acyltransferase (ACAT) of both newly

TABLE 5. IDL apoB-100 (S_f 12–20) metabolism in 10 control subjects

Number	IDL ApoB-100	Synthesis	Flux	Transfer	Direct Catabolism	Total FCR
	mg	*mg/day*			*pools/day*	
1	288	182	538	1.8	0.7	2.5
2	360	172	416	2.0	0.0	2.0
3	210	211	83	1.4	0.0	1.4
4	353	19	581	1.7	0.0	1.7
5	190	48	693	3.9	0.0	3.9
6	160	0	865	5.4	0.0	5.4
7	172	0	940	5.4	0.0	5.4
8	181	206	753	4.1	1.2	5.3
9	256	5	789	3.1	0.0	3.1
10	187	61	855	3.4	1.5	4.9
Mean ± SEM	236 ± 24	90 ± 29	651 ± 81	3.2 ± 0.5	0.3 ± 0.2	3.6 ± 0.5

Flux is VLDL-2 apoB-100 transfer (pools/day) × VLDL-2 apoB-100 pool size.

TABLE 6. LDL apoB-100 (S_f 0–12) metabolism in 10 control subjects

Number	LDL ApoB-100	Synthesis	Flux	Total FCR	Total ApoB-100 Production
	mg	*mg/day*		*pools/day*	*mg/day*
1	1,462	213	518	0.5	1,807
2	1,459	155	720	0.6	1,720
3	927	170	294	0.5	1,481
4	1,556	22	600	0.4	1,491
5	891	239	741	1.1	980
6	1,405	120	864	0.7	998
7	1,916	29	929	0.5	969
8	1,690	272	742	0.6	1,231
9	1,405	330	794	0.8	1,124
10	1,041	301	636	0.9	985
Mean ± SEM	1,375 ± 105	185 ± 34	684 ± 58	0.6 ± 0.0	1,279 ± 102

Flux is IDL apoB-100 transfer (pools/day) × IDL apoB-100 pool size.

synthesized and recycled LDL cholesterol. In line with this hypothesis is the observation that ACAT-generated cholesterol ester plays a modulatory role in VLDL production (41, 42). These data fit with the current two-step model of VLDL assembly. Based upon substrate availability (free fatty acid or cholesterol) in the liver, a VLDL-1- or VLDL-2-sized apoB-100 free lipid particle is formed that fuses with the apoB-100-containing VLDL precursor (43) before secretion.

It has been suggested that apoE is important for regulation of apoB-100-containing lipoproteins. Welty et al. (44) showed in healthy normolipidemic subjects with apoE3/E4 genotype a 30% reduction in the VLDL apoB-100 production rate compared with healthy subjects with the apoE3/E3 genotype. In this study where both VLDL-1 apoB-100 and VLDL-2 apoB-100 kinetics were studied, the subjects with the apoE3/E4 genotype did not have lower production rates for VLDL-1 apoB-100 or VLDL-2 apoB-100; this was putatively explained by a different study design. In the study by Welty et al., the subjects were continuously fed and total VLDL apoB-100 production was studied, whereas our subjects were fasting and the VLDL apoB-100 pool was subdivided in VLDL-1 apoB-100 and VLDL-2 apoB-100.

No correlation was observed between plasma cholesterol concentration and VLDL-1 apoB-100, or VLDL-2 apoB-100 production rate in this study, or as reported in other studies (9, 40, 45). The absence of a correlation might be explained by the fact that plasma cholesterol concentration reflects several processes including diet, synthesis, and elimination as fecal sterols and bile acids. Furthermore, it has been suggested that triglyceride availability, essential for the intracellular VLDL apoB-100 assembly, plays a central role in apoB-100 targeting, either for intracellular degradation, or assembly and production as VLDL particles. This implies that, based upon triglyceride availability, the liver secretes either small, triglyceride-poor VLDL-2 or large, triglyceride-rich VLDL-1 particles. These particles serve subsequently as precursors for the formation of buoyant or small, dense LDL particles by lipid transfer protein- and hepatic lipase-mediated processes (46). However, in this study, no association was found between plasma triglycerides and VLDL-1 apoB-100 production or VLDL-2 apoB-100 production, likely explained by the low number of subjects included.

In conclusion, this study shows that VLDL-2 apoB-100 production is a process predominantly driven by endogenous cholesterol synthesis. It remains to be established at the clinical level whether standardized measurements of the L-C ratio could be used as a marker for prediction of coronary heart disease.

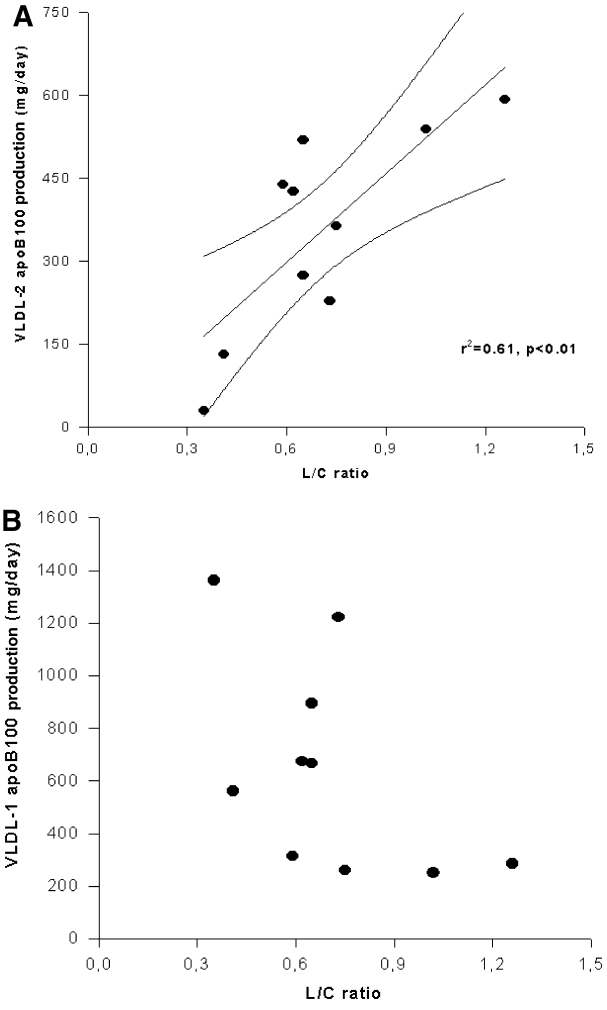

Fig. 3. A: Association between VLDL-2 apoB-100 production and lathosterol-cholesterol ratio (L-C ratio) in healthy control subjects (n = 10). B: Association between VLDL-1 apoB-100 production and L-C ratio in healthy control subjects (n = 10).

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