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Splanchnic Metabolism of Plasma Apolipoprotein B: *STUDIES OF ARTERY-HEPATIC VEIN DIFFERENCES OF MASS AND RADIOLABEL IN FASTED HUMAN SUBJECTS*

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STUDIES OF ARTERY-HEPATIC VEIN DIFFERENCES OF MASS AND RADIOLABEL IN FASTED HUMAN SUBJECTS

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ABSTRACT The metabolism of apoprotein B-containing plasma lipoproteins by human splanchnic tissues has been studied in 29 men undergoing coronary angiography. Before catheterization autologous radioiodinated lipoproteins were infused into a peripheral vein: 10 subjects received ^{125}I-labeled Sf 12-60 lipoproteins; 12 received ^{125}I-labeled Sf 12-60 plus ^{131}I-labeled Sf 100-400 lipoproteins; and 7 received ^{125}I-labeled Sf 12-60 plus ^{131}I-labeled Sf 0-12 lipoproteins. Paired arterial and hepatic vein blood samples were subsequently collected for replicate measurements of apoprotein B (apo B) mass, radioactivity and specific activity in each lipoprotein class. Splanchnic plasma flow was measured with indocyanine green. All studies were conducted after a 14-h overnight fast.

Newly synthesized apo B was shown to be secreted by splanchnic tissues as a component of Sf 100-400 lipoproteins, with no detectable uptake of apo B from this class. Sf 12-60 apo B was extracted by the splanchnic bed, with no detectable secretion. After continuous intravenous infusion of ^{125}I-labeled Sf 12-60 for five or more hours, 41–67% (mean 55%) of extracted Sf 12-60 apo B radioactivity reappeared in hepatic vein Sf 0-12 apo B. There was no detectable splanchnic catabolism of Sf 0-12 apo B.

The rates of Sf 100-400 apo B secretion, calculated as the product of artery-hepatic vein concentration difference and splanchnic plasma flow, were greater than the previously reported rates of very low density lipoprotein apo B turnover in fed subjects obtained by kinetic analysis of plasma specific radioactivity decay curves, suggesting that there may be a diurnal variation in hepatic apo B synthesis. They also exceeded the splanchnic extraction rates of Sf 12-60 apo B, suggesting there was some extrasplanchnic catabolism of the apo B of Sf > 60 lipoproteins.

INTRODUCTION

The kinetics of plasma very low density lipoprotein (VLDL)[1] and low density lipoprotein (LDL) metabolism in man have been the subject of numerous investigations. It is now established that a precursor-product relationship exists between VLDL-apolipoprotein B (apo B) and LDL-apo B, and that lipoproteins of intermediate density and flotation rate are intermediate in this metabolic sequence (1–4). Normally, all LDL-apo B is derived from VLDL-apo B (2–4), but in familial hypercholesterolemia there is evidence of LDL-apo B synthesis not involving VLDL as a precursor, in both the homozygous (5) and heterozygous (6) forms. Oversecretion of VLDL-apo B has been reported in hypertriglyceridemia (3, 7, 8), particularly in hypertriglyceridemia due to familial combined hyperlipidemia (9). Catabolic defects may underlie other disorders characterized by elevated plasma triglyceride levels (4, 5, 7, 10).

Most studies have used preparations of autologous VLDL, LDL and/or intermediate lipoprotein classes (Sf 12-20 or Sf 12-60) labeled in vitro with radioiodine. After intravenous injection of such radiolabeled lipo-

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[1] *Abbreviations used in this paper*: Apo B, apolipoprotein B; C_a, concentration in artery; C_{hv}, concentration in hepatic vein; LDL, low density lipoprotein (Sf 0-12); SPF, splanchnic plasma flow; VLDL, very low density lipoprotein (Sf 20-400).

proteins, sequential peripheral blood samples have been assayed for apo B specific activity in different lipoprotein fractions, and the specific activity time curves analyzed to give values for the pool sizes, production rates, interconversion and fractional rates of catabolism of the lipoproteins. Other procedures to label apo B have also been used (11). The use of apo B as a marker of the metabolism of these lipoproteins appears to be particularly apt, as this protein is quantitatively conserved during the metabolism of VLDL and LDL, playing a structural role in the composition of the particles (1, 4, 12).

Possible discrepancies exist between certain of the published accounts of VLDL-apo B metabolism in man. Though the transfer of apo B from larger to smaller VLDL subclasses appears to be quantitative in normolipidemic subjects, it is unclear whether all apo B is transferred to LDL. Sigurdsson et al. (2) concluded that more than 90% of VLDL-apo B is normally transferred to the Sf 0-20 range (2), and other studies have suggested that most VLDL-apo B normally reappears in Sf 0-12 lipoproteins (4). Other investigations, however, have suggested that some apo B in VLDL, or in the Sf 12-20 range, may be catabolized by an alternative pathway (3, 5). The synthetic rates of VLDL-apo B and LDL-apo B are correlated, but only moderately so ($r = +0.57$) (7), which may favor the latter interpretation. Among the possible explanations for these varying reports is the heterogeneity of the assumed models for the VLDL-to-LDL cascade, which vary in their complexity and for which there is a dearth of physiological bases.

We have sought an alternative approach, not dependent on model building, to define the kinetics of the metabolism of apo B-containing lipoproteins in man. By making measurements of apo B mass and radiolabel in different lipoprotein fractions, isolated from samples of arterial and hepatic venous blood following intravenous injection of radioiodinated autologous lipoproteins, the synthesis, interconversion and catabolism of VLDL (Sf 100-400), LDL (Sf 0-12), and intermediate density lipoproteins (Sf 12-60) in the human splanchnic bed have been directly quantified. An analogous approach has been employed by Havel et al. (13) and Boberg et al. (14) for the measurement of splanchnic triglyceride secretion, and more recently by Sniderman et al. (15) to study LDL cholesterol metabolism by the splanchnic viscera.

A further objective of the study concerned the localization of various steps in the VLDL-to-LDL cascade. The paucity of information on regional lipoprotein metabolism in man contrasts with the wealth of animal data obtained, for example, by use of isolated perfused livers (16, 17), functional hepatectomy (18, 19), multiple indwelling catheters (20), and measurement of organ distribution of ^{125}I following injection of labeled lipoproteins (21).

METHODS

Subjects. 29 male patients were selected at random from those undergoing routine cardiac catheterization for suspected myocardial ischemia in St. Thomas' Hospital. Ages ranged from 26 to 68 yr, plasma cholesterol concentration from 5.0 to 8.3 mmol/liter, and plasma triglyceride from 0.90 to 5.60 mmol/liter. No patient had clinical or biochemical evidence of diabetes mellitus, alcoholism, renal disease, or liver disease. One patient (No. 16) was taking thyroxine for myxoedema; five had been taking lipid-lowering drugs (subject 13, cholestyramine; subjects 1, 22, 23, and 29, clofibrate), but these were withdrawn 3 d before catheterization. The majority of the patients were taking adrenergic beta-blocking agents for angina pectoris. Although some of these have been reported to produce small changes in plasma lipids, they were continued for ethical reasons. The clinical details of each patient are given in Table I. All were hospitalized 4–6 d before catheterization, and remained on an isocaloric solid diet (derived calories: 45, 40, 15; carbohydrate, fat, protein). Body weights remained essentially constant. Coronary angiography demonstrated normal arteries in three patients, and single, double, or triple vessel disease in the remainder.

Preparation of radioiodinated autologous lipoproteins. Peripheral venous blood (150 ml) was collected after a 14-h overnight fast, using disodium EDTA (1 mg/ml) as anticoagulant, and plasma was immediately separated by centrifugation at 4°C. When present, chylomicrons (Sf > 400), were removed by aspiration after overlayering plasma with NaCl solution of $d = 1.006$ g/ml and centrifugation at 20,000 g for 20 min (23). Subsequently lipoproteins of Sf 100-400, Sf 12-60 and Sf 0-12 were isolated by sequential preparative ultracentrifugation at 4°C in an MSE Prepspin 50 centrifuge, according to previously described procedures (23, 24); an MSE 900 rotor (MSE Scientific Instruments, Sussex, England) was used.

4 vol of plasma were overlayered with 2 vol of d 1.006 g/ml solution, and centrifuged at 87,000 g for 50 min (24). The supernatant lipoproteins (Sf 100-400) were removed by aspiration, and concentration by recentrifugation under similar conditions. The infranate was mixed and 4 vol again overlayered with 2 vol of d 1.006 g/ml solution. After centrifugation at 105,000 g for 110 min, the supernatant lipoproteins (Sf 60-100) were aspirated and discarded. After mixing the infranate, 5 vol were added to 1 vol of d 1.085 g/ml solution (to give a final d of 1.019 g/ml) and centrifuged at 105,000 g for 20 h. The supernatant lipoproteins (Sf 12-60, d 1.006–1.019 g/ml) were removed and concentrated by recentrifugation at 1.019 g/ml. Lipoproteins of d 1.019–1.063 g/ml (Sf 0-12) were then isolated by ultracentrifugation at 105,000 g for 20 h, after addition of 2 vol of d 1.151 g/ml solution to 4 vol of the d 1.019 g/ml infranate, and concentrated by recentrifugation at 1.063 g/ml.

The lipoprotein fractions were dialyzed against 0.15 M NaCl (pH 7.4) at 4°C before labeling. Sf 100-400 or Sf 0-12 lipoproteins were labeled with ^{131}I, and Sf 12-60 with ^{125}I, using the iodine monochloride method of McFarlane (25) as modified by Langer et al. (26).

Assuming a molecular weight of 300,000 for total VLDL apolipoprotein (4) and 100,000 for LDL apolipoprotein (2), a maximum of one iodine atom was incorporated per molecule of protein. Free iodide was partially removed from the preparation by passage through a column of Sephadex G-10 (20 × 0.5 cm). Sterile human serum albumin was added to the eluted lipoprotein (final concentration, 5% wt/vol) to minimize auto-irradiation damage. Residual free iodide was removed by dialysis against 0.15 M NaCl – 1 mg/dl Na_2EDTA (pH 7.4). The labeled lipoproteins were then made up to 10 ml with 0.15 M NaCl and passed through a 0.45-μm filter (Millipore Corp., Bedford, Mass.).

TABLE I
Clinical Details of Patients Studied

Study No.	Weight	Height	Age	Plasma cholesterol	Plasma triglyceride	VLDL triglyceride	LDL cholesterol	Lipoprotein phenotype*	Study protocol‡	Drug therapy
	kg	*cm*	*yr*		*mmol/liter*					
1	74	172	38	5.0	0.90	0.6	3.1	IV§	1	Propranolol, clofibrate
2	82	166	49	5.3	2.2	1.4	3.7	Normal	1	Verapamil, salbutamol
3	79	183	40	6.9	4.8	3.2	4.3	IV	1	Propranolol
4	72	170	58	6.5	1.5	0.9	4.6	Normal	1	Ibuprofen, nitrazepam, metoprolol
5	93	184	52	6.2	2.3	1.8	4.1	Normal	1	Propranolol, diazepam
6	78	175	68	6.0	1.7	0.9	4.1	Normal	1	Acebutalol, diazepam, glyceryl trinitrate
7	80	179	58	5.7	2.0	1.3	4.1	Normal	1	Metoprolol, diazepam
8	56	164	63	8.0	5.6	4.6	6.2	IIb	1	Prednisone, metoprolol
9	77	173	48	6.7	1.8	1.1	3.8	Normal	1	None
10	81	169	57	5.2	2.3	1.5	3.8	Normal	1	Propranolol
11	71	168	44	8.0	4.1	3.5	6.0	IIb	1	Propranolol, perhexiline, nifedipine, bendrofluazide
12	70	177	26	5.2	2.0	1.5	3.4	Normal	1	None
13	73	173	42	6.6	2.6	1.8	5.0	IIb	2	Propranolol, cholestyramine, nifedipine
14	75	183	34	8.1	2.1	1.3	6.6	IIa	2	None
15	76	179	58	6.7	0.9	0.4	4.7	Normal	2	Nifedipine, sprinolactone, metoprolol, digoxin
16	73	170	49	7.4	2.7	2.0	4.9	IIb	2	Thyroxine, bendrofluazide
17	73	168	37	7.3	4.5	3.6	4.9	IIb	2	Glyceryl trinitrate
18	84	171	46	6.4	2.9	2.2	4.5	IV	2	Propranolol
19	90	178	45	5.9	2.0	1.4	4.1	Normal	2	Propranolol, nifidipine, sorbide nitrate
20	79	181	59	7.4	2.1	1.3	5.4	IIa	3	Propranolol, oxacepam, glyceryl trinitrate
21	68	174	49	5.6	2.3	1.4	3.8	Normal	3	Metoprolol, chlorthalidone
22	74	171	43	7.7	3.3	2.1	4.8	III‖	3	Propranolol, clofibrate
23	77	173	49	8.3	3.2	2.3	6.5	IIb	3	Propranolol, clofibrate
24	74	180	52	4.6	1.0	0.5	3.4	Normal	3	Digoxin, frusemide
25	65	163	57	6.3	2.0	1.1	4.4	Normal	3	Sotalol, sorbide nitrate
26	82	182	36	8.3	1.4	0.8	5.7	IIa	3	Chlorpromazine
27	70	170	44	6.2	2.8	2.2	4.3	Normal	3	Propranolol
28	65	165	65	7.9	1.8	1.2	6.0	IIa	3	Acebutalol
29	87	180	36	6.6	2.7	2.1	3.6	III‖	3	Clofibrate, atenolol, isosorbide dinitrate

* Beaumont et al. (22).

‡ Radiolabeled lipoprotein(s) injected or infused: 1, ^{131}I-Sf > 100 and ^{125}I-Sf 12-60; 2, ^{125}I-Sf 12-60 and ^{131}I-Sf 0-12; 3, ^{125}I-Sf 12-60 alone. In studies 23–28 blood was sampled from the inferior vena cava as well as from the hepatic vein.

§ Plasma lipoproteins normalized during clofibrate therapy.

‖ Broad β band demonstrated on agarose gel electrophoresis of $d < 1.006$ g/ml lipoproteins.

Less than 4% of the radioactivity in the final preparations was present as free iodide, as determined by precipitation with 15% trichloroacetic acid (10 min, 21°C). The percentage of radiolabel in the lipid moiety averaged 4.5% in Sf 100-400, 4.7% in Sf 12-60, and 3.0% in Sf 0-12 lipoproteins, as determined by solvent extraction according to Scanu and Edelstein (27).

All procedures were carried out using sterile glassware. Inorganic aqueous solutions were made up in pyrogen-free distilled water, sterilized by autoclaving, and stored at 4°C. Cellulose nitrate ultracentrifuge tubes were sterilized with 70% isopropanol, and all lipoprotein manipulations were conducted, whenever possible, in a laminar flow cabinet.

Clinical procedures. The studies were carried out in patients undergoing routine cardiac catheterization in the course of investigation for suspected coronary heart disease. Elec-

tive studies for purely research purposes were not undertaken. The nature of the investigation was discussed with all patients, each of whom gave informed consent.

Each patient received 50 mg potassium iodide orally each morning from the day of admission until 3 d after catheterization, to minimize thyroidal uptake of radioiodine. Catheterization studies were conducted in the morning after a 14-h overnight fast.

Intravenous reinjection of radioiodinated lipoproteins was performed within 96 h of the initial blood collection. Three different protocols were used: (*a*) Bolus injection (in 0.15 M NaCl) of ^{131}I-labeled Sf 100-400 plus ^{125}I-labeled Sf 12-60 lipoproteins (10–30 μCi of each), followed by catheterization 40–60 min later (12 patients). (*b*) Bolus injection of ^{125}I-labeled Sf 12-60 plus ^{131}I-labeled Sf 0-12 lipoproteins (10–30 μCi of each), followed by catheterization 1–5 h later (seven patients). (*c*) Initial bolus injection of ^{125}I-labeled Sf 12-60 (40 μCi), followed by continuous intravenous infusion of this preparation at 10 μCi/h for 5–22 h up to catheterization (10 patients). On each occasion 5–6 mg of lipoprotein protein was injected. There were no pyrogenic or allergic reactions.

The catheterization procedure involved the standard Seldinger technique (28). A No. 7 French catheter was introduced under fluoroscopic guidance into the lower right hepatic vein via the right femoral vein under local anesthesia with lignocaine. A No. 8 Pigtail catheter was introduced into the aorta as far as the origin of the celiac axis, via the right femoral artery. Indocyanine green was infused via an indwelling cannula in the left antecubital vein. Blood samples (60 ml) were drawn simultaneously from the aorta and hepatic vein during a timed 90-s period, and divided into separate aliquots for: (*a*) measurement of packed cell volume in triplicate; (*b*) measurement of indocyanine green; and (*c*) lipoprotein analyses (1 mg Na_2EDTA/ml). Catheter lines were kept patent with sterile 0.15 M NaCl, and no heparin was introduced until completion of the lipoprotein studies, in view of the effect of heparin in releasing lipoprotein lipase and hepatic lipase into the circulation (29). The interval between the start of the catheterization and blood sampling did not exceed 30 min.

Splanchnic plasma flow measurement. After a bolus injection (15 mg, i.v.), indocyanine green (Cardio Green; Hynson, Westcott and Dunning Inc., Baltimore, Md.) was infused (0.5 mg/min) using a Harvard pump for at least 15 min before blood sampling. The concentrations of dye in arterial and hepatic venous plasma were determined spectrophotometrically at 815 nm, using as standard curve dilutions of the dye in plasma and a plasma blank. The Fick principle was applied to calculate the splanchnic plasma flow (SPF) (30):

$$SPF = \frac{I}{C_a - C_{hv}} \text{ milliliters per minute,}$$

where,

I = infusion rate (milligrams per minute),
C_a = concentration (milligrams per milliliter) in artery,
C_{hv} = concentration (milligrams per milliliter) in hepatic vein.

Lipoprotein analyses. Because of the high blood flow through the splanchnic bed, it was assumed that arteriovenous concentration differences would be small; hence, it was considered necessary to conduct multiple replicate analyses on the arterial and hepatic venous samples in order to obtain reliable data. To this end each plasma sample was ultracentrifuged in quadruplicate, apo B isolations were performed in triplicate, and apo B mass measurements were made in duplicate. Throughout the lipoprotein analyses arterial and venous samples were processed in parallel and in identical manner.

The lipoprotein fractions of Sf 100-400, Sf 12-60, and Sf 0-12 were isolated by sequential preparative ultracentrifugation, as already described. Mass and specific activity measurements of apo B in Sf 100-400, Sf 12-60, and Sf 0-12 lipoproteins were made by precipitation of apo B with 1,1,3,3-tetramethylurea (Sigma Chemical Co., St. Louis, Mo.) (31) that had been redistilled in glass at 176°C. Aliquots (0.5 ml) of the lipoprotein fractions were mixed with 0.5 ml tetramethylurea for 10 min at 25°C. When necessary, lipoproteins were first diluted with 0.15 M NaCl to a protein concentration of <1.5 mg/ml, to avoid coprecipitation of apoprotein C (31). Following centrifugation at 6,000 *g* for 20 min the tetramethylurea-soluble infranate was removed with a finely drawn-out Pasteur pipette. Lipid was removed from the pellet by overnight extraction in ethanol/ether (3:2, vol:vol) at 10°C, followed by two ether extractions. The residual apo B precipitate was dried and dissolved in 1 ml of 0.5 M NaOH. After radioactivity determination in an LKB (LKB Instruments, Inc., Rockville, Md.) Wallac gamma counter, the protein content was determined by the method of Lowry et al. (32), slightly modified (in that NaOH was admitted from the sodium carbonate reagent to compensate for the NaOH in which the apo B was dissolved); bovine serum albumin (Sigma Chemical Co.) was used as standard.

The mean coefficients of variation (percent) for replicate assays (n = 24) of apo B mass (m), ^{125}I radioactivity (r) and specific activity in the different lipoproteins were as follows; Sf 100-400: m 4.6, r 2.5, sp act 5.1; Sf 12-60: m 8.4, r 4.2, sp act 7.7; Sf 0-12: m 5.3, r 3.7, sp act 6.0. That for replicate assays of ^{131}I radioactivity in Sf 0-12 was 3.7%.

When ^{125}I-labeled Sf 100-400, ^{131}I-labeled Sf 12-60, or ^{125}I-labeled Sf 0-12 lipoproteins were added to samples of arterial (art) and hepatic venous (hv) plasma, and the corresponding fractions reisolated according to the ultracentrifugal procedures described above, the following percentage recoveries were obtained (mean±SD, n = 6); Sf 100-400: art 91.3±2.2, hv 90.3±2.3; Sf 12-60: art 88.3±3.8, hv 88.0±1.1; Sf 0-12: art 81.7±4.4, hv 84.2±2.6.

Values for the rate of splanchnic secretion or uptake of apo B (milligrams per kilogram per hour) were calculated as: $(C_{hv} - C_a)$. SPF/BW, where C_{hv} = mean hepatic vein apo B concentration (milligrams per milliliter), C_a = mean arterial apo B concentration (milligrams per milliliter), SPF = splanchnic plasma flow (milliliters per hour), and BW = body weight (kilograms).

RESULTS

Values obtained for splanchnic plasma flow (range, 7.7–11.8 ml/min per kg; mean, 9.0) in the 19 patients (Nos. 1–19) in whom this was measured were similar to previously reported values (30). Packed cell volumes in arterial and hepatic venous blood were not significantly different (43.8±0.6 vs. 43.3±0.6%). Thus, none of the recorded differences in lipoprotein mass and radioactivity across the splanchnic bed was attributable to hemodilution or hemoconcentration.

Sf 100-400 lipoproteins. The artery-hepatic vein differences in apo B mass and apo B radioactivity in plasma lipoproteins of Sf 100-400 are presented in Table II. In 11 of 12 subjects the concentration of Sf 100-400 apo B was significantly higher in hepatic vein plasma than in arterial plasma. In no patient, however, was there a statistically significant artery-hepatic vein difference in Sf 100-400 apo B radioactivity. In the

TABLE II

Sf 100-400 Apoprotein B Mass, Radioactivity, and Specific Activity in Arterial and Hepatic Venous Plasma following Intravenous Injection of ^{131}I-labeled Sf 100-400 Lipoproteins

Patient No.	Mass		Radioactivity		Specific activity	
	Artery	Hepatic vein	Artery	Hepatic vein	Artery	Hepatic vein
	mg/dl		*cpm/ml*		*cpm/mg*	
1	3.9±0.09	4.4±0.10*	1,142±90	1,247±40 (NS)	22,019±320	21,526±473‡
2	2.8±0.12	3.6±0.66‡	1,120±49	1,144±42 (NS)	40,795±4,368	32,968±3,045‡
3	10.9±0.71	12.4±0.32*	2,082±81	2,217±82 (NS)	19,266±778	17,879±566‡
4	9.3±0.22	9.9±0.31‡	3,750±64	3,833±54 (NS)	40,778±1,082	38,717±982‡
5	6.2±0.14	7.5±0.09‡	1,708±96	1,631±70 (NS)	27,468±1,994	21,221±2,698‡
6	3.2±0.17	4.0±0.23‡	1,467±27	1,408±69 (NS)	45,794±812	35,000±1,099*
7	5.1±0.14	5.5±0.17‡	1,128±13	1,141±21 (NS)	22,048±413	20,698±385‡
8	10.5±0.20	11.4±0.26*	354±7	349±11 (NS)	3,384±150	3,052±106‡
9	5.2±0.12	5.9±0.14*	2,258±16	2,254±20 (NS)	43,702±1,838	37,936±2,013*
10	6.8±0.29	7.5±0.33*	464±18	480±19 (NS)	6,792±93	6,453±81*
11	10.9±0.38	11.6±0.47 (NS)	1,038±36	1,078±85 (NS)	9,512±114	9,414±174 (NS)
12	6.8±0.32	7.7±0.41‡	896±39	923±22 (NS)	13,384±968	11,882±713‡

Results are given as mean±SEM of 24 measurements. Statistical comparisons of arterial and hepatic venous data were performed by Student's *t* test: * $P < 0.01$; ‡ $P < 0.05$; NS, $P > 0.05$.

pooled data from all patients there was a significant ($P < 0.001$) increase in concentration of 14.0±2.1% (mean±SEM) and a significant ($P < 0.001$) decrease in specific activity of 10.5±2.2% across the splanchnic organs, with no significant arteriovenous difference in apo B radioactivity. These results demonstrate secretion of newly synthesized apo B in Sf 100-400 lipoproteins by splanchnic tissues, with no detectable uptake within the region of Sf 100-400 apo B from arterial blood.

Values for the splanchnic secretion rate of Sf 100-400 apo B ranged from 1.9 to 7.0 mg/kg per h. Results in the hypertriglyceridemic patients (Nos. 3, 8, and 11) were higher than those in the normotriglyceridemic patients (Nos. 2, 4–7, 9, 10, and 12), but this difference failed to achieve statistical significance (6.0±0.78 vs. 4.2±0.51 mg/kg per h; $0.05 < P < 0.10$). Patient 1 was excluded from this comparison on account of the apparent residual triglyceride-lowering effect of clofibrate (see Table I).

Sf 12-60 lipoproteins. As shown in Table III, in 20 of the 29 patients both apo B concentration and apo B radioactivity in Sf 12-60 lipoproteins were significantly lower in hepatic vein plasma than in arterial plasma. Of the remaining nine patients, three showed a statistically significant decrease across the splanchnic bed in apo B concentration alone, and two others showed a significant decrease in radioactivity alone. In the pooled data, the decrease in apo B concentration averaged 6.4±2.9% and that in apo B radioactivity averaged 6.1±3.3%. Accordingly, there was no significant change across the splanchnic bed in the specific activity of Sf 12-60 apo B. Thus, an uptake of Sf 12-60 lipoproteins from arterial plasma was demonstrated within the splanchnic region, without any detectable secretion of newly synthesized apo B into this fraction.

Values for splanchnic removal of Sf 12-60 apo B, calculated in patients 1–19, ranged from 0.90 to 6.3 mg/kg per h. Results in the hypertriglyceridemic patients (again excluding subject 1) did not differ significantly from those in the normotriglyceridemic patients (2.3 ±0.5 vs. 2.7±0.5 mg/kg per h).

Conversion of Sf 12-60 apo B to Sf 0-12 apo B. A proportion of the radioactivity that was extracted from the Sf 12-60 lipoproteins in the splanchnic bed was found to reappear in the apo B of the Sf 0-12 fraction of hepatic venous plasma (Table IV). In the first seven patients (Nos. 13–19), in whom this was quantified following a bolus injection of ^{125}I-labeled Sf 12-60, the incorporation of ^{125}I into hepatic vein Sf 0-12 apo B (expressed as a percentage of the total radioactivity removed from Sf 12-60 apo B) appeared to depend upon the interval between injection and blood sampling, increasing from 22 to 53% as the interval was increased from 2 to 5 h. These results are presented in Fig. 1. Since the recovery of radiolabeled Sf 0-12 apo B from plasma was a little lower than that of radiolabeled Sf 12-60 apo B (see Methods) these values will be slight underestimates.

These findings were explored further in patients 20–29, in whom ^{125}I-Sf 12-60 lipoproteins were infused continuously for 4.5 to 22 h prior to blood sampling. In seven normotriglyceridemic patients (Nos. 20, 21, 24–28) and one hypertriglyceridemic patient (No. 23) the percentage incorporation of extracted Sf 12-60 apo B radioactivity into Sf 0-12 apo B reached a plateau after

TABLE III

Sf 12-60 Aproprotein B Mass, Radioactivity, and Specific Activity in Arterial and Hepatic Venous Plasma following Intravenous Injection of ^{125}I-labeled Sf 12-60 Lipoproteins

Patient No.	Mass		Radioactivity		Specific activity	
	Artery	Hepatic vein	Artery	Hepatic vein	Artery	Hepatic vein
	mg/dl		*cpm/ml*		*cpm/mg*	
1	4.4±0.11	3.9±0.23*	2,648±54	2,544±85*	46,449±1,327	43,632±1,876*
2	5.2±0.27	4.5±0.35*	2,592±145	2,504±251 (NS)	51,844±698	53,348±1,072 (NS)
3	7.5±0.15	7.7±0.18 (NS)	6,362±64	6,377±90 (NS)	84,804±1,943	82,629±1,881 (NS)
4	6.3±0.38	5.5±0.24*	9,605±219	8,677±122*	153,110±2,861	157,636±2,998 (NS)
5	2.1±0.09	1.8±0.08‡	6,633±142	6,135±78*	315,667±4,771	335,137±3,819*
6	6.9±0.13	6.6±0.13*	11,009±227	8,925±352*	159,651±3,845	135,921±3,303‡
7	3.0±0.09	2.8±0.06*	8,973±115	8,431±96*	294,098±6,714	301,071±7,807 (NS)
8	6.2±0.19	5.8±0.11*	3,514±56	3,303±34*	56,850±246	56,995±128 (NS)
9	5.0±0.17	4.6±0.21*	1,932±119	1,791±78*	39,411±328	39,345±514 (NS)
10	5.2±0.14	4.7±0.24*	2,701±76	2,538±71*	52,237±1,132	53,510±968 (NS)
11	6.3±0.11	6.1±0.09 (NS)	4,700±68	4,636±56 (NS)	74,691±844	75,068±967 (NS)
12	3.5±0.08	3.2±0.09*	3,116±47	2,857±115*	88,352±632	88,334±1,944 (NS)
13	5.2±0.14	4.9±0.11*	2,182±63	1,939±50*	41,683±1,246	39,808±1,023 (NS)
14	10.2±0.16	9.6±0.12*	4,890±35	4,664±56*	47,769±583	48,788±821 (NS)
15	4.0±0.08	3.7±0.06*	1,910±40	1,790±53‡	47,178±796	48,241±685 (NS)
16	10.2±0.24	9.6±0.30*	2,194±23	2,015±15*	21,429±361	21,872±405 (NS)
17	10.6±0.21	10.2±0.10*	7,794±196	7,472±116*	73,275±252	73,158±388 (NS)
18	11.2±0.44	10.6±0.25*	822±32	736±26*	7,293±197	7,021±208 (NS)
19	11.1±0.36	10.0±0.48‡	2,097±67	1,845±86‡	18,865±168	19,073±142 (NS)
20	5.4±0.32	4.7±0.29*	2,281±50	2,168±24*	42,351±1,769	45,330±2,054 (NS)
21	2.5±0.06	2.3±0.04*	2,807±48	2,495±50*	114,754±3,991	106,138±3,262*
22	16.3±0.43	16.1±0.39 (NS)	11,827±263	11,667±218 (NS)	72,561±1,358	72,875±984 (NS)
23	6.0±0.29	5.7±0.21 (NS)	9,771±128	9,329±269*	151,960±3,656	162,596±4,931*
24	5.6±0.41	5.4±0.50 (NS)	2,080±30	1,985±13*	36,932±1,728	36,789±2,051 (NS)
25	2.7±0.06	2.5±0.03*	3,504±66	3,381±97 (NS)	128,112±2,547	132,588±3,866 (NS)
26	7.5±0.39	6.8±0.34*	2,120±53	2,001±46*	28,126±1,843	30,164±1,327 (NS)
27	5.2±0.11	4.9±0.17*	7,427±146	7,091±132*	145,036±8,219	145,703±7,961 (NS)
28	3.5±0.18	3.2±0.11*	2,758±37	2,687±60 (NS)	75,550±2,843	82,360±3,115*
29	8.0±0.24	7.8±0.43 (NS)	3,784±30	3,629±38 (NS)	46,667±1,547	46,339±2,159 (NS)

Results are given as mean±SEM of 24 measurements. Statistical comparisons of arterial and hepatic venous data were performed by Student's t test: * $P < 0.05$; ‡ $P < 0.01$; NS, $P > 0.05$.
The segregation of the patients corresponds to the three protocols used (see Table I).

5 h of infusion, averaging 55% beyond this time (range, 41–67%). For technical reasons splanchnic plasma flow was not measured in these patients. Using the mean flow obtained in studies 1–19, their absolute rates of conversion of Sf 12-60 apo B to Sf 0-12 apo B averaged 1.07 mg/kg per h (range, 0.56–2.52). Two patients with Type III hyperlipoproteinemia showed much lower fractional rates of conversion (Fig. 1).

Sf 0-12 lipoproteins. In no patient was there a statistically significant difference between the arterial and hepatic vein concentrations of Sf 0-12 apo B (Table IV), although in the pooled data there was a trend for the mean concentrations to be greater in the hepatic vein than in the artery (paired t test: $0.05 < P < 0.10$). There was no detectable uptake of ^{131}I-apo B by the splanchnic organs when ^{131}I-labeled Sf 0-12 lipoproteins were injected intravenously 5 h before blood sampling (Table IV).

DISCUSSION

In these studies radioiodinated apo B was used as a marker of the metabolism of Sf 0-400 lipoproteins. There is good evidence to support the validity of this approach. Unlike other lipoprotein components, apo B is quantitatively conserved during the conversion of VLDL via IDL to LDL (4, 33). Secondly, following intravenous injection of radioiodinated LDL no significant transfer of apo B radioactivity occurs to less dense lipoproteins (26). Thirdly, lipolysis of VLDL triglyceride in vitro results in the quantitative transfer of apo B to LDL-like particles (34).

TABLE IV

Sf 0-12 Apoprotein B Mass, Radioactivity, and Specific Activity in Arterial and Hepatic Venous Plasma following Intravenous Injection of ^{125}I-labeled Sf 12-60 Lipoproteins and ^{131}I-labeled Sf 0-12 Lipoproteins

Patient No.	Mass		^{131}I Radioactivity		^{131}I Specific activity		^{125}I Radioactivity	
	Artery	Hepatic vein	Artery	Hepatic vein	Artery	Hepatic vein	Artery	Hepatic vein
	mg/dl		*cpm/ml*		*cpm/mg*		*cpm/ml*	
13	72.5±6.63	78.8±7.20 (NS)	3,470±51	3,557±80 (NS)	4,772±363	4,629±347 (NS)	275±11	320±34*
14	105.6±4.91	109.2±4.71 (NS)	412±7	417±6 (NS)	401±61	388±48 (NS)	51±8	57±12 (NS)
15	70.0±3.40	71.9±2.80 (NS)	789±23	773±18 (NS)	1,114±206	1,094±231 (NS)	144±12	171±20*
16	110.5±6.74	118.9±5.21 (NS)	437±26	451±16 (NS)	387±47	391±65 (NS)	166±17	193±14*
17	97.5±4.10	97.3±3.81 (NS)	439±17	446±28 (NS)	441±54	457±63 (NS)	1,746±17	1,854±27*
18	88.1±5.22	92.6±6.47 (NS)	822±32	874±26 (NS)	946±73	932±56 (NS)	273±20	314±13*
19	83.5±4.63	78.6±4.45 (NS)	307±15	312±8 (NS)	388±24	399±53 (NS)	910±22	1,044±48*
20	96.8±5.83	90.4±4.74 (NS)					1,018±21	1,066±18*
21	94.5±3.39	93.4±2.92 (NS)					1,184±29	1,389±60*
22	67.5±2.18	69.1±3.41 (NS)					4,025±138	4,039±121 (NS)
23	94.8±5.07	95.3±4.91 (NS)					7,528±64	7,731±90*
24	71.4±5.42	75.3±4.71 (NS)					4,923±56	4,977±49 (NS)
25	57.1±2.90	57.9±3.62 (NS)					994±28	1,058±19*
26	94.0±3.79	96.2±4.11 (NS)					1,083±24	1,164±32*
27	55.7±4.15	57.7±6.61 (NS)					2,630±48	2,803±67*
28	106.5±4.79	108.1±3.88 (NS)					3,947±23	3,986±19*
29	82.3±6.12	80.0±5.80 (NS)					3,137±38	3,157±51 (NS)

Subjects 20–29 received ^{125}I-labeled Sf 12-60 lipoproteins only (protocol 3); subjects 13–19 were given both isotopes (protocol 2). Results are given as mean±SEM of 24 measurements. Statistical comparisons of arterial and hepatic venous data were performed by Student's *t* test: * $P < 0.05$; NS, $P > 0.05$.

A second assumption was that the measurements were made under steady-state conditions. To this end the patients spent several days in a metabolic ward prior to catheterization, so as to ensure constancy of energy balance and dietary composition, and all studies were conducted in the fasted state. During the precatheterization period body weights remained essentially constant.

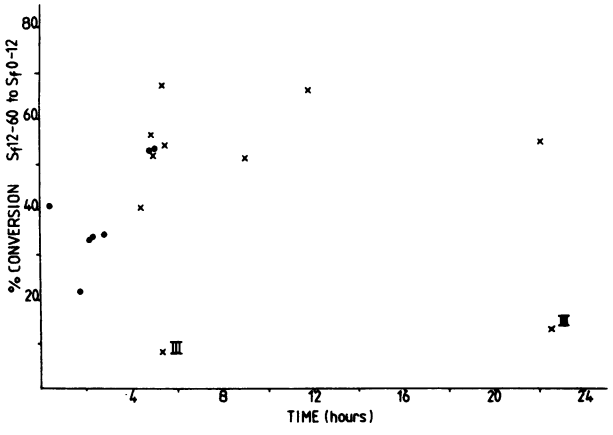

FIGURE 1 Percentage conversion by splanchnic tissues of extracted Sf 12-60 apo B to Sf 0-12 apo B. Values on the horizontal axis are the time intervals between intravenous bolus injection (●) or commencement of a continuous intravenous infusion (×) of radioiodinated Sf 12-60 lipoproteins and blood sampling. The two Type III hyperlipoproteinemic patients are indicated.

The results showed that newly synthesized apo B is secreted by splanchnic tissues as a component of Sf 100-400 lipoproteins in man. Using similar techniques, net secretion of triglyceride by human splanchnic viscera has been demonstrated (13, 14), and VLDL production by the perfused rat liver (17, 35, 36) is well documented. No splanchnic uptake of Sf 100-400 apo B could be detected in any of the patients studied, on the basis of either mass or radioactivity measurements. It remains possible, however, that some Sf 100-400 triglyceride was hydrolyzed by splanchnic tissues without producing a detectable shift of ^{131}I-apo B to Sf < 100 lipoproteins.

In contrast to the results obtained for Sf 100-400 lipoproteins, no splanchnic secretion of apo B in Sf 12-60 lipoproteins could be detected. This finding is compatible with the evidence from kinetic studies that it is the VLDL subclasses of greatest flotation rate that are the primary secreted forms, the smaller more dense subclasses being largely catabolic products (3, 4, 37). The results of in vitro studies, in which the hydrolysis of VLDL triglyceride by lipoprotein lipase has been shown to generate progressively smaller and denser particles (34, 38), have supported this concept.

On the basis of the known composition of VLDL (24), the artery-hepatic vein differences in Sf 100-400 apo B concentration were in agreement with those in VLDL

cholesterol concentration reported by Sniderman et al. (15). The calculated splanchnic secretion rates of Sf 100-400 apo B in the fasted state were also compatible with the rates of splanchnic triglyceride secretion obtained by Boberg et al. (14) by measurement of artery-hepatic vein differences in triglyceride concentration under similar experimental conditions. Our secretion rates of apo B exceeded, however, the rates of VLDL apo B synthesis in the fed state (low-fat diets) previously obtained in this and other laboratories by kinetic analysis of plasma apo B specific activity decay curves after intravenous injection of radiolabeled VLDL (3–9). This suggests that there may be a diurnal variation in the hepatic production of VLDL apo B in man, perhaps in response to the documented diurnal variation in plasma free fatty acid concentrations (39). It is also possible that there is some recycling of apo B, derived from extracted Sf > 100 particles, into new Sf 100-400 lipoproteins by the liver.

Another notable aspect of this study was the demonstration that the human splanchnic organs extract Sf 12-60 lipoproteins from the circulation. The role of the liver in the uptake of VLDL "remnants" (19) and chylomicron remnants (18) in the rat is well documented, but there is no published evidence for a similar process in man. The rates of splanchnic Sf 12-60 apo B extraction in eight normotriglyceridemic patients were lower ($P < 0.05$) than the rates of Sf 100-400 apo B secretion, suggesting that there was some extrasplanchnic catabolism of VLDL particles not resulting in their conversion to Sf 12-60 lipoproteins. The reason for the discrepancy between this result and the evidence from kinetic studies for the quantitative transfer of apo B from VLDL to Sf 0-20 lipoproteins in normotriglyceridemic individuals (2, 4) is not clear, but may be related to the differing experimental conditions under which the measurements were made.

Our results also indicated that a proportion of the Sf 12-60 lipoproteins extracted by the human splanchnic bed is converted to Sf 0-12 lipoproteins. The percentage transfer of extracted Sf 12-60 radioactivity to hepatic vein Sf 0-12 lipoproteins increased in curvilinear manner with increasing time interval between the injection of radiolabeled Sf 12-60 and blood sampling, perhaps reflecting the presence of a slowly turning-over intermediate pool in the splanchnic tissues. The greatest conversion rates, observed after continuous intravenous infusion of radiolabel for 5–22 h, averaged 55%.

Our measurements failed to detect any splanchnic extraction of Sf 0-12 lipoproteins. Evidence obtained from tissue culture (40) and animal experiments (41) suggests that LDL is catabolized partly in the liver and partly in extrahepatic tissues. Although our findings do not support a role of the liver in LDL catabolism, they do not exclude this possibility: artery-hepatic vein differences in radioactivity too small to be demonstrated by the procedure used for isolating Sf 0-12 apo B could nevertheless be physiologically significant.

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