Metabolism of apoprotein B in selectively bred baboons with low and high levels of low density lipoproteins

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Abstract Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) apoprotein (apo)-B turnover rates were measured simultaneously by injecting ^{131}I-labeled VLDL and ^{125}I-labeled LDL into fasting baboons (*Papio* sp.) selectively bred for high serum cholesterol levels and having either low or high LDL levels. The radioactivities in VLDL, intermediate density lipoprotein (IDL), LDL apoB, and urine were measured at intervals between 5 min and 6 days. Kinetic parameters for apoB were calculated in each baboon fed a chow diet or a high cholesterol, high fat diet (HCHF). VLDL apoB residence times were similar in the two groups of animals fed chow; they were increased by HCHF feeding in high LDL animals, but not in low LDL animals. Production rates of VLDL apoB were decreased by the HCHF diet in both high and low LDL animals. Most of the radioactivity from VLDL apoB was transferred to IDL. However, a greater proportion of radioactivity was removed directly from IDL apoB in low LDL animals than in high LDL animals, and only about one-third appeared in LDL. In high LDL animals, a greater proportion of this radioactivity was converted to LDL (61.4 ± 7.2% in chow-fed animals and 49.2 ± 10.9% in animals fed the HCHF diet; mean ± SEM, n = 5). Production rates for LDL apoB were higher in high LDL animals than those in low LDL animals on both diets. The HCHF diet increased residence times of LDL apoB without changing production rates in both groups. VLDL apoB production was not sufficient to account for LDL apoB production in high LDL animals, a finding that suggested that a large amount of LDL apoB was derived from a source other than VLDL apoB in these animals. —**Kushwaha, R. S., G. M. Barnwell, K. D. Carey, and H. C. McGill, Jr.** Metabolism of apoprotein B in selectively bred baboons with low and high levels of low density lipoproteins. *J. Lipid Res.* 1986. **27:** 497–507.

Supplementary key words lipoproteins • atherosclerosis • lipoprotein metabolism

Epidemiologic data from humans and experimental evidence from animal models have suggested that an elevated low density lipoprotein (LDL) concentration in plasma is a major cause of atherosclerosis (1–5). Levels of these lipoproteins in the plasma vary considerably among humans and among experimental animals, even while they are consuming identical diets. The baboon (*Papio* sp.), a large nonhuman primate, varies in its plasma lipoprotein response to diet within a range similar to that of humans (6), and antisera to human apoproteins cross-react with baboon lipoproteins (7). These similarities suggest that the baboon may be useful as a model with which to investigate the metabolic basis of elevated plasma LDL concentrations.

We have selectively bred baboons by positive assortative mating of high and low responders to an atherogenic diet, and have demonstrated that the response in total serum cholesterol concentration is heritable (8, 9). Furthermore, preliminary evidence indicates that the LDL cholesterol concentration in the baboons fed an atherogenic diet is subject to a major gene effect (C. Kammerer et al., unpublished data). Since apolipoprotein B (apoB) from very low density lipoproteins (VLDL) is the precursor for LDL apoB, VLDL apoB catabolism may play a significant role in the regulation of plasma LDL levels. Production and clearance rates of LDL apoB also may contribute to control of plasma LDL levels. The present studies were conducted to determine whether production, or catabolism, or both, of either VLDL or LDL apoB are responsible for the contrasting high and low plasma concentrations of LDL in selectively bred pedigreed baboons.

MATERIALS AND METHODS

Animals and diets

To search for genetic dyslipoproteinemias, we surveyed about 750 pedigreed baboon offspring (*Papio* sp.) for their lipoprotein profiles after they were fed a chow diet or an atherogenic diet for 7 weeks (10). Among 187 adult male feral baboons surveyed to establish the original breeding colony, the average serum VLDL + LDL cholesterol concentration for males on chow was 40.4 ± 12.3 (mean

Abbreviations: apo, apoprotein (apolipoprotein); VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HCHF, high cholesterol, high fat diet; TMU, tetramethylurea.

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± SD) mg/dl, and on an atherogenic diet, 78.4 ± 41.5 mg/dl. Corresponding values for 191 females on chow were 48.5 ± 11.9 mg/dl, and on an atherogenic diet, 82.5 ± 40.3 mg/dl. High and low responders from this group were bred by positive assortative mating. There were wide differences in serum LDL cholesterol concentrations among the different sire groups of progeny.

We selected from among these progeny six baboons (three males and three females) with low LDL levels and six baboons (four males and two females) with high LDL levels. Of the low LDL animals, four were progeny of sire X84 and two were progeny of sires X98 and A776. The serum VLDL + LDL cholesterol level for the six low LDL animals fed chow was 18.2 ± 12.7 mg/dl, and on an atherogenic diet, 44.7 ± 3.6 mg/dl. Of the high LDL animals, four were progeny of sire X113 and two were progeny of sires X116 and X1126. The mean serum VLDL + LDL cholesterol level in chow-fed baboons was 74.5 ± 10.2 mg/dl, and on an atherogenic diet, 205.7 ± 50.6 mg/dl. The plasma lipoprotein profile, body weight, age, and sex of each animal are described in **Fig. 1** and **Table 1**. The lipoprotein profiles of the sires were similar to those of their progeny selected for this experiment.

Animals were studied first while consuming the chow diet (Monkey Chow 25, Ralston Purina Co., Indianapolis, IN), and again after consuming the atherogenic diet enriched in cholesterol and saturated fat (HCHF) for 8 weeks. The compositions of these diets are given in **Table 2**. Four animals (two high LDL and two low LDL animals) were studied at each of three times.

This experiment was approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research.

Isolation and labeling of lipoproteins

To isolate VLDL and LDL for labeling and reinjection, fasting (12–14 hr) baboons under ketamine immobilization (10 mg/kg) were bled from the femoral vein with a syringe. Blood (25 ml from each animal) was collected into tubes containing EDTA (1 mg/ml) and was centrifuged in a low speed refrigerated centrifuge (TJ6, Beckman Instruments, Inc., Palo Alto, CA) to obtain plasma. The plasma was then ultracentrifuged to separate VLDL using a 50 Ti rotor and L5-50 ultracentrifuge (Beckman Instruments, Inc.) (6). The VLDL fractions were washed once under similar conditions and pooled. Solid potassium bromide (KBr) was added to the infranatant from individual animals to adjust the density to 1.019 g/ml. The infranatant was then layered with KBr solution (d 1.019 g/ml) and ultracentrifuged at 45,000 rpm at 6°C for 22 hr. After ultracentrifugation, IDL was removed by slicing the top 3.5-ml layer and discarded. The infranatant was once again ultracentrifuged under similar conditions to remove any contaminating IDL. Following removal of IDL, the infranatant was adjusted to density 1.063 g/ml by adding solid KBr and layered with KBr solution (d 1.063 g/ml). It was then ultracentrifuged under similar conditions for 24 hr and the LDL (d 1.019–1.063 g/ml) was obtained by slicing off the top 3.5-ml layer. The LDL from individual animals was washed by ultracentrifuging once again under similar conditions and it was then dialyzed immediately against saline (d 1.006 g/ml) containing EDTA to remove KBr.

The protein content of pooled VLDL and individual samples of LDL was measured by the method of Lowry et al. (11) with a slight modification (6). VLDL (1–2 mg of protein) and LDL (2–4 mg of protein) were iodinated by the iodine monochloride procedure of McFarlane (12) as modified by Bilheimer, Eisenberg, and Levy (13) and as we have described for baboons (6), rabbits (14), and humans (15). The pooled VLDL was labeled with ^{131}I (ICN Chemicals and Radioisotope Division, Irvine, CA) and individual LDL fractions were labeled with ^{125}I.

Labeled lipoproteins were characterized for radioactivity in free iodide, trichloroacetic acid-precipitable fraction, lipid, and apoB (6, 14). The radioactivity in apoB was measured by the tetramethylurea (TMU) method described by Kane (16) and by using 3.5% polyacrylamide gels containing sodium dodecyl sulfate (SDS) (17). Labeled VLDL and LDL did not have any appreciable free

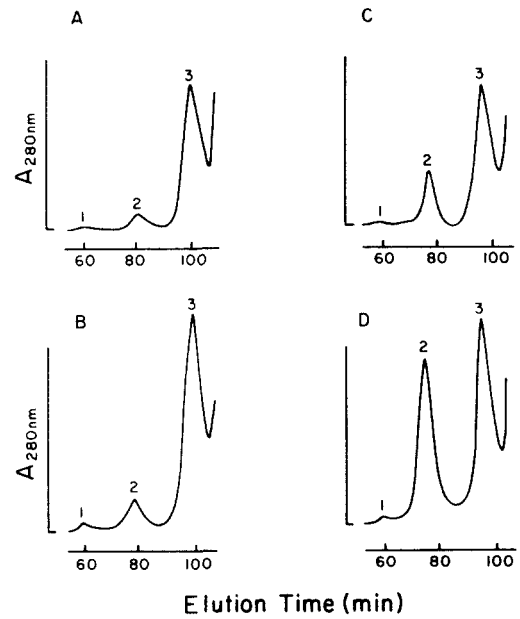

Fig. 1. Elution patterns monitored at 280 nm for plasma lipoproteins separated by high performance gel exclusion chromatography (HPLC) for representative animals, one having low levels of plasma LDL on chow (A) and on a high cholesterol, saturated fat diet (B), and another with high LDL levels on chow (C) and on a high cholesterol, saturated fat diet (D). Numbers 1, 2 and 3 correspond to VLDL, LDL, and HDL, respectively. HPLC used a combination of TSK columns 4000 PW and 3000 PW with a flow rate of 0.2 ml/min. The retention time is given in minutes.

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iodide (<0.5%) and most of the radioactivity was precipitated with trichloroacetic acid (>95%). Most of the radioactivity of VLDL and LDL was present in apoB (**Table 3**).

Lipoprotein turnover procedures

Each animal was injected simultaneously with 0.2–0.3 mg of pooled VLDL protein in a volume of 1–2 ml of saline and 1–2 mg of autologous LDL protein in a volume of 2–4 ml. The specific activity of VLDL and LDL varied from 20–100 μCi/mg and 26–50 μCi/mg, respectively.

For the injection of labeled lipoproteins and collection of blood samples during the first 12 hr, the animals were immobilized with ketamine (10 mg/kg) after an overnight fast and maintained under intravenous anesthesia by infusing sodium pentobarbital at 30–45 mg/hr. Animals were maintained in stage three of anesthesia. We monitored eyelash reflex and jaw muscle tension to assess the appropriate level of anesthesia. At subsequent intervals from 24 to 144 hr, the blood was collected while the animals were under ketamine immobilization. Six-ml blood samples were drawn into tubes containing EDTA (1 mg/ml) at 5, 15, and 45 min and 1, 2, 4, 6, 8, 10, 12, 24, 30, 48, 72, 96, 120, and 144 hr. In the last study, samples were drawn up to 196 hr. Each blood sample was centrifuged to obtain plasma. Plasma samples (2.5 ml) were ultracentrifuged sequentially in L5-50 and L8-70 ultracentrifuges (Beckman Instruments, Inc.) using 50.3 Ti rotors (6).

Small samples (200 μl) of plasma and of each lipoprotein fraction were counted to determine the radioactivity using a gamma spectrometer (Nuclear-Chicago, Des Plaines, IL). The raw counts were corrected for overlapping ^{131}I-counts into ^{125}I-channel and isotopic decay. After the radioactivity was counted, the same sample was used to extract the lipids by the method of Folch, Lees, and Sloane Stanley (14, 18). The chloroform phase was dried before counting for the radioactivity. Another sample (200 μl) from each fraction was used for measuring TMU-insoluble radioactivity by treatment with an equal volume of TMU and incubation at 37°C for 0.5 hr (16). The mixture was then passed through a glass wool filter fitted in a Pasteur pipet as described earlier and the precipitate was washed with saline. The TMU-soluble fraction was counted and the apoB radioactivity was determined by subtracting lipid and TMU-soluble radioactivity from the total radioactivity. In the first study, the apoB radioactivity was also measured by separating the delipidated lipoproteins in 3.5% polyacrylamide gels containing 0.1% SDS (17). Delipidation was carried out by extracting the lyophilized lipoproteins (500 μl) with chloroform–methanol 2:1 or ethanol–diethyl ether 3:1 and finally with diethyl ether alone. The tube gels were stained with Coomassie blue and destained with methanol–acetic acid–water 5:2:13 using destainer (Hoefer Scientific Instruments, San Francisco, CA). The protein band corresponding to apoB was sliced and counted. Tube gels were not overloaded to avoid aggregation on the top of the gel as seen in overloaded lanes in slab gels. The apoB from injected lipoproteins did not aggregate on the top of the gel. The radioactivity in apoB measured by the TMU method and that measured by the SDS gel method were similar; therefore, in subsequent experiments, radioactivity in apoB was measured by the TMU method. The apoB radioactivity in three different samples of VLDL

TABLE 1. Characterization of study animals and their plasma lipids and lipoproteins on chow and on high cholesterol, saturated fat (HCHF) diets

Variables	Units	Low LDL Baboons	High LDL Baboons
Age	yr	5.8 ± 0.48[a]	4.0 ± 0.45
Sex			
Male		3	4
Female		3	2
Body weight	kg	20.6 ± 2.90	10.6 ± 1.39
Plasma cholesterol	mg/dl		
Chow		70.0 ± 2.97	131.0 ± 8.63
HCHF		129.8 ± 7.28	246.5 ± 13.99
Plasma triglycerides	mg/dl		
Chow		59.0 ± 16.74	63.9 ± 25.4
HCHF		67.0 ± 17.35	75.4 ± 15.3
VLDL cholesterol	mg/dl		
Chow		5.2 ± 0.8	2.2 ± 0.7
HCHF		7.2 ± 1.3	5.1 ± 1.6
VLDL apoB	mg/dl		
Chow		3.21 ± 0.81	4.31 ± 1.04
HCHF		2.71 ± 0.37	3.36 ± 0.43
IDL cholesterol	mg/dl		
Chow		3.58 ± 0.63	2.92 ± 0.76
HCHF		4.10 ± 0.82	10.83 ± 1.86
LDL cholesterol	mg/dl		
Chow		13.3 ± 3.7	45.7 ± 4.8
HCHF		60.7 ± 4.0	151.0 ± 13.7
LDL apoB	mg/dl		
Chow		12.9 ± 1.5	44.2 ± 5.3
HCHF		21.6 ± 3.4	74.4 ± 8.2
HDL cholesterol	mg/dl		
Chow		47.9 ± 2.9	57.9 ± 4.5
HCHF		80.0 ± 6.8	79.8 ± 8.9

[a]Mean ± SEM, n = 6.

TABLE 2. Composition of chow and high cholesterol, saturated fat (HCHF) diets

Nutrients	Chow Diet[a]	HCHF Diet[b]
Carbohydrates (% calories)	62	40
Protein (% calories)	28	20
Fat (% calories)	10	40
Energy (Kcal per 100 g diet)	329	377
Cholesterol (mg/Kcal)	0.03	1.7

[a]Monkey chow 25, manufactured by Ralston Purina Co., Indianapolis, IN.

[b]HCHF diet was prepared by mixing 81.4% (dry weight basis) of Purina monkey meal 5-5045-6 (a special mix with no added fat, dehydrated alfalfa, sodium chloride, ascorbic acid, or retinyl acetate) with lard (16.5%), sodium chloride (1.1%), retinyl acetate (0.005%), ascorbic acetate (0.2%), and cholesterol (0.74%).

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TABLE 3. Percent of total radioactivity in labeled lipoproteins from baboons fed chow and high cholesterol, saturated fat (HCHF) diets

	VLDL		LDL	
	Chow	HCHF	Chow	HCHF
	%			
Trichloroacetic acid-precipitable	88.27 ± 3.06*	88.17 ± 1.80	95.071 ± 1.76	97.20 ± 0.76
Free iodine	<0.1	<0.1	<0.1	<0.1
Lipid	12.74 ± 1.42	16.07 ± 6.19	2.53 ± 0.55	5.11 ± 0.42
ApoB	51.31 ± 7.41	65.27 ± 4.42	82.38 ± 2.72	87.60 ± 1.50

*Mean ± SEM, n = 3.

(which were labeled separately) measured by the TMU method was 45.7%, 56.9%, and 69.5% of the total radioactivity. The VLDL apoB radioactivity for the same samples measured by SDS gel methods was 44.0%, 56.7%, and 71.8%, respectively. Similarly, very close values were obtained for LDL apoB (88.1%, 91.8%, and 88.6% by the TMU method versus 92.0%, 90.3%, and 87.8%, respectively, by SDS gels).

Lipid and protein analyses

Cholesterol and triglycerides in plasma and lipoproteins were measured by enzymatic methods using cholesterol and triglyceride measurement kits (Sigma Chemical Co., St. Louis, MO). ApoB was measured by electroimmunoassay (19) using monospecific antibody against baboon apoB (20). Total protein in lipoproteins was measured by the method of Lowry et al. (11).

Plasma lipoprotein profiles of low and high LDL animals were characterized by HPLC (10). A Waters Associates HPLC Model 204 with pump (Model 6000A) and UV detector (Model 440) was used for lipoprotein separation. Lipoprotein peaks were detected by monitoring absorbance at 280 nm using a Sigma 15 recorder-integrator (Perkin-Elmer Corp., Norwalk, CT). A combination of gel permeation columns TSK 4000 PW and TSK 3000 PW (600 × 7.5 mm) were used with a flow rate of 0.2 ml/min (10).

Kinetic analyses

The kinetic data for VLDL apoB turnover were fitted to the integrated kinetic model for human VLDL apoB (21) using the CONSAM program (22). The model is described in **Fig. 2**. According to this model, the first phase is rapid and consists of a delipidation chain with four compartments (1, 6, 7, and 8). The residence time (RT) for VLDL apoB particles in the chain was calculated as given below in a manner similar to that described for human α_2-VLDL (23).

$$RT_{chain} = \frac{[1-P(21)]^4}{P(1)\times[1-P(21)]}$$

where P(1) is the total turnover of each compartment in the delipidation chain and P(21) is the fraction of P(1) diverted to compartment 21.

The second phase was represented by a slowly turning over compartment 21. However, a very small proportion of radioactivity decayed by the slower second phase. The residence time for whole VLDL was therefore calculated by the following relationship.

$$RT_{VLDL} = \frac{mass_{VLDL}}{U(1)}$$

where $mass_{VLDL}$ is the total amount of VLDL in compartment 1 and U(1) is the amount of VLDL going into compartment 1 per unit time.

Residence times for IDL and LDL were calculated from the relations:

$$RT_{IDL} = \frac{1}{P(2)}$$

where P(2) is the turnover rate of compartment (2).

$$RT_{LDL} = \frac{1}{L(0,3)}$$

where L(0,3) is the rate of loss of LDL from compartment 3.

The percent of VLDL transferred to IDL and LDL was calculated by the relationships described by Berman et al. (24).

$$\%\ \text{VLDL to IDL} = \frac{R(2,22)}{U(1)} \times 100$$

where R(2,22) is the amount flowing into compartment 2 from 22.

$$\%\ \text{VLDL to LDL} = \frac{R(3,2)}{U(1)} \times 100$$

where R(3,2) is the amount going into compartment 3 from 2.

The data for LDL turnover studies were analyzed using the conventional two-pool model proposed by Langer, Strober, and Levy (25). The production rates (turnover rates) were calculated by multiplying the fractional catabolic rate by the apoB pool in VLDL and LDL. The pro-

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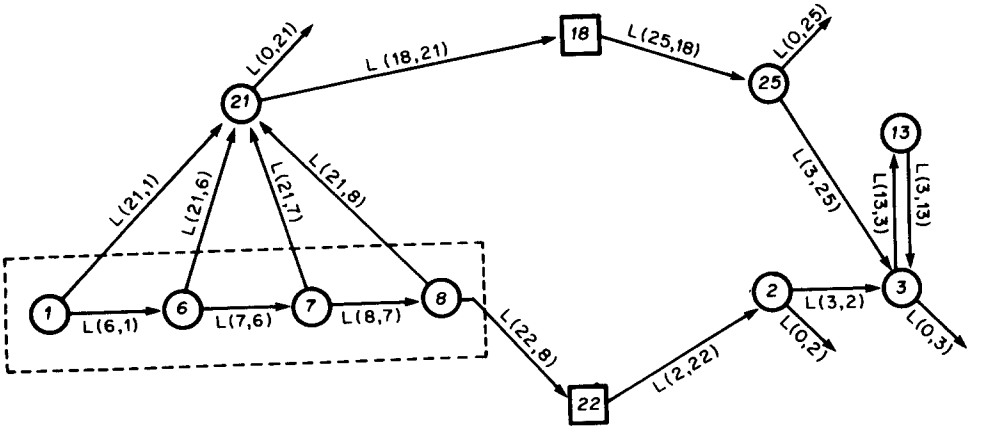

Fig. 2. Kinetic model for VLDL apoB metabolism used for these studies. The model consists of a four-compartment delipidation chain (compartments 1, 6, 7, and 8) and a slowly turning over pool (compartment 21). The model also has an IDL subsystem, containing a rapidly turning over pool (compartment 2), a slowly turning pool (compartment 25), and a two-pool LDL subsystem consisting of compartments 3 and 13. There are two delay compartments (22 and 18) in the conversion of VLDL to IDL. Rate constant L(x,y) is defined as the rate of material going into x from y per unit time. The rate constant L(o,z) is defined as the removal of material from compartment z per unit time.

duction rates were expressed as mg/kg per day.

Statistical analysis

Metabolic parameters obtained from the kinetic analysis for each animal and plasma lipid and lipoprotein levels in both groups of animals on the chow and HCHF diet were analyzed using repeated measures analysis of variance design. The repeated measures analysis of variance simultaneously tested for differences between low and high LDL groups and between chow and atherogenic diets. After detecting significant differences by analysis of variance, individual means were compared by using Duncan's New Multiple Range Test to see which pairs of means were significantly different. Data in tables have been presented as mean ± standard error.

RESULTS

Effect of HCHF diet on plasma lipoproteins

As shown in Table 1 and Fig. 1, both high and low LDL baboons responded to the HCHF diet with similar proportional increases in plasma cholesterol concentrations. Plasma cholesterol levels were increased 2-fold; LDL cholesterol, 3- to 4-fold; LDL apoB, two-fold; and HDL cholesterol, 1.5-fold. The ratio of cholesterol to apoB in LDL increased on the HCHF diet in both high and low LDL animals, an observation that suggests that LDL was enriched in cholesterol. These observations are similar to those previously reported for baboons (6).

Fig. 3 shows the apoprotein composition of VLDL, IDL, and LDL analyzed by 3.5% SDS-polyacrylamide gel electrophoresis. All these fractions contained apoB-100 as the only apoB component. Other apoproteins in LDL were present to a very limited extent. ApoE content was increased slightly in all fractions from animals fed the HCHF diet.

VLDL turnover

The decay of radioactivity in VLDL, IDL, and LDL apoB in baboons on chow and on the HCHF diet is shown in **Fig. 4**. The radioactivity in VLDL apoB decayed in a biphasic manner in both low and high LDL animals on both diets. In chow-fed baboons, the residence time for VLDL apoB in the delipidation chain (first phase) was very rapid (**Table 4**). Most of the radioactivity decayed by the end of the first phase, and only a small proportion decayed by the second phase (compartment 21). However, in both low and high LDL animals consuming the HCHF diet, a relatively greater proportion of radioactivity decayed in the second phase. In low LDL animals, diet had no effect on the residence times for apoB radioactivity in whole VLDL (residence time 1.36 ± 0.59 hr, chow; and 1.53 ± 0.32 hr, HCHF). In high LDL animals, the HCHF diet significantly increased ($P < 0.05$) the residence time for VLDL apoB radioactivity (7.5 ± 1.94 hr) compared to the residence time for the chow-fed animals (1.73 ± 0.45 hr). The residence time for VLDL apoB in high LDL animals was higher ($P < 0.05$) than that in low LDL animals either on chow or on an atherogenic diet (Table 4).

Radioactivity from VLDL apoB appeared rapidly in the IDL fraction, and almost all the VLDL apoB radioactivity transferred into IDL (Table 4). Subsequently, radioactivity in IDL apoB decayed very rapidly and, like

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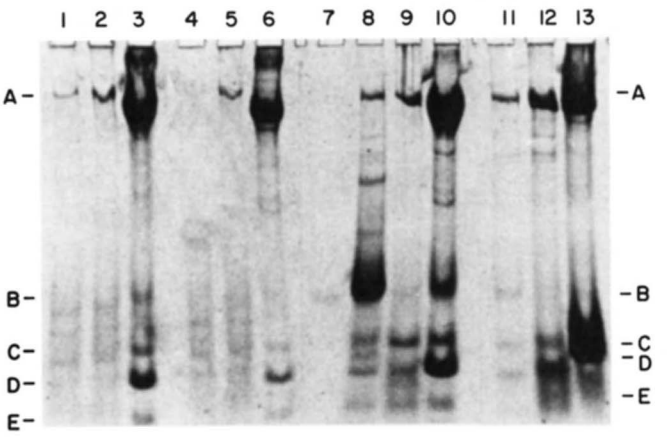

Fig. 3. Electrophoretic patterns of VLDL, IDL, and LDL on 3.5% polyacrylamide gels containing SDS. Numbers 1, 2, and 3 correspond to VLDL, IDL, and LDL, respectively, from a low LDL animal on chow diet; 4, 5, and 6 correspond to VLDL, IDL, and LDL, respectively, from a high LDL animal on chow diet; 7 corresponds to albumin standard; 8, 9, and 10 correspond to VLDL, IDL, and LDL, respectively, from a low LDL animal on HCHF diet; and 11, 12, and 13 correspond to VLDL, IDL, and LDL, respectively, from a high LDL animal on HCHF diet. The bands A, B, C, D, and E correspond to apoB-100, albumin, apoE, apoA-I and apoCs, respectively. Gels were overloaded in order to see the minor bands. The aggregation on the top in some lanes is due to overloading.

VLDL apoB, this decay was biphasic (Fig. 4).[2] There were no significant effects of diet on residence time of IDL apoB in either low or high LDL animals, but the residence time of IDL apoB was longer in the high LDL animals.

VLDL apoB radioactivity also appeared rapidly in LDL, but to a variable extent. In low LDL animals, the transfer of VLDL radioactivity to LDL was 35.2 ± 8.6% when they consumed chow and 36.2 ± 14.2% when they consumed the HCHF diets. In high LDL animals consuming the chow diet, a significantly ($P < 0.05$) greater proportion of VLDL radioactivity was transferred to LDL than was transferred in low LDL animals fed the same diet. In high LDL animals, the transfer was higher in chow-fed baboons (61.4 ± 7.2%) than in those fed the HCHF diet (49.2 ± 10.9%). The radioactivity in LDL then decayed slowly and biphasically. The residence time for LDL did not differ significantly between low and high LDL animals on either diet (Table 4).

The production rate of VLDL apoB in low LDL animals on the chow diet was 27.5 ± 3.1 mg/kg per day. The HCHF diet significantly decreased the production rate for VLDL apoB in both low LDL and high LDL animals (Table 4). Regardless of diet, the production rate of VLDL apoB was similar in high and low LDL animals.

LDL turnover

The radioactivity from ^{125}I-labeled LDL apoB decayed biphasically in all animals (**Fig. 5**). The pattern of decay was similar to that of ^{131}I-labeled LDL apoB derived from VLDL (Fig. 4). The residence time for ^{125}I-labeled LDL apoB in low LDL animals on chow was 19.3 ± 2.1 hr and was increased by the HCHF diet (**Table 5**). Likewise, the residence time for ^{125}I-labeled LDL apoB in high LDL animals on chow was 22.3 ± 2.1 hr and the HCHF diet increased these rates significantly ($P < 0.01$) to 37.6 ± 5.1 hr.

LDL apoB production rates, as measured by direct injection of ^{125}I-labeled LDL, were not affected by diet in either animal group (Table 5). However, the production rates for LDL apoB were significantly different between the two groups. Production rates were approximately 3-fold higher ($P < 0.01$) in high LDL animals than those in low LDL animals on both chow and the HCHF diet (Table 5).

Since only about one-third of the VLDL apoB in VLDL in chow-fed low LDL animals was transferred to LDL, it appeared that most of the LDL apoB was derived from VLDL. HCHF feeding of low LDL animals did not affect the production of LDL apoB. It did, however, decrease VLDL apoB production. Since there was a decrease in VLDL apoB production due to cholesterol and saturated fat feeding, the LDL apoB production could not be accounted for by the VLDL apoB production in these animals on the HCHF diet. Up to approximately 43% of the LDL apoB was derived from sources other than VLDL apoB in low LDL animals. In high LDL animals, on the other hand, the VLDL apoB production rate was not enough to account for the LDL production either in the chow-fed or the saturated fat- and cholesterol-fed state. Approximately 45% of the LDL apoB in chow-fed animals and 83% of the LDL apoB in HCHF-fed animals were derived from sources other than VLDL apoB. On the HCHF diet, the production of LDL apoB in high LDL animals was approximately 3-fold higher than that of VLDL apoB.

DISCUSSION

LDL is an important atherogenic lipoprotein and varies substantially between individuals. One would like to know what accounts for this variation. The two groups of

[2]There was a rapid transfer of VLDL apoB into IDL and LDL. The values (expressed as cpm/ml plasma) corresponding to 0.25, 0.5, 0.75, 1, and 2 hr time points in Fig. 4 are as follows: For A: VLDL, 11289, 2589, 2117, 1662, and 1302; IDL, 5374, 4539, 4894, 3449, and 2013; LDL, 4800, 10243, 12312, 11029, and 10511; for B: VLDL, 324, 322, 206, 263, and 243; IDL, 19867, 13273, 13139, 11151, and 9084; LDL, 1268, 1483, 1722, 1033, and 2286; for C: VLDL, 109630, 58833, 50706, 30068, and 5524; IDL, 16029, 21665, 25226, 24466, and 19465; LDL, 4811, 11496, 13064, 15826, and 22544; and for D: VLDL, 4430, 2381, 2001, 1717, and 1067; IDL, 1753, 1060, 1947, 1984 and 1928; LDL, 1500, 789, 1265, 1905, and 2159.

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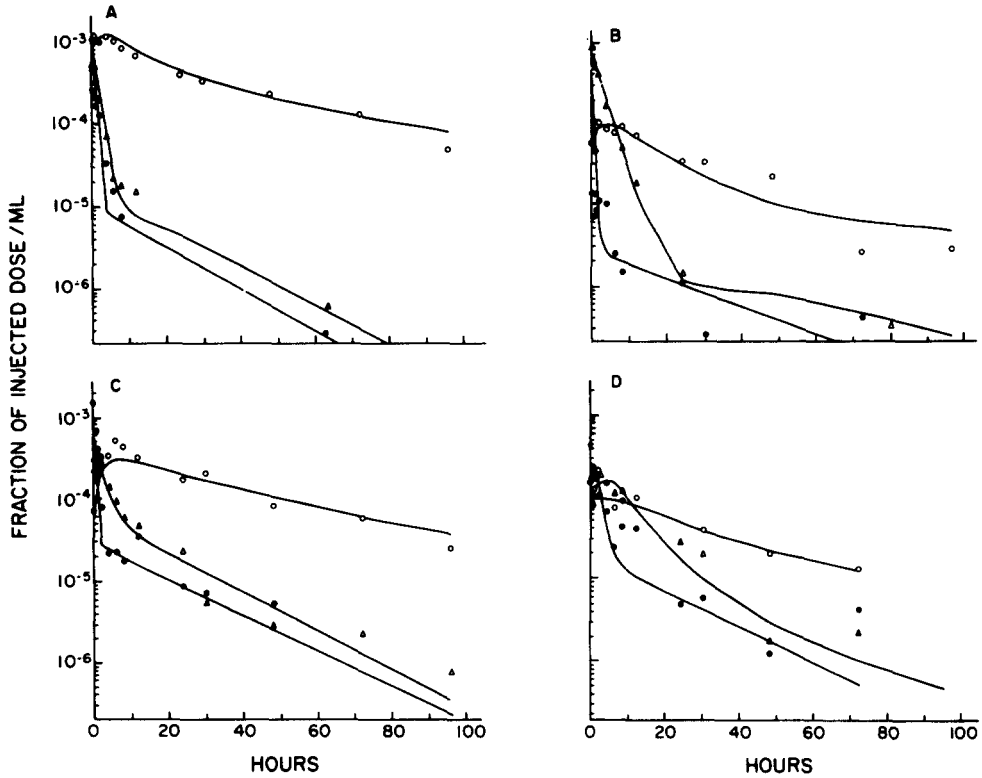

Fig. 4. Decay of radioactivity in apoB in VLDL (●), IDL (△), and LDL (○) following the injection of ^{131}I-labeled VLDL in representative animals with low LDL level on chow (A) and on a high cholesterol, saturated fat diet (B); and with high LDL level on chow (C) and on a high cholesterol, saturated fat diet (D). Chow-fed animals received VLDL isolated from chow-fed animals and animals studied on a high cholesterol, saturated fat diet received the VLDL from animals fed a similar diet.

selectively bred pedigreed baboons used in this study differed in their LDL cholesterol levels both when consuming chow and when challenged by a fat- and cholesterol-enriched diet. The diet increased LDL cholesterol in high LDL animals by about 100 mg/dl, and increased LDL cholesterol in the low LDL animals by only about 50 mg/dl. Likewise, the diet increased LDL apoB in high LDL animals by 30 mg/dl, and LDL apoB in low LDL animals by only about 10 mg/dl. Among the mechanisms that could account for the greater diet-induced increase in LDL in high LDL animals are greater LDL production, lesser LDL catabolism, or more rapid conversion of precursor to LDL. The high LDL animals, in comparison to the low LDL animals, seem to convert VLDL apoB more rapidly to LDL and to secrete more LDL apoB into the plasma independently of VLDL apoB, and these differences are exaggerated when they are challenged by the HCHF diet.

The similar VLDL apoB production rates for low and high LDL animals on either chow or the HCHF diet suggest that VLDL apoB production rate does not contribute to the variation in LDL levels. Since one-third of VLDL apoB was converted to LDL, VLDL apoB production was sufficient to account for the total LDL apoB production in low LDL animals on chow. The VLDL apoB production rate, however, was inadequate to account for the total production of LDL apoB in high LDL animals either on chow or on the HCHF diet. Thus, sources other than VLDL apoB must have contributed LDL apoB into the plasma of high LDL animals. This direct production of LDL apoB in high LDL animals was increased considerably by HCHF feeding. These observations suggest that both the metabolic fate of VLDL apoB and direct secretion of LDL apoB into the plasma play important roles in determining plasma LDL levels of high LDL baboons.

VLDL apoB metabolism has been studied in humans (26–28) and in a number of experimental animals (13, 28–32) including baboons (6). The flux of VLDL apoB into LDL apoB varies considerably. Studies by Sigurdsson, Nicoll, and Lewis (28) in normal human subjects have suggested that VLDL apoB is transferred quantitatively into LDL. In hypertriglyceridemic subjects, however, a lower amount of VLDL apoB is converted to LDL (28, 33, 34). Goldberg et al. (32) reported that in cynomolgus monkeys only 33–59% of VLDL apoB was converted to LDL apoB. Most baboons convert a proportion of VLDL apoB to LDL as do cynomolgus monkeys. In this experiment, however, the low LDL baboons converted a much smaller proportion of their VLDL apoB into LDL than did high LDL animals.

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TABLE 4. Metabolic parameters of apoB in lipoproteins following injection of iodinated VLDL into baboons fed chow and high cholesterol, saturated fat (HCHF) diets

Group	Diet	Animal Number	Residence Time (hr)				Conversion (%)		VLDL Production Rate mg/kg per day
			VLDL in Chain	Whole VLDL	IDL	LDL	VLDL to IDL	IDL to LDL	
Low LDL	Chow	1	0.32	0.93	3.33	20.0	99.8	20.6	27.0
		2	0.57	3.68	10.00	50.0	96.1	47.6	20.7
		3	1.00	1.08	2.50	10.0	99.9	36.7	35.6
		4	0.25	0.41	2.00	20.0	99.2	11.9	33.7
		5	0.17	0.69	1.09	20.0	99.9	59.0	20.3
			0.46 ± 0.15[a]	1.36 ± 0.59	3.78 ± 1.60	24.0 ± 6.8	99.0 ± 0.7	35.2 ± 8.6	27.5 ± 3.1
Low LDL	HCHF	1	1.52	1.72	2.86	20.0	99.9	10.0	13.2
		2	0.20	0.81	3.33	20.0	98.6	80.8	11.9
		3	0.42	1.11	2.50	20.0	99.9	28.0	18.1
		4	1.50	2.68	2.58	12.5	99.8	55.9	7.0
		5	0.30	1.35	5.00	33.3	99.9	6.5	18.7
			0.79 ± 0.30[a]	1.53 ± 0.32	3.25 ± 0.46	21.2 ± 3.4	99.6 ± 0.3	36.2 ± 14.2	13.8 ± 2.2[d]
High LDL	Chow	1	0.57	2.25	10.00	20.0	98.0	57.6	10.1
		2	0.49	3.00	10.00	21.5	98.0	88.5	18.4
		3	0.50	2.01	2.50	20.0	99.9	45.0	26.1
		4	0.17	0.69	1.25	33.3	99.9	58.4	26.6
		5	0.17	0.71	2.50	20.0	99.9	57.4	20.1
			0.38 ± 0.09[a]	1.73 ± 0.45	5.25 ± 1.95	23.0 ± 2.61	99.1 ± 0.5	61.4 ± 7.2[c]	20.3 ± 3.4
High LDL	HCHF	1	3.09	10.36	5.88	12.5	99.0	39.6	6.5
		2	1.65	12.95	6.25	28.6	98.4	16.3	5.9
		3	0.45	7.54	7.39	16.7	99.5	49.8	6.3
		4	0.98	4.44	2.00	20.0	97.6	57.4	10.1
		5	0.40	2.19	10.00	16.7	96.8	82.8	14.2
			1.31 ± 1.11[a]	7.50 ± 1.94[b]	6.30 ± 1.30	18.9 ± 2.7	98.3 ± 0.5	49.2 ± 10.9	8.6 ± 1.6[d]

[a] Mean ± standard error, n = 5.
[b] Significantly different ($P < 0.05$) from those for low LDL animals or for high LDL animals on chow diet.
[c] Significantly different ($P < 0.05$) from those for low LDL animals.
[d] Significantly different ($P < 0.05$) from those for chow diet.

The physiological mechanism responsible for the differences in the conversion of VLDL apoB to LDL is not obvious from our studies. Goldberg et al. (32) have suggested that the conversion of VLDL to LDL may in part be related to VLDL particle size and composition. Autologous VLDL from cynomolgus monkeys, which had a larger particle size (flotation rate, S_f 20-60), had less VLDL apoB transfer to LDL compared with that of homologous VLDL from humans. The VLDL from humans had a smaller particle size (flotation rate, S_f 60-400 and poor in cholesterol) and this smaller homologous VLDL transferred most of its apoB to LDL (32). Packard et al. (35) have reported that, in human subjects, large triglyceride-rich VLDL particles of flotation rates S_f 100-400 were quantitatively converted to IDL (S_f 12-100) with only a minor fraction converted to LDL (S_f 0-12). Most IDL was removed directly. VLDL of smaller particle size (S_f 20-60) was rapidly and substantially converted to LDL. VLDL particle size may differ between low and high LDL baboons. Most VLDL apoB was, however, converted to IDL in both low and high LDL animals on both diets. These observations are consistent with those recently reported by Packard et al. (35).

The differences in VLDL apoprotein composition, especially in apoE, may also affect conversion to LDL because apoE is bound by hepatic receptors. ApoE-rich VLDL particles may be removed without being converted to LDL. Analysis of VLDL particles from animals with low and high LDL levels would disclose whether any of these structural and compositional differences are responsible for the differential conversion of VLDL apoB to LDL. Likewise, a difference in the activity of remnant receptors in the liver may also affect the conversion of VLDL apoB to LDL (36, 37).

VLDL apoB production rates in normal humans suggest that most LDL apoB is derived from VLDL apoB (26, 27). Direct production of LDL apoB occurs in subjects with familial hypercholesterolemia (26). Simultaneous turnover studies of VLDL and LDL apoB in cynomolgus monkeys have suggested a direct secretion of LDL apoB into the plasma independently of VLDL apoB (32). Baboons seem to vary considerably from animal to animal in secretion of LDL directly into the plasma. On a chow diet, low LDL animals produce most of their LDL from VLDL apoB. An HCHF diet in low LDL animals decreases VLDL apoB production without affecting LDL apoB production and therefore approximately 43% of the LDL apoB cannot be accounted for by the VLDL catabolism. In high LDL animals the HCHF diet also decreases VLDL apoB production and exaggerates the production of LDL apoB that is independent of VLDL apoB. Thus high LDL animals have a much higher secretion of LDL

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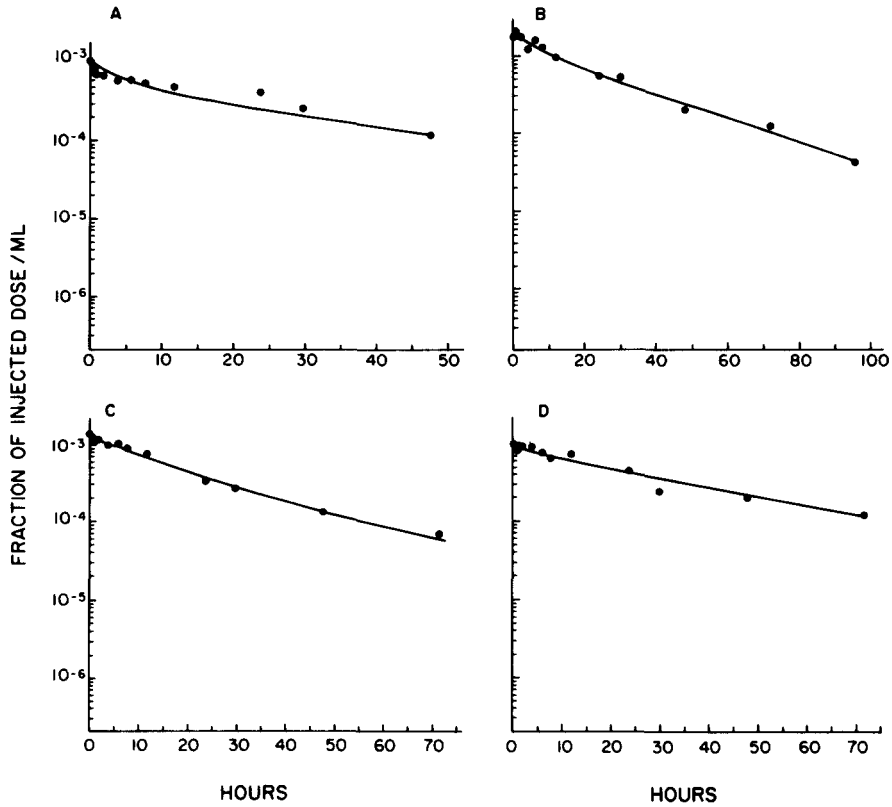

Fig. 5. Decay of radioactivity in apoB in LDL following the injection of their own ^{125}I-labeled LDL in representative animals with low LDL level on chow (A) and on a high cholesterol, saturated fat diet (B); and with high LDL level on chow (C) and on a high cholesterol, saturated fat diet (D).

apoB that cannot be accounted for by VLDL catabolism in both chow-fed and HCHF-fed animals. Huff and Telford (38) have suggested a direct synthesis of LDL apoB in the miniature pig similar to that in cynomolgus monkeys (32). Huff et al. (39) have further reported that mevinolin and cholestyramine inhibit the direct synthesis of LDL apoB in miniature pigs. Total LDL apoB production in high LDL animals is approximately 3-fold higher than in low LDL animals. HCHF feeding does not change the production of LDL apoB but it does decrease the catabolic rate. Thus, the increased pool of LDL apoB in these animals while they are consuming a HCHF diet is mainly due to decreased catabolic rate. It is, therefore, possible that receptor activity is down-regulated by HCHF feeding in baboons as it is in rabbits. It is also possible that HCHF feeding may affect the composition of LDL, and the change in composition may in turn decrease its catabolism. In addition to increasing residence time of LDL apoB, HCHF feeding in high LDL animals also increased resident times of VLDL and IDL apoB. Such slowing in clearance of VLDL apoB may also be due to alteration of particle composition.

The radioactivity from VLDL apoB decays rapidly and appears in IDL, as has been reported in cynomolgus monkeys (32) and pigtail monkeys (29). It is possible that, due to a rapid conversion of VLDL into LDL in these animals, a significant proportion of VLDL isolated for turnover studies has already been converted into LDL and, therefore, VLDL apoB production measured by the kinetic analysis of altered or partially catabolized VLDL would not account for the LDL apoB production. VLDL catabolic rates did not differ between low and high LDL animals, and yet there were differences in the amounts of apoB secreted directly in the plasma of these animals. These differences are, therefore, not likely due to rapid VLDL catabolism to LDL prior to sampling of VLDL for iodination and injection.

The residence times for LDL apoB measured by the direct injection of ^{125}I-labeled LDL and those measured indirectly by monitoring the conversion of ^{131}I-labeled VLDL to LDL were similar for both groups fed the chow diet. However, on the atherogenic diet, the residence times for VLDL-derived apoB and for directly injected LDL apoB differed. ^{125}I-Labeled LDL apoB had higher residence times than ^{131}I-labeled LDL derived from ^{131}I-labeled VLDL. These differences in residence times for LDL apoB, measured by direct and indirect injections, could be accounted for by two reasons. First, the measurement of VLDL-derived LDL apoB residence time is indirect and may be less precise and, therefore, may differ

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TABLE 5. Metabolic parameters of LDL apolipoprotein B following injection of iodinated LDL in baboons fed chow and high cholesterol, saturated fat (HCHF) diets

Group	Residence Time (hr)		Production rate (mg/kg/day)	
	Chow	HCHF	Chow	HCHF
Low LDL	19.3 ± 2.1[a]	27.2 ± 5.4	7.5 ± 0.6	8.7 ± 1.5
High LDL	22.3 ± 2.0	37.6 ± 5.1[b]	22.6 ± 2.8[c]	22.3 ± 1.0[c]

[a]Mean ± SEM, n = 6.
[b]Significantly different ($P < 0.05$) from those for chow diet.
[c]Significantly different ($P < 0.01$) from those for low LDL animals.

from that measured by the direct injection. Alternatively, the residence time of VLDL-derived LDL apoB (measured by injected ^{131}I-labeled VLDL) may be different from the residence time of total LDL apoB (both derived from VLDL and secreted independently, measured by injected ^{125}I-labeled LDL). Since both low and high LDL animals fed the HCHF diet produce LDL apoB in excess of VLDL apoB production, the latter explanation seems more plausible.

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