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Low-density lipoprotein apolipoprotein B production differs between laboratory opossums exhibiting high and low lipemic responses to dietary cholesterol and fat

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Abstract

Two partially inbred strains of laboratory opossums exhibit extremely high or low levels of low-density lipoprotein (LDL) cholesterol concentrations, respectively, when challenged with a high-cholesterol and high-fat (HCHF) diet. The present studies were conducted to determine whether the catabolism or the production of LDL apolipoprotein B (apoB) is responsible for the variability in plasma LDL cholesterol and apoB concentrations. Iodinated LDL prepared from plasma of donor opossums consuming HCHF diet was injected into high- and low-responding recipients maintained on the HCHF diet. Blood was drawn at intervals beginning at 3 minutes and ending at 24 hours. At the end of the study, animals were necropsied, and livers were removed for isolation of RNA. Plasma LDL apoB was separated by sodium dodecyl sulfate–electrophoresis, and the level of radioactivity was determined. Hepatic LDL receptor and apoB mRNA levels were measured by Northern blotting. Radioactivity decay curves were plotted by using the radioactivity at each time point as percentage of the radioactivity recovered at 3 minutes. Fractional catabolic rates (FCRs) were calculated by the curve peeling technique. Steady-state production rates were calculated by multiplying the FCR values with apoB concentrations. LDL apoB FCR was slightly higher (1.63-fold) in low responders than in high responders. On the other hand, LDL apoB production was much higher (5.5-fold) in high responders than in low responders. There was no difference in hepatic mRNA levels for either the LDL receptor or apoB. The differences in LDL apoB FCR may be explained on the basis of differences in pool size between the 2 strains. Therefore, LDL apoB production is the major determinant of diet-induced hyperlipidemia in laboratory opossums. Because LDL apoB production was not associated with hepatic mRNA levels, the production of LDL apoB is regulated posttranscriptionally or posttranslationally.

1. Introduction

Laboratory opossums (*Monodelphis domestica*) have quite uniform plasma and lipoprotein cholesterol levels on a low-cholesterol and low-fat basal diet, but exhibit extreme variability in plasma cholesterol levels when challenged with a high-cholesterol and high-fat (HCHF) diet [1]. The plasma cholesterol variability on the HCHF diet is primarily due to very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol. Low-responding opossums show a slight increase in VLDL+LDL cholesterol, whereas high-responding opossums show up to a 50-fold increase in VLDL+LDL (mainly in LDL) cholesterol [2]. Genetic analyses have provided evidence that a major gene for VLDL+LDL cholesterol concentration on the HCHF diet is responsible for 80% of the variability in VLDL+LDL cholesterol on the challenge diet [3]. Thus, the laboratory opossum is a unique model for identifying genetic markers for responsiveness to dietary lipids. We conducted a number of metabolic studies to determine the differences in lipoprotein metabolism and the expression of cholesterol-responsive genes between high- and low-responding opossums to identify markers associated with responsiveness to diet [4,5]. Rainwater and VandeBerg [1] have described plasma lipoproteins of laboratory opossums and found high degree of similarity with human plasma lipoproteins. Like humans, opossums carry most of their plasma cholesterol in LDL

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under dietary conditions that lead to elevated plasma cholesterol levels, and apolipoprotein constituents of various subclasses of their lipoproteins have similarities with human apolipoprotein constituents. Because variability in the accumulation of LDL cholesterol and apolipoprotein B (apoB) on the HCHF diet is the major difference between high- and low-responding opossums, we conducted the present studies to determine whether the synthesis or the catabolism of LDL is responsible for the variability in plasma LDL cholesterol and apoB concentrations. We also measured the expression of LDL receptor and apoB genes to determine if the expression of either of these genes is associated with high and low responsiveness to dietary lipids in opossums.

2. Materials and methods

2.1. Experimental animals

We used 2 partially inbred strains of the gray short-tailed opossum (*M domestica*), which were developed at the Southwest Foundation for Biomedical Research (SFBR). Animals from the ATHH strain, which was selectively bred for hyperresponsiveness to dietary lipids, had had inbreeding coefficients in the range of 0.72 to 0.78. Animals from the hyporesponsive strain, ATHE, had inbreeding coefficients of 0.75 to 0.78. We used animals (5-7 months old) from ATHE (a hyporesponding line) and ATHH (a hyperresponding line) stocks. The animals were maintained in polycarbonate rodent cages under laboratory conditions that have been standardized for this species [6,7].

The protocol of these experiments was approved by the institutional animal care and use committee of the SFBR. The SFBR is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and is registered in the US Department of Agriculture.

2.2. Experimental diets

Laboratory opossums were fed a basal or the HCHF diet ad libitum during these studies. The basal diet is a commercially pelleted fox food (Reproduction Diet, Nutritionally Complete Fox Food Pellets, Milk Specialties Co, New Holstein, Wis) and contains 10% fat and 0.16% cholesterol by weight. The HCHF diet contains 18.8% fat and 0.71% cholesterol by weight and is prepared from the fox chow by adding 7.5 L tap water to 22.7 kg of pellets, together with lard (liquefied) and crystalline cholesterol (USB Corporation, Cleveland, Ohio). The ingredients are mixed in a Hobart food mixer (Hobart Corporation, Troy, Ohio) until all of the liquid is absorbed. Then the blend is passed through a mist grinder (Rodriguez Butcher Supplies, San Antonio, Tex) to produce soft pellets and frozen at −20°C to prevent spoilage and oxidation.

2.3. Experimental design

We conducted these studies by using 9 high-responding and 9 low-responding 5- to 7-month-old adult male and female opossums. All the animals had been maintained on the basal diet since weaning at 2 months of age. Animals were challenged with the HCHF diet for 6 weeks to confirm their phenotype. Based on their plasma cholesterol levels on the HCHF diet, 4 high-responding (plasma cholesterol >150 mg/dL) and 4 low-responding (plasma cholesterol <150 mg/dL) opossums were selected as the recipients. The remaining animals were used as blood donors for the isolation of lipoproteins to be used for turnover studies. The animals were maintained on the HCHF diet and were used in the turnover studies at the end of week 7. During the turnover experiment, 1 ATHH and 1 ATHE animal did not receive the full amount of isotope and were not included in the study; therefore, data for 3 high- and 3 low-responding opossums are presented. The animals were maintained on a 14-hour light/10-hour dark cycle, with the light coming on at 7:00 AM. The animals were fasted (12-14 hours) before blood drawings.

2.4. Isolation and labeling of LDL

Donor animals were fasted overnight and anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ). Blood from each animal (1.0 mL) was collected by cardiac puncture and placed in 2 mL EDTA Vacutainer tube (Becton Dickinson Labware, Franklin Lakes, NJ) [7-9]. Blood was pooled from high- and low-responding animals, and plasma (6.2 mL) was obtained by centrifugation in a low-speed centrifuge (TJ6, Beckman Instruments, Palo Alto, Calif). LDL (density 1.019-1.063 g/mL) was isolated by sequential ultracentrifugation in an ultracentrifuge (L8-70M, Beckman Instruments) by methods described for baboons and rabbits [10,11]. LDL was dialyzed against saline (0.15 mol/L sodium chloride) containing EDTA (0.05 mol/L, pH 7.4) to remove potassium chloride. The protein concentration of LDL was determined by the method of Bradford [12] (Protein Assay Kit, Bio-Rad, Richmond, Calif) using bovine plasma albumin as a standard. LDL (1 mg protein) was iodinated by the iodine monochloride procedure [13] with iodine 125 (Amersham Biosciences Corporation, Piscataway, NJ) as described previously for baboon LDL [10]. Free iodide was removed by passing the labeled lipoproteins through a column of Sephadex G-100 packed in a 20-mL syringe and by further dialysis against saline (0.15 mol/L sodium chloride containing EDTA [0.05 mol/L, pH 7.4]) at 6°C with 6 to 8 changes. Before injection into the recipients, iodinated LDL was characterized for trichloroacetic acid precipitate, lipids, and free iodide as described for rabbit VLDL [11]. Most of the radioactivity in the iodinated LDL was recovered in trichloroacetic acid precipitate fraction (>94%); there was little detectable radioactivity (<1%) in the free iodide fraction. The radioactivity in the lipid fraction was <5%.

2.5. LDL apoB turnover procedure

For LDL apoB turnover studies, opossums were housed in individual cages and fasted overnight. The turnover studies were performed with 1 animal at a time. The animal was

anesthetized lightly in a covered chamber containing cotton balls sprinkled with 1 mL of halothane and covered with paper towels. The animal was removed from the chamber by lifting up the skin of the lower abdomen. With the needle pointing toward the animal's head, 0.1 mL of ketamine (Phoenix Scientific, St Joseph, Mo)/diazepam (Abbot Labs, North Chicago, Ill) anesthesia prepared by mixing 1 volume of stock solution of ketamine hydrochloride (100 mg/mL), 1 vol of stock solution of diazepam (5 mg/mL) and 8 volumes of sterile saline. Some animals required 0.15 mL of the anesthesia mixture. After the animal was fully anesthetized, the animal was placed onto the underside of a Styrofoam tube rack, which had a notch removed for placing the head of the animal to prevent movement and to insure the correct angle for the needle insertion. With forceps, the skin was lifted over the area in the animal's upper left neck, and an incision (2-2.5 cm long parallel to the jugular vein) was made. The flaps of the skin were opened, and any fat or extraneous tissue covering the vein was removed. The iodinated LDL was drawn into a syringe, and after expelling any air bubbles, the needle was inserted into the vein with the needle pointing toward the animal's abdomen. When it was clear that the needle was positioned inside the jugular vein, iodinated LDL in the syringe was injected slowly. Afterward, the needle was removed, and the vein was covered with skin and glued (VetBond glue, 3M Animal Care Products, St Paul, Minn). Animals were placed into their cages after complete recovery. Animals were injected with 0.045 mg LDL protein in 0.3 mL saline. The specific activity of iodinated LDL was 55 μCi/mg protein. Blood samples (0.25 mL) were collected at 3 and 30 minutes and 1, 2, 4, 8, 12, and 24 hours by cardiac puncture as described above. Animals were fed after the blood collection at 4 hours.

2.6. Determination of radioactivity in plasma, LDL, and apoB

Blood samples collected at various time points were centrifuged at low speed to obtain plasma. Radioactivity in a small sample of plasma (100 μL) was determined using a gamma spectrometer (LKB Wallace, Inc, Gaithersburg, Md). The same plasma samples were used to precipitate VLDL+LDL by heparin–manganese chloride according to the Lipid Research Clinics procedure [14]. The radioactivity in both the precipitate VLDL+LDL and the supernatant (high-density lipoprotein [HDL]) was determined. The precipitate was delipidated with acetone/ethanol (1:1, at −20°C overnight) 1 time, and then with ether alone 3 times. The protein precipitate was dried under nitrogen and then dissolved in buffer and separated by 3.5% sodium dodecyl sulfate (SDS) slab gel electrophoresis [15,16]. The apoB band was cut out of the gel, and the radioactivity was measured.

2.7. Tissue collection

Animals were exsanguinated by cardiac puncture under halothane (Halocarbon Laboratories) anesthesia. The liver, lungs, kidneys, and brain were removed, placed in vials, and frozen in liquid nitrogen. Samples were stored at −80°C before their use.

2.8. Plasma and HDL cholesterol analysis

Total plasma cholesterol was measured by enzymatic methods using commercial kits. When the cholesterol level in a sample exceeded the value of the highest calibrator, 358 mg/dL, the sample was diluted with saline to bring it into the range of the calibrators and analyzed again to obtain an accurate measurement. HDL cholesterol was measured after precipitation of VLDL and LDL by heparin–manganese chloride according to the Lipid Research Clinics procedure [14]. The VLDL+LDL cholesterol concentration was calculated as the difference between the total plasma cholesterol and HDL cholesterol concentrations. For samples that were diluted for the total cholesterol assay, the same dilutions were used for the precipitation procedure.

2.9. Measurement of LDL apoB

Plasma samples from all time points from each animal were pooled, and lipoproteins were separated by density gradient ultracentrifugation using an SW 41Ti rotor in a Beckman Ultracentrifuge Model L8-70M (Beckman Instruments). The density gradient procedure was a modification of that reported by Redgrave et al [17] and described in detail previously for baboon lipoproteins [18]. The relative index of each fraction was measured, and fractions were pooled on the basis of density. The densities of fractions pooled for LDL corresponded 1.019 to 1.063 g/mL.

LDL was dialyzed, and the volume was measured. An aliquot of LDL (1.0 mL) was used to precipitate apoB by adding equal volume of isopropanol (Sigma Chemical Company, St Louis, Mo) as described by Egusa et al [19]. The precipitate was washed with isopropanol, dissolved in 1 N sodium hydroxide, and the suspension incubated at 37°C until the pellet was completely dissolved. The protein content of the pellet was then measured by the method of Bradford (Protein Assay Kit, Bio-Rad) using bovine plasma albumin as a standard [12] and expressed as microgram per deciliter of plasma.

2.10. Measurement of hepatic mRNA abundance

Total RNA from 50 to 100 mg of frozen liver sample was isolated using the TRIzol kit (Invitrogen, Carlsbad, Calif). The quality and the quantity of the RNA were determined using a spectrophotometer (Biophotometer, Eppendorf, Westbury, NY). Northern blot analysis was used to measure the mRNA abundance of LDL receptor and apoB. Total RNA (10 μg) was fractionated on 1% agarose-formaldehyde gels, transferred on to a Nitroplus 2000 membrane (Micron Separations, Inc, Westborough, Mass) in 10× SSC buffer (1.5 mol/L NaCl, 0.15 mol/L sodium citrate), and cross-linked to the nylon membrane using UV cross linker (Spectronicks, Lincoln, Neb). We used *GAPDH* as a housekeeping gene for these studies.

DNA probes were radiolabeled with [α-^{32}P]dCTP using High Prime (Roche, Indianapolis, Ind). Unincorporated nucleotides were removed from radiolabeled DNA probes using Quick Spin columns (Roche). Membranes were hybridized with 2×10^6 cpm/mL of radioactive DNA probe in ULTRAhyb at 42°C overnight. After hybridization, membranes were washed once in 2× SSC and 0.1% SDS at 42°C for 5 minutes, then once in 2× SSC and 0.1% SDS at 65°C for 10 minutes, and twice in 0.2× SSC and 0.1% SDS at 65°C for 20 minutes. Blots were exposed to a storage phosphor screen (Molecular Dynamics, Sunnyvale, Calif) for 2 hours and were scanned on a PhosphorImager (Amersham). The blots were further exposed to BioMax MR films (Kodak, Rochester, NY) overnight at −80°C. The expression of these genes was expressed in relative units.

To create a probe for the LDL receptor, total RNA isolated from the liver of an opossum was reverse transcribed and amplified with primers based on the human LDL receptor cDNA sequence. An 820–base pair (bp) reverse transcription (RT)–polymerase chain reaction (PCR) product was synthesized using primers LDLR-2F (5′-GGGAATATGACTGCAAGGACAT-3′) and LDLR-2R (5′-AAGCCATGAACAGGATCCAC-3′). The RT-PCR product was sequenced and found to be 75% identical to the human LDL receptor cDNA. The opossum LDL receptor cDNA sequence has been submitted to GenBank (accession numberAY871266). The 820-bp RT-PCR product was cloned into pCR4-TOPO (Invitrogen) to generate pCR4-LDLR. DNA for the opossum LDL receptor probe was amplified from pCR4-LDLR using LDLR-2F and LDLR-2R primers.

To create a probe for apoB, RT-PCR amplification was performed using primers based on the published opossum apoB cDNA sequence. Primers apoB-F (5′-CGCTTACGATCCCTGAAATG-3′) and apoB-R (5′-TGACCTGAAATGAGCCACAA-3′) amplified a 722-bp RT-PCR product from liver cDNAs, which was subsequently cloned into pCR4-TOPO (Invitrogen) to generate pCR4-apoB. DNA for the opossum apoB probe was amplified from pCR4-apoB using apoB-F and apoB-R primers.

2.11. Calculation of residence time and fractional catabolic rate

Decay curves for LDL apoB were plotted by using the percentage of radioactivity recovered at the 3-minute time point. The fractional catabolic rates (FCRs) for apoB (fraction of LDL apoB pool catabolized per hour) were calculated using 2-pool model and curve peeling technique described by Langer et al [20]. According to this model, LDL apoB is removed from the extravascular compartment, which equilibrates with the intravascular compartment [20]. The first phase of decay represents the equilibrium of LDL apoB radioactivity with extravascular compartment and a rapid removal [20]. The FCR was calculated by the expression, FCR as 1/(c1/b1 + c2/b2), where c1 and c2 are Y-intercepts and b1 and b2 are slopes of the LDL apoB decay curve and its peeled exponentials, respectively. The residence times were calculated as the inverse of the FCRs and expressed in hours. LDL apoB synthetic/production rates were calculated (not measured) by multiplying the LDL apoB fraction catabolic rate by the mass of LDL apoB in the plasma. To determine the mass of apoB, blood volume was determined by the dilution of radioactivity at 3-minute point. LDL apoB production rate was expressed as milligram per kilogram body weight per day. To determine if the pool of apoB was stable, we measured plasma cholesterol concentration at several time points between 0- and 24-hour time points for each animal.

2.12. Data analysis

Values in the tables are expressed as mean ± SEM. The values for high- and low-responding opossums were compared by a standard *t* test. Associations among the bile acid concentrations and plasma lipoprotein cholesterol concentrations were determined by using Pearson correlation. Significance was set at $P < .05$.

3. Results

3.1. Characteristics of low- and high-responding laboratory opossums used as recipients for LDL apoB turnover studies

Plasma and lipoprotein cholesterol levels and body weights of laboratory opossums with low and high responses to dietary lipids are given in Table 1. Low- and high-responding laboratory opossums consuming the HCHF diet (7 weeks) did not differ in their body weights, plasma triglyceride concentrations, or HDL cholesterol concentrations, but differed significantly in total plasma ($P < .05$) and LDL ($P < .06$) cholesterol concentrations. Plasma cholesterol concentration in high-responding animals was 12.3-fold higher than in low-responding animals, and the LDL cholesterol concentration in high-responding animals was 30.5-fold higher than in low-responding animals. Consistent with LDL cholesterol concentration differences between high- and low-responding opossums, apoB was

Table 1
Characteristics of low- and high-responding laboratory opossums used for LDL apoB turnover studies and maintained on the HCHF diet for 7 weeks

Characteristic	Low responding (n = 3)	High responding (n = 3)
Plasma cholesterol (mg/dL)	87.3 ± 1.5*	1073.3 ± 171.9
Plasma triglyceride (mg/dL)	25.7 ± 3.8	43.3 ± 8.1
LDL cholesterol (mg/dL)	53.3 ± 7.6*	1038.0 ± 180.8
HDL cholesterol (mg/dL)	34.0 ± 6.3	35.3 ± 10.1
Body weight (g)	99.0 ± 3.5	97.2 ± 8.8

* Values are significantly different from those of high-responding group ($P < .05$).

Table 2
Values for metabolic variables of LDL apoB in low- and high-responding opossums consuming HCHF diet for 7 weeks

Metabolic variable	Low responding (n = 3)	High responding (n = 3)
ApoB (mg/dL)	3.67 ± 0.87*	37.67 ± 5.46
Residence time (h)	31.94 ± 0.76*	54.16 ± 5.26
FCR (pool per hour)	0.031 ± 0.001*	0.019 ± 0.002
Production rate (mg/kg per day)	1.39 ± 0.25*	7.70 ± 0.60

* Values are significantly different from those of high-responding group ($P < .05$).

also 25-fold higher in high-responding animals than in low-responding animals (Table 2).

3.2. Differences in kinetic variables of LDL apoB metabolism between low- and high-responding opossums consuming HCHF diet

The average decay of radioactivity in plasma LDL apoB is presented in Fig. 1. There was a biphasic decay of LDL apoB radioactivity in both low- and high-responding opossums. However, the decay of apoB radioactivity was much faster in low-responding animals than in high-responding animals. At the end of the 24-hour period, 24.60% ± 1.91% of the total injected radioactivity in apoB remains in the plasma of low-responding opossums, whereas 54.90% ± 4.22% of the total injected radioactivity in apoB remains in the plasma of high-responding opossums.

Values for metabolic variables for LDL apoB in low- and high-responding opossums maintained on the HCHF diet are given in Table 2. Residence times for LDL apoB were higher in high-responding opossums than in low-responding opossums, whereas FCRs were higher in low-responding opossums than in high-responding opossums. In low-responding opossums, approximately 3% pool of plasma LDL apoB was catabolized per hour, whereas in high-responding opossums, it was approximately 2% per hour. Low-responding opossums catabolized LDL apoB 1.5 times faster than high-responding opossums. The difference in LDL apoB production rate was much greater than the difference in LDL apoB catabolic rate between high- and low-responding lines. LDL apoB production rate was 5.54 times higher in high-responding opossums than in low-responding opossums.

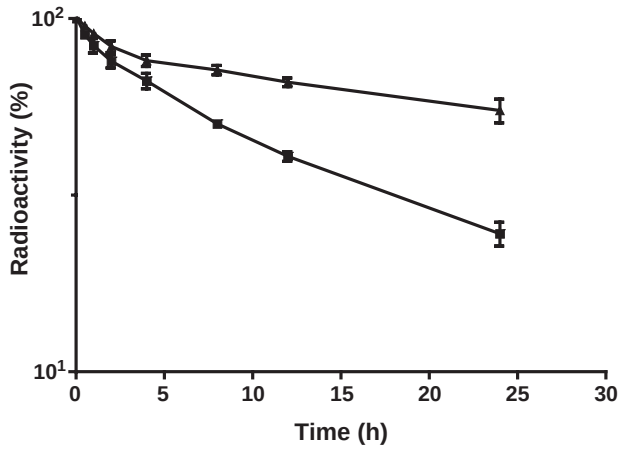

Fig. 1. Figure shows the decay of radioactivity in LDL apoB (mean ± SE, n = 3) with time after injection of iodinated LDL in low-responding (■) and high-responding (▲) laboratory opossums. Delipidated LDL apoproteins were separated by 3.5% SDS gel electrophoresis; the band corresponding to apoB was cut out, and the radioactivity was measured. The decay of radioactivity in apoB at each time point has been plotted as a percent of radioactivity recovered at the 3-minute time point.

Table 3
Hepatic mRNA abundance of LDL receptor and apoB in high- and low-responding opossums maintained on the HCHF diet

Hepatic mRNA abundance[a]	Low responding (n = 3)	High responding (n = 3)
LDL receptor	0.800 ± 0.0023	0.846 ± 0.038
ApoB	0.995 ± 0.041	0.867 ± 0.030

[a] The units are relative units as compared with a control sample.

3.3. Differences in LDL receptor and apoB mRNA levels between high- and low-responding opossums consuming HCHF diet

Hepatic mRNA abundances for LDL receptor and apoB are given in Table 3. There was very little variability in hepatic mRNA values for both LDL receptor and apoB, and there was no significant difference in hepatic mRNA levels for either the LDL receptor or the apoB between high- and low-responding opossums maintained on the HCHF diet.

4. Discussion

The expression of a number of lipid-responsive genes is affected by increased delivery of cholesterol to the liver. Variations in any of these cholesterol-responsive genes will affect hepatic and extrahepatic lipoprotein metabolism and, in turn, will affect responsiveness to diet. The major difference between high- and low-responding opossums is that high-responding opossums accumulate a large amount of LDL in their plasma. Therefore, high- and low-responding opossums differ in their hepatic apoB metabolism and thus in the expression of genes affecting hepatic apoB metabolism. Because LDL apoB is removed from the circulation without being exchanged to VLDL, we measured LDL apoB metabolism to determine whether apoB synthesis or catabolism or both are affected by the dietary cholesterol and fat in opossums. The present studies suggested that high- and low-responding opossums differ in both LDL apoB catabolism and production. The difference between high- and low-responding opossums in FCR (1.63-fold) was much less by comparison with production rate (5.54-fold). Thus, as in baboons and humans, the apoB production seems associated with responsiveness to diet in opossums [10,21,22].

The LDL receptor in the liver plays a major role in the removal of circulating LDL cholesterol from the plasma

[23]. The hepatic LDL receptor not only removes LDL cholesterol, but it also removes cholesterol from remnant lipoproteins that are enriched with apo E. Because remnant lipoproteins are converted into LDL, a decrease in the removal of remnant lipoproteins will result in increased LDL production. Thus, the hepatic LDL receptor also contributes to the production of LDL. In the present study, the hepatic mRNA levels for LDL receptor did not differ between high- and low-responding opossums. Thus, the differences in LDL apoB FCR between high- and low-responding opossums cannot be explained on the basis of hepatic LDL receptor expression. It is possible that LDL receptor in opossums is regulated at the level of translation or at the level of receptor distribution to the plasma membrane of cells and may be responsible for these differences in LDL apoB catabolic rate. Alternatively, the differences in FCR are due to the differences in LDL apoB pool size in the circulation. Because there was no difference in hepatic LDL receptor mRNA between high- and low-responding opossums, the plasma levels of apoB are not related to its catabolism. Similarly, the several-fold difference in LDL apoB production rates between high- and low-responding opossums cannot be explained by either the expression of the LDL receptor or apoB genes. Thus, LDL apoB production rates must be due to induction of some other genes by the dietary cholesterol and fat.

In spite of large differences in plasma LDL apoB concentrations and LDL apoB production rates on the HCHF diet, hepatic apoB mRNA levels were not different between high- and low-responding opossums. These results suggest that LDL apoB production rates are not related to hepatic LDL apoB mRNA levels. These observations are consistent with studies in baboons, in which apoB mRNA levels were not associated with LDL apoB production rates [21]. Because hepatic mRNA levels are not associated with LDL apoB production in plasma, it is likely that hepatic LDL apoB production is regulated posttrancriptionally in opossums as suggested by Dixon and Ginsberg's [24] observation of cultured liver cells. In vitro studies in rat hepatocytes and HepG2 cells have suggested that intracellular degradation of apoB may be an important posttranslational process involved in the regulation of apoB production [25,26]. Oleate stimulates the secretion of apoB by protecting the degradation of newly synthesized apoB in HepG2 cells [26]. Similarly, the availability of cholesteryl esters in hepatocytes also increases the secretion of apoB [27]. Hepatic triglyceride concentration does not differ between high- and low-responding opossums, whereas hepatic cholesterol concentration differs considerably between high- and low-responding opossums. Therefore, cholesterol concentration may affect apoB production [5]. The increased amount of intestinal cholesterol delivered to the liver or the decreased amount of cholesterol routed into the bile may affect the posttranslational modification and secretion of apoB and affect the responsiveness to diet in opossums.

Our previous studies have suggested that high-responding opossums, as compared with low-responding opossums, have much higher percentage of cholesterol absorption on the HCHF diet, and thus the increased amount of cholesterol delivered to the liver of high-responding opossums may increase apoB production [5]. However, the differences in cholesterol absorption between high- and low-responding opossums alone do not explain the differences in responsiveness to diet in opossums. Therefore, another mechanism may be affecting the apoB posttranscriptional modification and secretion of apoB in opossums. Our previous studies have also suggested that low-responding opossums, as compared with high-responding opossums, exhibit an increased expression of 27-hydroxylase in their hepatic and extrahepatic tissues [4]. The increased expression of 27-hydroxylation in low-responding opossums, as compared with high-responding opossums, may affect hepatic cholesterol concentration in 3 ways. First, because 27-hydroxycholesterol is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A–reductase, increased expression of 27-hydroxylase would decrease cholesterol synthesis in hepatic and extrahepatic tissues; second, increased expression of 27-hydroxylase would affect the conversion of cholesterol into 27-hydroxycholesterol in hepatic and extrahepatic tissues and would affect bile acid formation; and third, increased expression of 27-hydroxylase would induce the expression of ABCG5 and ABCG8, which increase the secretion of cholesterol into the bile and also reduce the cholesterol absorption [28-30]. Further studies of opossums are needed to determine whether there is a relationship between 27-hydroxylase and ABC transporters as has been described in baboons with high and low lipemic responses to dietary lipids.

In summary, the present studies suggest that both catabolic and production rates of LDL apoB are involved in responsiveness to diet in opossums. However, the hepatic overproduction of apoB in high-responding opossums is the major determinant of diet-induced hyperlipidemia. Because hepatic levels of apoB mRNA were not associated with the apoB production rates, apoB production in opossums is regulated posttranscriptionally or posttranslationally. The posttranscriptional modification of apoB production may be affected by the differences in cholesterol absorption and 27-hydroxylase activities between high- and low-responding opossums.

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