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Differential expression of hepatic genes involved in cholesterol homeostasis in high- and low-responding strains of laboratory opossums

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Abstract

Plasma very low-density lipoprotein and low-density lipoprotein (VLDL + LDL) cholesterol levels of 2 partially inbred strains of opossums (*Monodelphis domestica*) differ markedly when they are fed a high-cholesterol and low-fat (HCLF) diet. High-responding opossums exhibit a dramatic increase (>10-fold) in VLDL + LDL cholesterol, whereas low-responding opossums exhibit a minimal increase (<2-fold) in VLDL + LDL cholesterol. The genes responsible for the accumulation of high levels of plasma VLDL + LDL cholesterol in high-responding opossums have not yet been identified. In this study, we analyzed the expression of genes encoding for (1) 4 bile acid synthesis enzymes (*CYP7A1*, *CYP27A1*, *CYP8B1*, and *CYP7B1*); (2) 3 cholesterol synthesis enzymes (*HMGCR*, *HMGCS1*, and *SQLE*); (3) the LDL receptor (*LDLR*); (4) 2 sterol transporters (*ABCG5* and *ABCG8*); and (5) 2 bile acid transporters (*ABCB11* and *SLC10A1*) to determine how the expression of these genes was affected by dietary cholesterol in the 2 strains of opossums. We found differences between high and low responders in the expression of cholesterol synthesis genes on the basal diet, as well as differences in the expression of the *CYP27A1*, *ABCG5*, *ABCG8*, and *SLC10A1* genes on the HCLF diet. *CYP27A1* messenger RNA levels were lower in the livers of high responders compared with low responders, whereas *CYP27A1* messenger RNA levels in extrahepatic tissues were similar in high and low responders on the HCLF diet. Low levels of *CYP27A1*, *ABCG5*, and *ABCG8* expression in the liver may contribute to hypercholesterolemia in high-responding opossums.

1. Introduction

Two partially inbred strains of opossums (*Monodelphis domestica*) have been developed to study diet-induced hypercholesterolemia [1,2]. In low-responding opossums, diets enriched in cholesterol alone (high cholesterol and low fat; HCLF) or enriched in cholesterol and fat (high cholesterol and high fat; HCHF) have a minimal effect on plasma very low-density lipoprotein and low-density lipoprotein (VLDL + LDL) cholesterol [3]. In high-responding opossums, in contrast, the HCLF diet induces a marked increase (>10-fold) in plasma VLDL + LDL cholesterol; and the HCHF diet induces an even greater increase (>30-fold) in plasma VLDL + LDL cholesterol [3]. However, a diet enriched in fat alone has little effect on plasma VLDL + LDL cholesterol in high- or low-responding opossums [3].

Plasma cholesterol concentration is the balance of cholesterol absorption, cholesterol synthesis, and cholesterol excretion. Metabolic studies conducted to measure cholesterol absorption revealed that fractional cholesterol absorption was 2-fold higher in high responders than in low responders fed the HCHF diet [4]; and biochemical studies revealed that activity of the bile acid synthesis enzyme sterol 27-hydroxylase was lower in high responders fed the HCHF diet [5], suggesting that differences in cholesterol absorption and bile acid synthesis contribute to increased plasma VLDL + LDL cholesterol in high responders. In this study,

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Table 1
Primer sequences and accession numbers

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Accession no.
CYP7A1	AGGCGAATGGGAGAGCCAC	AAACTCGATATCATTTGTTGGTG	EF599652
CYP8B1	CAGGCGTTATGGGAACATCT	ACCACCAGGCGTAATGTCTC	EF599651
CYP27A1	TGGAGCAGCTGCTGAGGCAG	AGAGTTTCCTTAAGCACTGCTTTG	EF601083
CYP7B1	AGACCTGGGGAGCCTCCTT	GCTGTCCATTGTTCTCTGGTGA	EF599114
HMGCR	ATGTTGTCAAGACTCTTCCGAAT	CTTGGAAAGATAAAATTGCCAGA	EF599116
HMGCS1	AGGACTTCGTGGGACACACAT	GCTGCTTCAGGTTCTGCTGT	EF599117
SQLE	GGCCTTGATGCCCATGTT	TCATCATAAAGATCAGGGATGC	EF599115
LDLR	GGGAATATGACTGCAAGGACAT	AAGCCATGAACAGGATCCAC	AY871266
ABCG5	ATGTTGTTCGATGAGCCAACC	AATCCAGACCCAACAAGGATG	EF599647
ABCG8	TCCAGGACAGTGTGTTCCTCTC	AGAAGTCCGCAGGATTGCTA	EF599648
ABCB11	TTCTTTTAAGTTTCGATTTTCTTCA	GTGAATATTCGCCTTCTTCCA	EF601084
SLC10A1	CAGCATTGTGATGACCACCT	CCTTGGGAGTCTTGATTTTATTG	EF599649
GAPDH	AAGGGCACTGTAAAGGCAGA	AGAAGAGTGGGTGTCGCTGT	EF599650

we examined the expression of genes in liver that are involved in the biosynthesis of bile acids or the biosynthesis of cholesterol as well as the expression of genes in liver that are involved in the transport of cholesterol or bile acids to investigate if there are differences in the expression patterns of these genes between high- and low-responding opossums after a dietary challenge.

2. Materials and methods

2.1. Animals and diets

The 2 partially inbred strains (ATHH and ATHE) of laboratory opossums (*M domestica*) used for this investigation were produced at the Southwest Foundation for Biomedical Research (SFBR), and had inbreeding coefficients greater than 0.7. They were selectively bred for high and low responsiveness to dietary lipids. High-responding opossums were from the ATHH strain, and low-responding opossums were from the ATHE strain. Animals were maintained in polycarbonate cages under laboratory conditions as described previously [6]. Opossums were fed either the basal diet that contained 0.16% cholesterol (in grams per kilogram; dry weight basis) or the HCLF diet that had the same fat content as the basal diet but a cholesterol content of 0.71% [3].

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 Assessment and Accreditation of Laboratory Animal Care International, and is registered with the US Department of Agriculture.

2.2. Measurement of lipoprotein cholesterol and hepatic cholesterol

Plasma VLDL + LDL cholesterol was calculated as total cholesterol minus high-density lipoprotein cholesterol as described previously [3]. After consuming the HCLF diet for 4 weeks, plasma VLDL + LDL cholesterol of high responders in this study ranged from 273 to 389 mg/dL, whereas plasma VLDL + LDL cholesterol of low responders ranged from 19 to 40 mg/dL. Hepatic cholesterol was measured as described previously [4].

2.3. Measurement of bile acids

Bile acids were analyzed by high-performance liquid chromatography negative ion electrospray tandem mass spectrometry as described [7]. Sample preparation for analysis of bile acids in plasma was performed as described [8]. For analysis of bile acids in bile, the bile was diluted 100 times in water.

2.4. Northern blot analysis

To study expression of the genes listed in Table 1, we cloned *Monodelphis* complementary DNAs (cDNAs) of these genes. Human cDNAs were used as queries in discontiguous MegaBLAST searches against the *Monodelphis* whole genome sequence trace archive database, and primers were selected from *Monodelphis* sequences that aligned with human sequences using the online program Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3-www.cgi). *Monodelphis* cDNAs were synthesized by reverse transcription–polymerase chain reaction using their respective forward and reverse primers (Table 1), and cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA). The cDNAs were sequenced on an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA), and their identities were verified by BLAST searches based on homology to sequences of human and other species.

Tissues were collected from animals fed the basal diet or from animals fed the HCLF diet for 4 weeks. Livers, lungs, and kidneys were quickly cut into small pieces and snap-frozen in liquid nitrogen. The small intestine was divided into 6 equal segments; the mucosa was scraped from the third segment from the proximal end and snap-frozen. Total RNA was isolated from frozen, pulverized tissues using TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. The RNA was quantified using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Total RNA (4 μg) from

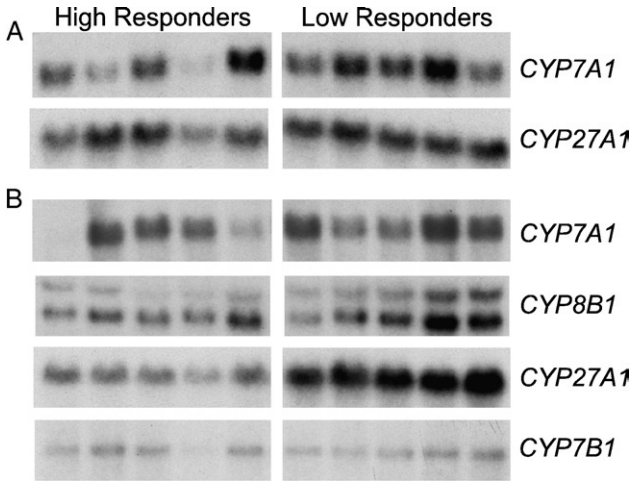

Fig. 1. Expression of genes encoding for enzymes in bile acid synthesis. Northern blots of total RNA (4 μg) from high- and low-responding opossums fed the basal (A) or HCLF diet (B) were hybridized with ^{32}P-labeled cDNA probes. The mRNA levels were quantified as described in the Materials and methods section. Expression of *CYP27A1* on the HCLF diet (B) was significantly lower ($P < .0001$) in high responders (17.7 ± 6.63 U) compared with low responders (74.1 ± 11.7 U). *CYP7A1* gene encodes cholesterol 7α-hydroxylase, *CYP8B1* gene encodes sterol 12α-hydroxylase, *CYP27A1* gene encodes sterol 27-hydroxylase, and *CYP7B1* gene encodes oxysterol 7α-hydroxylase.

each sample was fractionated by electrophoresis on 1% agarose-formaldehyde gels, and transferred to Hybond-XL membranes (Amersham Biosciences, Piscataway, NJ).

Probes were synthesized by amplifying the cDNA insert from each clone with gene-specific primers, and the polymerase chain reaction products were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; Perkin Elmer, Shelton, CT) using a megaprime DNA labeling kit (Amersham Biosciences). Blots were hybridized with radiolabeled cDNA probes in ULTRAhyb (Ambion, Austin, TX) according to the manufacturer's instructions. After an overnight hybridization at 42°C, blots were washed once in 2× SSC at 42°C for 5 minutes, once in 2× SSC at 65°C for 10 minutes, and twice in 0.2× SSC at 65°C for 20 minutes. After washing, blots were exposed to BioMax MR films (Kodak, Rochester, NY) or to phosphor screens to quantify the hybridization signals using the Storm 840 Imaging System (Amersham Biosciences). To correct for differences in the amount of RNA loaded on the gels or transferred to membranes, blots were stripped of the labeled probes by washing in 0.1% sodium dodecyl sulfate at 80°C for 15 minutes, and probed with radiolabeled glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) cDNA. All quantifications of gene expression were normalized to the *GAPDH* hybridization signal.

2.5. Statistical analysis

Results are expressed as mean ± SD. Statistical significance was determined with Student *t* test. A value of $P < .05$ was considered significant.

3. Results

3.1. Expression of bile acid synthesis genes

We examined the expression of 4 bile acid synthesis genes to investigate if any of these genes were affected by dietary cholesterol in high and low responders. The first gene was *CYP7A1*. The *CYP7A1* gene encodes cholesterol 7α-hydroxylase, which is the first and rate-limiting enzyme in the classic pathway [9]. The classic pathway is the major pathway for bile acid synthesis in the liver [9]. Variable expression of *CYP7A1* messenger RNA (mRNA) was observed in the 2 strains of opossums on the basal diet (Fig. 1A) as well as on the HCLF diet (Fig. 1B), and there was no difference in *CYP7A1* mRNA expression between high and low responders on the basal and HCLF diets.

The second gene was *CYP8B1*. The *CYP8B1* gene encodes sterol 12α-hydroxylase, which is the third enzyme in the classic pathway. Sterol 12α-hydroxylase produces 12α-hydroxylated intermediates for cholic acid synthesis, and thereby determines the ratio of cholic acid to chenodeoxycholic acid in the composition of bile acids [9]. Two of the low responders appeared to have higher *CYP8B1* mRNA levels than those in high responders on the HCLF diet (Fig. 1B), but the difference in *CYP8B1* expression between high and low responders was not statistically significant.

The third gene was *CYP27A1*. The *CYP27A1* gene encodes sterol 27-hydroxylase, which initiates bile acid synthesis in the alternate pathway to produce 27-hydroxycholesterol [9]. In addition, sterol 27-hydroxylase catalyzes oxidation of the steroid side chain in later steps of the classic and alternate biosynthesis pathways [9]. There was no difference in *CYP27A1* expression on the basal diet (Fig. 1A), but *CYP27A1* mRNA was expressed 4-fold higher ($P < .0001$) in low responders (74.1 ± 11.7 U) compared with high responders (17.7 ± 6.33 U) on the HCLF diet (Fig. 1B).

The fourth gene was *CYP7B1*. The *CYP7B1* gene encodes oxysterol 7α-hydroxylase, the enzyme that hydroxylates 27-hydroxycholesterol at the 7α position in the second step of the alternate pathway [9]. *CYP7B1* mRNA was expressed at very low levels in high and low responders on the HCLF diet (Fig. 1B), and similar levels of *CYP7B1* mRNA were detected in high and low responders.

In another experiment, we compared *CYP27A1* mRNA levels in opossums fed the HCLF diet with those in opossums fed the basal diet to determine how *CYP27A1* expression was affected by a cholesterol-enriched diet. The HCLF diet had little effect on *CYP27A1* expression in low responders, but down-regulated *CYP27A1* expression in high responders such that high responders fed the HCLF diet had the lowest level of *CYP27A1* mRNA compared with other groups of opossums (Fig. 2A). Analysis of bile acids in the gall bladder revealed that the composition of bile acids in high responders was similar to that in low responders (data not shown), indicating that there is no defect in the biosynthesis of bile acids in high responders.

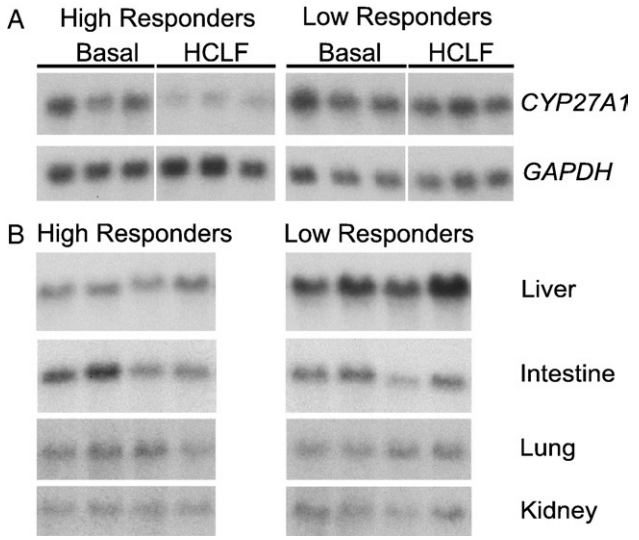

Fig. 2. Expression of the *CYP27A1* gene in liver and extrahepatic tissues. A, High- and low-responding opossums were fed the basal or HCLF diet. Total RNA isolated from the liver was subjected to electrophoresis on a formaldehyde-agarose gel, and analyzed by Northern blotting with a ^{32}P-labeled *CYP27A1* probe. After stripping the *CYP27A1* probe, the membrane was hybridized with a ^{32}P-labeled *GAPDH* probe. The mRNA levels were quantified as described in the Materials and methods section. B, Northern blots of total RNA from different tissues of high and low responders fed the HCLF diet were hybridized with a ^{32}P-labeled *CYP27A1* probe.

The *CYP27A1* gene is also expressed in extrahepatic tissues [10-12]; so we examined *CYP27A1* expression in small intestines, lungs, and kidneys of high and low responders fed the HCLF diet to determine if the pattern of gene expression in extrahepatic tissues was the same as that in the liver. We collected livers and extrahepatic tissues from another 4 high responders and 4 low responders. As shown in Fig. 2B, high and low responders differed in *CYP27A1* expression in the liver, which is consistent with the results in Fig. 1B; but the difference in *CYP27A1* expression was not detected in small intestine, lung, and kidney.

3.2. Expression of cholesterol synthesis genes

We investigated the effect of dietary cholesterol on the expression of cholesterol synthesis genes in high and low responders. The major rate-limiting enzyme in cholesterol synthesis is hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase. Mevalonic acid, the precursor of cholesterol, is synthesized from acetyl-CoA by 2 enzymes, HMG-CoA synthase and HMG-CoA reductase. HMG-CoA synthase catalyzes the production of the intermediate HMG-CoA from acetyl-CoA, and HMG-CoA reductase catalyzes the formation of mevalonic acid from HMG-CoA [13,14]. Squalene epoxidase, the enzyme that catalyzes the oxidation of squalene, is the secondary rate-limiting enzyme in cholesterol synthesis [15]. To determine if dietary cholesterol affects endogenous cholesterol synthesis in high and low responders, we measured mRNA levels of the genes encoding HMG-CoA reductase (*HMGCR*), HMG-CoA synthase (*HMGCS1*), and squalene epoxidase (*SQLE*) in opossums fed the basal or high-cholesterol diet. We also measured LDL receptor (*LDLR*) mRNA levels because *LDLR* expression is regulated by the sterol regulatory element-binding protein–2, the same transcription factor that regulates expression of cholesterol synthesis genes [16].

In opossums fed the basal diet, *HMGCR* (56.5 ± 20.8 U for low responders vs 18.3 ± 11.1 U for high responders; $P = .007$) and *SQLE* (5.96 ± 2.35 U for low responders vs 1.03 ± 0.66 U for high responders; $P = .002$) mRNAs were expressed at higher levels in low responders than in high responders (Fig. 3A). *HMGCS1* expression varied among low responders, and one high responder expressed a high level of *HMGCS1* mRNA (Fig. 3A). Despite variability in *HMGCS1* expression within the 2 groups of opossums, the average *HMGCS1* mRNA level was higher in low responders (19.5 ± 9.7 U for low responders vs 4.2 ± 2.9 U for high responders; $P = .01$). *LDLR* mRNA expression did not show a significant difference in high and low responders (Fig. 3A).

Plasma VLDL + LDL cholesterol concentrations of high and low responders do not differ on the basal diet [2,3]. Measurement of cholesterol in the liver revealed that hepatic

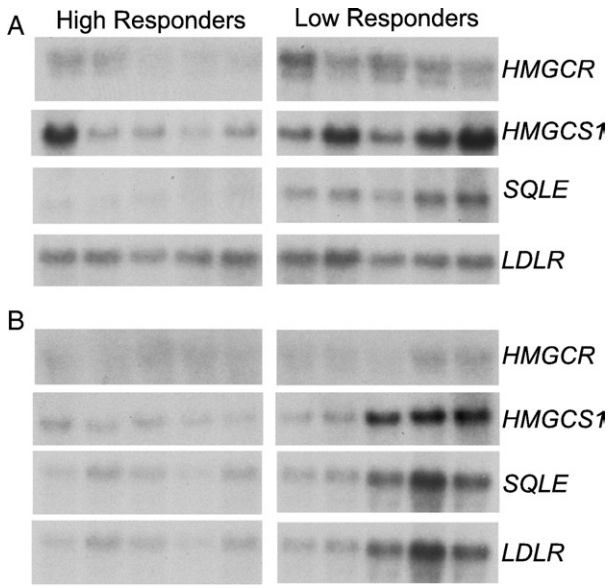

Fig. 3. Northern blots showing expression of the *HMGCR*, *HMGCS1*, *SQLE*, and *LDLR* genes. Total RNA from high- and low-responding opossums fed the basal (A) or HCLF diet (B) were hybridized with ^{32}P-labeled cDNA probes. The mRNA levels were quantified as described in the Materials and methods section. *HMGCR* (56.5 ± 20.8 U for low responders vs 18.3 ± 11.1 U for high responders; $P = .007$), *HMGCS1* (19.5 ± 9.7 U for low responders vs 4.2 ± 2.9 U for high responders; $P = .01$), and *SQLE* (5.96 ± 2.35 U for low responders vs 1.03 ± 0.66 U for high responders; $P = .002$) mRNAs were expressed significantly higher in low responders compared with high responders on the basal diet.

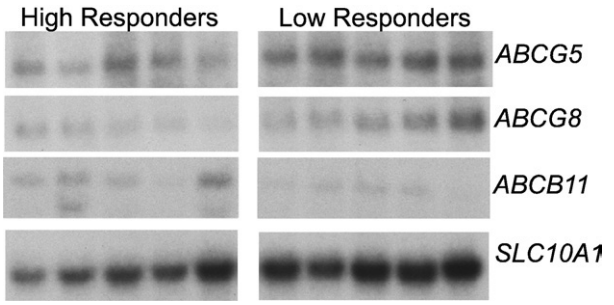

Fig. 4. Expression of sterol transporter genes (*ABCG5* and *ABCG8*) and bile acid transporter genes (*ABCB11* and *SLC10A1*). Northern blots of total RNA (4 μg) from high and low responders fed the HCLF diet were hybridized with ^{32}P-labeled cDNA probes. The mRNA levels were quantified as described in the Materials and methods section. *ABCG5* (19.9 ± 5.4 U for high responders vs 45.8 ± 14.3 U for low responders; P = .005), *ABCG8* (7.9 ± 2.8 U for high responders vs 12.7 ± 2.8 U for low responders; P = .026), and *SLC10A1* (16.4 ± 14.2 U for high responders vs 54.8 ± 20.8 U for low responders; P = .009) mRNAs were expressed significantly lower in high responders compared with low responders.

cholesterol concentrations were slightly elevated in high responders (3.61 ± 0.15 mg/g liver for high responders vs 2.81 ± 0.53 mg/g liver for low responders; P = .012). Therefore, lower mRNA levels of the cholesterol biosynthesis genes in high responders reflect that endogenous cholesterol synthesis is suppressed to maintain normal concentrations of plasma cholesterol.

When the diet was changed to one enriched with cholesterol, hepatic cholesterol was increased in low responders (2.81 ± 0.53 mg/g liver on basal diet vs 4.34 ± 0.42 mg/g liver on HCLF diet) as well as in high responders (3.61 ± 0.15 mg/g liver on basal diet vs 8.86 ± 2.74 mg/g liver on HCLF diet); and the increase was greater in high responders. *HMGCR* expression was hardly detectable in high and low responders fed the HCLF diet (Fig. 3B), implying that endogenous cholesterol is synthesized at a very low rate to counteract the increase in hepatic cholesterol. *HMGCS1*, *SQLE*, and *LDLR* mRNAs were also expressed at very low levels in all of the high responders (Fig. 3B). However, there were 2 distinct patterns of expression of these genes in low responders. The *HMGCS1*, *SQLE*, and *LDLR* genes were coordinately down-regulated in 2 of the 5 low responders. Plasma VLDL + LDL cholesterol in these 2 low responders (27 and 40 mg/dL) was slightly higher than that in the other 3 low responders (~19 mg/dL), which may explain the difference in expression of the *HMGCS1*, *SQLE*, and *LDLR* genes.

3.3. Expression of sterol transporter genes

The adenosine triphosphate–binding cassette (ABC) transporter genes, *ABCG5* and *ABCG8*, encode proteins that are involved in sterol transport [17,18]. *ABCG5* and *ABCG8* are half-size transporters that dimerize to form a functional transporter at the apical membrane of liver to remove sterol from the liver into bile [19,20]. We investigated if expression of the *ABCG5* and *ABCG8* genes differed between the 2 strains of opossums on the HCLF diet. Lower levels of *ABCG5* (19.9 ± 5.4 U for high responders vs 45.8 ± 14.3 U for low responders; P = .005) and *ABCG8* (7.9 ± 2.8 U for high responders vs 12.7 ± 2.8 U for low responders; P = .026) mRNAs were detected in high responders compared with low responders (Fig. 4).

3.4. Expression of bile salts transporter genes

After bile salts are synthesized in the liver, they are first secreted into the bile canaliculi by the bile salt export pump, then secreted into the intestinal lumen. Eventually, most of the excreted bile acids are reabsorbed in the lower intestine and go to the hepatic portal circulation. Finally, uptake of most of the conjugated bile acids and some of the unconjugated bile acids from the portal circulation back to hepatocytes is mediated by the sodium-taurocholate cotransporting protein to complete the enterohepatic circulation of bile salts [21,22].

We analyzed expression of the *ABCB11* gene, which encodes the bile salt export pump. The bile salt export pump mediates the excretion of taurine- or glycine-conjugated bile salts from hepatocytes [21,22]. Northern blots showed very weak signals for *ABCB11* expression, and there was no consistent difference in *ABCB11* mRNA levels between high- and low-responding opossums on the HCLF diet (Fig. 4).

We also analyzed expression of the *SLC10A1* gene, which encodes the sodium-taurocholate cotransporting protein. As shown in Fig. 4, high responders expressed lower levels of *SLC10A1* mRNA (16.4 ± 14.2 U for high responders vs 54.8 ± 20.8 U for low responders; P = .009). Analysis of bile acids in plasma revealed that the levels were markedly elevated in the plasma of high responders (205-480 μmol/L) relative to those in low responders (12-58 μmol/L). The difference was predominantly caused by increased levels of the mature bile acids, taurocholic acid, and taurochenodeoxycholic acid. Decreased *SLC10A1* expression in high responders may reduce the uptake of bile acids from the hepatic portal circulation and lead to an accumulation of bile acids in plasma.

4. Discussion

High- and low-responding laboratory opossums were developed to study the effect of dietary cholesterol on plasma lipoprotein cholesterol. To investigate how some of the important hepatic genes that control cholesterol homeostasis respond to dietary cholesterol in the opossum model, we compared their expression in high- and low-responding opossums by Northern blot analysis. A notable difference is the expression of the *CYP27A1* gene, which was down-regulated in the livers of high responders after they were fed a high-cholesterol diet; but the same diet had little effect on *CYP27A1* expression in low responders (Fig. 2A).

The *CYP27A1* gene encodes the mitochondrial enzyme sterol 27-hydroxylase that is expressed in a wide variety of tissues. In the liver, sterol 27-hydroxylase catalyzes several reactions in bile acid synthesis [9]. Therefore, decreased expression of the *CYP27A1* gene may impede synthesis and excretion of bile acids in high responders. Sterol 27-hydroxylase is also expressed in extrahepatic tissues, where it converts cholesterol to 27-hydroxycholesterol or 3β-hydroxy-5-cholestenoic acid. The 27-oxygenated products are then transported to the liver, where they are metabolized to bile acids [23,24]. Northern blots showed that in kidney, lung, and intestine, high and low responders had similar levels of *CYP27A1* expression, which were different from the pattern expressed in liver (Fig. 2B). Thus, in high responders, the effect of dietary cholesterol on *CYP27A1* expression in the liver was different from that in extrahepatic tissues.

Another difference is the expression of the *ABCG5* and *ABCG8* genes in opossums fed the HCLF diet. Repa et al [25] reported that *ABCG5* and *ABCG8* mRNAs were up-regulated by cholesterol feeding in mice, and the response was mediated by the ligand-activated transcription factor liver X receptor (LXR). The naturally occurring ligands for LXR are oxysterols. One of the oxysterols that activates LXR is 27-hydroxycholesterol, which is produced from cholesterol by sterol 27-hydroxylase. It is conceivable that lower levels of *ABCG5* and *ABCG8* mRNAs in high responders reflect lower levels of 27-hydroxycholesterol because of lower levels of *CYP27A1* mRNA in high responders. Lower *ABCG5* and *ABCG8* expression implies less cholesterol is secreted into bile, so excess cholesterol accumulates in the livers of high responders.

A third difference is lower expression of the bile salt transporter gene *SLC10A1* in high responders. The sodium-taurocholate cotransporting protein encoded by the *SLC10A1* gene is expressed at the basolateral membrane of hepatocytes and extracts bile acids from the portal circulation back to the liver [21,22]. *SLC10A1* mRNA expression in the liver has been shown to be down-regulated by the retention of biliary constituents in bile-obstructed rats as a defense mechanism to protect the liver against toxic bile acids [26]. High plasma levels of bile acids are most likely due to low *SLC10A1* expression in high responders.

Plasma cholesterol response to dietary cholesterol and fat differs considerably among species and among individuals of the same species including humans. A number of animal species such as rabbits [27], mice [28], rats [29], rhesus monkeys [30], baboons [31,32], and humans [33,34] also exhibit individual variability in lipoprotein cholesterol in response to dietary cholesterol. Increased cholesterol absorption has been suggested to be a possible mechanism for high and low responsiveness to dietary lipids in some animals and humans [35]. Our previous studies have demonstrated that high-responding opossums as compared with low-responding opossums have a 2-fold higher percentage of cholesterol absorption on a cholesterol- and fat-enriched diet [4]. Several genes (*ABCG5, ABCG8, ACAT2, NPC1L1, SR-BI*, and *CD36*) play a role in intestinal cholesterol absorption [36,37]. The expression pattern of these genes in the intestines of high- and low-responding opossums will be reported in a separate manuscript. The present studies were conducted to determine whether high- and low-responding opossums differ in the expression of hepatic cholesterol–responsive genes and thus hepatic cholesterol metabolism on the high-cholesterol diet. The results clearly demonstrate that high-responding opossums as compared with low-responding opossums express lower levels of 4 genes (*CYP27A1*, *ABCG5*, *ABCG8*, and *SLC10A1*) that affect hepatic cholesterol and bile acid metabolism. Similar studies were conducted in high- and low-responding baboons that were produced by selective breeding based on variability in LDL cholesterol to a high-cholesterol and high-fat diet [31,32]. In the baboon model, high-responding baboons have moderately elevated LDL cholesterol. High-responding baboons also express lower levels of *CYP27A1*, *ABCG5*, and *ABCG8* mRNAs in their livers compared with low-responding baboons after the dietary challenge [32,38]. However, there is a difference in the regulation of *CYP27A1* expression between baboons and opossums. The difference in *CYP27A1* expression in the baboon model is due to up-regulation of the *CYP27A1* gene to higher levels in low-responding baboons relative to those in high-responding baboons [32], whereas the difference in *CYP27A1* expression in the opossum model is due to down-regulation of the *CYP27A1* gene in high-responding opossums, although *CYP27A1* gene expression is not affected by the high-cholesterol diet in low-responding opossums. The genes that control responsiveness to dietary cholesterol in opossums and baboons may be different and have not yet been identified. Thus, the opossum and baboon models provide an opportunity for a comparative study of the genetic basis of variation in the responsiveness of lipoprotein cholesterol to dietary cholesterol.

In conclusion, the *CYP27A1*, *ABCG5*, *ABCG8*, and *SLC10A1* genes were expressed at lower levels in high-responding opossums compared with low-responding opossums on the HCLF diet. Down-regulation of the *CYP27A1* gene may affect *ABCG5* and *ABCG8* expression, and contribute to the accumulation of plasma VLDL + LDL cholesterol and hepatic cholesterol. Down-regulation of the *SLC10A1* gene may contribute to the accumulation of bile acids in plasma.

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