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RESEARCH ARTICLES

Expression levels of ACAT1 and ACAT2 genes in the liver and intestine of baboons with high and low lipemic responses to dietary lipids

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Abstract

Acyl–coenzyme A:cholesterol acyltransferase (ACAT) 1 and ACAT2 play an important role in cellular cholesterol esterification and thus modulate intestinal cholesterol absorption and hepatic lipoprotein secretion. The relative expression levels of ACAT1 and ACAT2 in human tissues differ from those in other animals, including nonhuman primates. The present study compared the relative expression levels of ACAT1 and ACAT2 in baboons with high and low lipemic responses to dietary lipids. We isolated RNA and prepared cDNA from frozen liver and small intestine from high- and low-responding pedigreed baboons necropsied after consuming a high-cholesterol and high-fat diet for 18 months. The expression of ACAT1 and ACAT2 was measured by TaqMan real-time quantitative PCR normalized to 18s ribosomal RNA. The expression of ACAT1 was higher than that of ACAT2 in the liver, whereas the expression of ACAT2 was higher than that of ACAT1 in the duodenum and jejunum. There was no difference in the expression of ACAT1 or ACAT2 in the liver and intestine between high- and low-responding baboons except that the expression of ACAT1 was higher in the duodenum of high responders than in that of low responders. Western blot analysis also showed a higher level of ACAT1 protein in the duodenum of high responders than in that of low responders. There was a significant correlation between duodenal ACAT expression levels and total plasma cholesterol concentration in baboons. These results suggest that differences in ACAT1 expression may affect plasma cholesterol concentration and partly affect diet-induced hyperlipidemia.

Keywords: Lipid metabolism; Duodenum; Jejunum; Ileum; Diet; Cholesterol metabolism; Diet-induced hyperlipidemia

1. Introduction

Acyl–coenzyme A:cholesterol acyltransferase (ACAT) is responsible for synthesizing cholesteryl esters in the cell from cholesterol and fatty acyl–coenzyme A [1,2]. The cholesteryl esters produced in hepatocytes can be stored in the cell as droplets, secreted into bile or incorporated into apolipoprotein B100-containing particles for their secretion as very low-density lipoproteins (VLDLs) [3–5]. The cholesteryl esters produced in intestinal cells are incorporated into apolipoprotein B48-containing particles and secreted as chylomicrons [6,7]. Hepatic ACAT activity is highly correlated with plasma low-density lipoprotein (LDL) cholesteryl ester concentration and coronary atherosclerosis [8]. ACAT activity and expression are often up-regulated in the liver of high-responding animals on a high-cholesterol and high-fat (HCHF) diet [9–11]. The oral administration of ACAT inhibitors in animals decreases cholesterol absorption, hepatic lipoprotein secretion, plasma lipoprotein cholesterol levels and the extent of atherosclerosis [5,12,13]. Thus, ACAT plays an important role in lipoprotein metabolism and atherosclerosis.

ACAT is present in two forms—ACAT1 and ACAT2 [2,6,14]. ACAT1 has been found in each cell type in humans and is the major isoenzyme of the adult human liver [6,15]. On the other hand, mice and monkeys have ACAT2 as the major isoenzyme in their liver [16,17]. The present study was conducted to measure tissue distribution of ACAT1 and ACAT2 in baboons to determine whether they resembled humans or monkeys and mice in the hepatic distribution of ACAT1 and ACAT2. Because ACAT plays an important role in cholesterol absorption and lipoprotein secretion, we also wanted to investigate whether the expression of ACAT1 and ACAT2 in the liver and intestine of baboons differs between those with high and those with low lipemic responses to dietary cholesterol and fat. If such differences exist, they may contribute to the differences in their responsiveness to dietary lipids.

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2. Materials and methods

2.1. Animals and diets

The protocol of this study was approved by the Institutional Animal Care and Use Committee of the Southwest Foundation for Biomedical Research.

We used frozen liver and intestinal samples from 19 high-LDL-responding and 13 low-LDL-responding baboons necropsied in an experiment conducted to determine the relationship of postprandial lipoproteins to atherosclerosis. High- and low-responding baboons were the progeny of high- and low-responding sires, respectively. After weaning, the baboons were fed a low-cholesterol and low-fat basal diet (Ralston Purina Monkey Chow). At age 27 months, they were fed an HCHF diet prepared by adding lard, cholesterol, salt and vitamins to a basic meal (Ralston Purina Monkey Meal 25-045-6) [18]. The HCHF diet provided 40% of calories from fat, of which 43% was derived from saturated fatty acids, and 1.7 mg cholesterol/1000 kcal.

2.2. Cholesterol and lipoprotein analysis

Total serum and high-density lipoprotein (HDL) cholesterol were measured by enzymatic methods with an ABA 100 Bichromatic Analyzer (Abbot Laboratories, South Pasadena, CA, USA). VLDL and LDL were precipitated by the Lipid Research Clinics procedure and HDL cholesterol was measured in the supernatant. The analytic methods met the criteria of the Centers for Disease Control Lipid Standardization Program.

2.3. Liver and intestinal sampling

At age 45 months (18 months on the HCHF diet), the baboons were necropsied after euthanasia with ketamine and pentobarbital. Liver samples were removed and quick frozen with liquid nitrogen and stored at −80°C. The small intestine was removed and opened longitudinally. The duodenum, jejunum and ileum were scraped separately. All the scrapings from each section were pooled and transferred to a plastic bag, quick frozen in liquid nitrogen and stored at −80°C.

2.4. Isolation of RNA

Total RNA was isolated from tissues (liver and small intestine scrapings) using a TRIzol Reagent kit from Invitrogen (Invitrogen, Carlsbad, CA, USA). Liver tissue and intestinal scrapings were kept on dry ice and small pieces were chiseled out. The chiseled pieces were placed on a microscope slide on dry ice in a tray and were minced after the addition of 100 μl of TRIzol Reagent. The contents were transferred to a microfuge tube and placed on ice. A volume of 900 μl of TRIzol Reagent was added again (a total of 1 ml) and the tissue was homogenized to completeness. After homogenization, 200 μl of chloroform was added to the homogenate. The RNA was precipitated with 500 μl of isopropanol and the precipitate was washed with 1 ml of 75% ethanol. The RNA was dissolved with 100 μl of DNase- and RNase-free water (Invitrogen). The RNA samples were then ethanol precipitated and resuspended with the initial volume using DNase- and RNase-free water. The quality and quantity of the isolated RNA were measured using a spectrophotometer (Biophotometer, Eppendorf, Westbury, NY, USA). The samples were stored in small aliquots at −80°C.

2.5. Preparation of cDNA

cDNA was prepared using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). The reaction mixture consisted of a reverse transcriptase (RT) buffer, RT random primers, dNTP mixture, DNase- and RNase-free water, MultiScribe RT enzyme and RNA samples (2 μl/50 μl reaction). The reaction ran at 25°C for 10 min and at 37°C for 120 min in a thermocycler (Applied Biosystems).

2.6. Quantification of gene expression

The expression of ACAT1 and ACAT2 was measured using primers and probes designed for human genes. Primers and probes (FAM labeled) for ACAT1 and 18s ribosomal RNA designed for human genes were purchased from Applied Biosystems (Assay-on-Demand Gene Expression Products). The primers and probe for ACAT2 were designed based on a recent report by Smith et al. [19]. The sequences of the primers and probe were as follows: forward primer, 5′-GCAAGTCCCTGCTTGATGAGC-3′; reverse primer, 5′-CCAGCGATGAA CATGTGG TAGAT-3′; probe, 5′-(FAM)-TGCGGAAATGCTGCACCTCCAT-(TAMRA)-3′. Primers were synthesized by Sigma-Genosys (The Woodlands, TX, USA), and the probe was synthesized by the Custom Oligo Synthesis Service of Applied Biosystems. The assay mixture consisted of a probe (labeled with FAM dye) and unlabeled PCR primers for human ACAT1 and ACAT2 genes. The components of a 20-μl gene expression assay reaction included 10 μl of a TaqMan Universal PCR Master Mix (AmpErase UNG), 1 μl of 20× Assays-on-Demand Gene Expression Assay Mix or 0.7 μl of primers and 0.3 μl of probe, 3 μl of diluted cDNA and 6 μl of water. All components were added to a well in the optical reaction plate covered with optical adhesive cover (Applied Biosystems). The reaction was conducted in an ABI Prism 7000 Sequence Detection System (Applied Biosystems). The mixture was incubated at 50°C for 2 min and at 95°C for 10 min, and 40 cycles of PCR were performed at 95°C for 15 s and at 60°C for 1 min.

To determine the linear range for the expression of each cDNA, a standard curve was run using 5- to 40-fold dilutions of the cDNA. Based on the linear range, we used 1:10 dilutions of cDNA for each expression assay. The assays were conducted in duplicate. The expression of 18s ribosomal RNA was used to normalize the RNA concentration. The change in threshold cycle (ΔCt) for each ACAT isoenzyme was calculated as ΔCt = (Ct of ACAT isoenzyme) − (Ct of 18s ribosomal RNA). The ratio of ACAT isoenzyme to 18s ribosomal RNA was calculated as $2^{-\Delta Ct}$ and expressed as $2^{-\Delta Ct} \times 10^5$. The relative abundance of ACAT2 to ACAT1 was calculated by ΔΔCt using the

Table 1
Plasma lipoprotein cholesterol concentrations (mean±S.E.) of high- and low-responding baboons maintained on an HCHF diet

Phenotype	*n*	Total serum cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)
High responders	19	7.01±0.35[a]	4.25±0.29[a]	2.75±0.10[b]
Low responders	13	3.72±0.13	1.31±0.05	2.39±0.12

[a] Significantly different ($P<.001$).
[b] Significantly different ($P=.032$).

equation described by Smith et al. [19], ΔΔCt = (ΔCt of ACAT2) − (ΔCt of ACAT1). The ratio of ACAT2 to ACAT1 was calculated as $2^{-\Delta\Delta Ct}$.

2.7. Western blotting

Liver samples (300 mg) and intestinal (duodenum) mucosal scrapings (100 mg) were homogenized with 10% sodium dodecyl sulfate (SDS; 5 ml for liver sample and 2 ml for duodenum sample) and 20 μl of Protease Inhibitor Cocktail from Sigma (St. Louis, MO, USA) containing 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin and aprotinin [15]. The protein concentration of the homogenate was measured by using a Bio-Rad Protein Assay kit with albumin as a standard (Bio-Rad Laboratories, Hercules, CA, USA). An aliquot of the homogenate (50-μg protein) was mixed with an equal volume of sample buffer containing 120 mM Tris, pH 6.8, 20% (vol/vol) glycerol, 4% (wt/vol) SDS, 0.01% (wt/vol) bromophenol blue and dithiothreitol with a final concentration of 0.1 M [10]. The samples were incubated at 37°C for 30 min and applied onto 10% Tris–HCl gels (Bio-Rad Laboratories). After electrophoresis, the proteins were blotted to nitrocellulose membrane (Immun-Blot PVDF membrane, Bio-Rad Laboratories) in Tris-glycine transfer buffer containing SDS at 85 V overnight. Nonspecific binding sites on the membrane were blocked by 5% nonfat dry milk dissolved in phosphate-buffered saline. Lyophilized affinity-purified rabbit IgG (50 μg) raised against either human ACAT1 or ACAT2 protein (kindly provided by Dr. Catherine C.Y. Chang, Department of Biochemistry, Dartmouth Medical School, Hanover, NH, USA) was dissolved in 100 μl of sterile phosphate-buffered saline containing 0.02% sodium azide and used as primary antibody (1:500 for liver and 1:250 for duodenum samples). A donkey anti-rabbit horseradish peroxidase-conjugated secondary antibody (Amersham Pharmacia Biotech, Piscataway, NJ, USA) was used (1:250 for liver and 1:100 for duodenum samples). Peroxidase signal was detected using ECL plus Western Blotting Detection System (Amersham Pharmacia Biotech). The protein mass was compared after quantifying the intensity of protein bands by Chem-iImager 4000 (Alpha Innotech, San Leandro, CA, USA).

2.8. Data analysis

Values in the tables and figures are expressed as mean±S.E. Values for the high-and low-responding groups were compared by a standard *t* test. Associations among the variables were determined by using Pearson's correlation. Significance was set at $P<.05$.

3. Results

3.1. Serum lipoprotein cholesterol concentrations in high- and low-responding baboons

Table 1 presents the serum lipoprotein cholesterol concentrations of high- and low-responding baboons. Total serum ($P<.001$), LDL ($P<.001$) and HDL ($P=.032$) cholesterol concentrations were significantly higher in high-responding baboons than in low-responding baboons on the HCHF diet.

3.2. Standard curves

Fig. 1 shows the relationship between Ct numbers and the dilution of cDNA for ACAT1, ACAT2 and 18s ribosomal

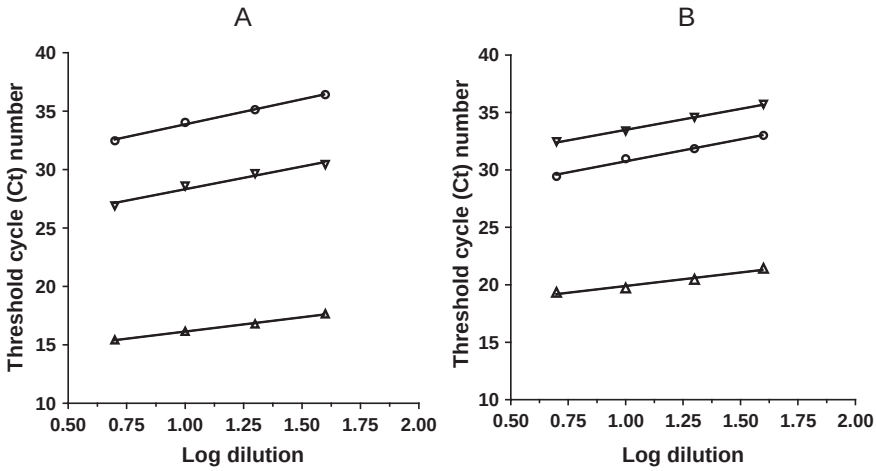

Fig. 1. Relationship of Ct number and log dilution of cDNA in the expression assay of ACAT1 (∇), ACAT2 (○) and 18s ribosomal RNA (Δ) in the liver (A) and intestine (jejunum) (B) of a represented baboon maintained on an HCHF diet. The measurements were done using an ABI Prism 7000 Sequence Detection System. The cDNA was diluted from 5- to 40-fold and the assay was conducted in duplicate.

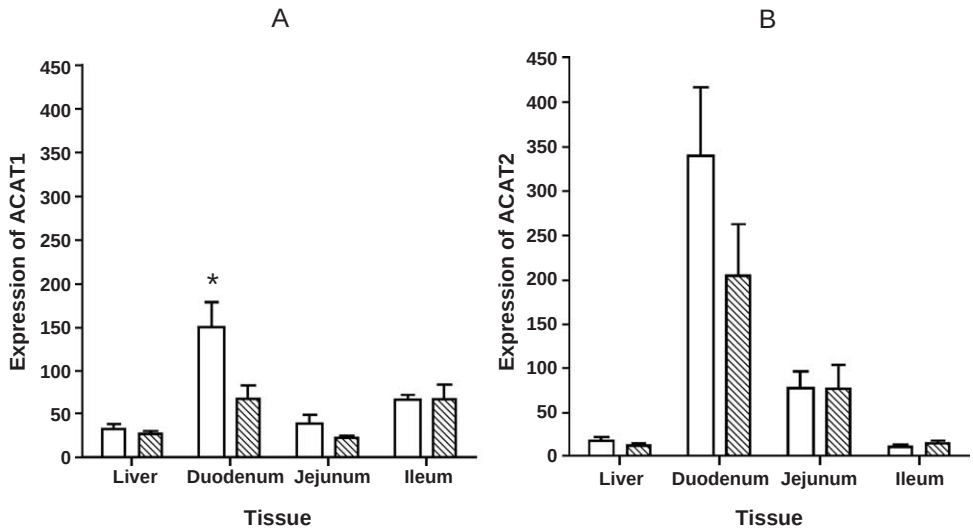

Fig. 2. Expression levels (mean±S.E.) of ACAT1 (A) and ACAT2 (B) in the liver and intestine of 12 high-responding baboons (open bars) and 12 low-responding baboons (striped bars) maintained on an HCHF diet. The asterisk indicates significant difference between high- and low-responding baboons in the same tissue (P=.022). The expression of ACAT1 and ACAT2 was normalized with 18s ribosomal RNA and expressed as (ACAT1 or ACAT2/18s ribosomal RNA)×10^5.

RNA in the liver (Fig. 1A) and intestine (jejunum) (Fig. 1B) of a represented baboon. There was a log-linear relationship between the cDNA dilution from 5- to 40-fold and the Ct number for all genes in both the liver and intestine. Based on Ct numbers, the expression of ACAT1 was much higher than that of ACAT2 in the liver, whereas the expression of ACAT2 was higher than that of ACAT1 in the intestine (jejunum) in the representative baboon.

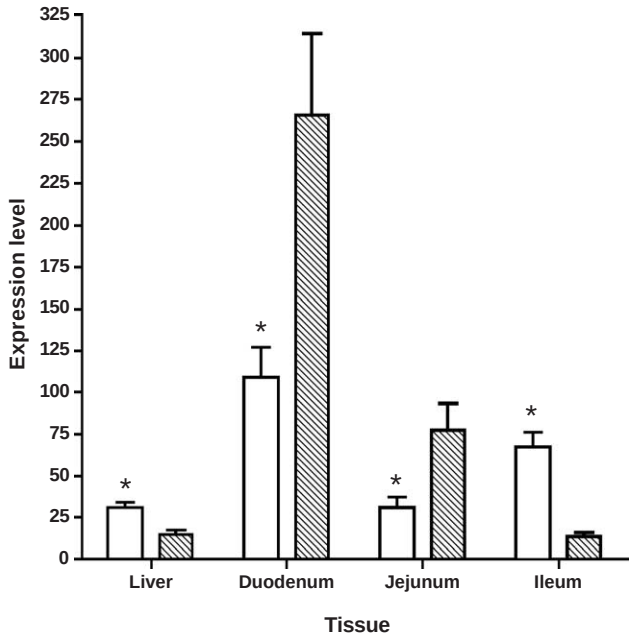

Fig. 3. Expression levels of ACAT1 (open bars) and ACAT2 (striped bars) in the liver and intestine of 24 baboons (pooled data for high and low responders) maintained on an HCHF diet. Asterisks indicate significant differences between the expression levels of ACAT1 and ACAT2 in the liver (P=.002), duodenum (P=.011), jejunum (P=.005) and ileum (P<.0001). The expression of ACAT1 and ACAT2 was normalized to 18s ribosomal RNA and expressed as (ACAT1 or ACAT2/18s ribosomal RNA)×10^5.

3.3. Expression of ACAT1 and ACAT2 genes in the liver of high- and low-responding baboons

Fig. 2 presents expression levels of ACAT1 and ACAT2 in the liver of high- and low-responding baboons. The quantities of expression levels were normalized to 18s ribosomal RNA. There was no difference in the expression levels of ACAT1 or ACAT2 between the livers of high- and low-responding baboons (Fig. 2). The expression of ACAT1 was much higher than that of ACAT2 in the livers of baboons (P=.002) (Fig. 3; pooled data for high and low responders). The ratio of ACAT2 to ACAT1 in the liver was

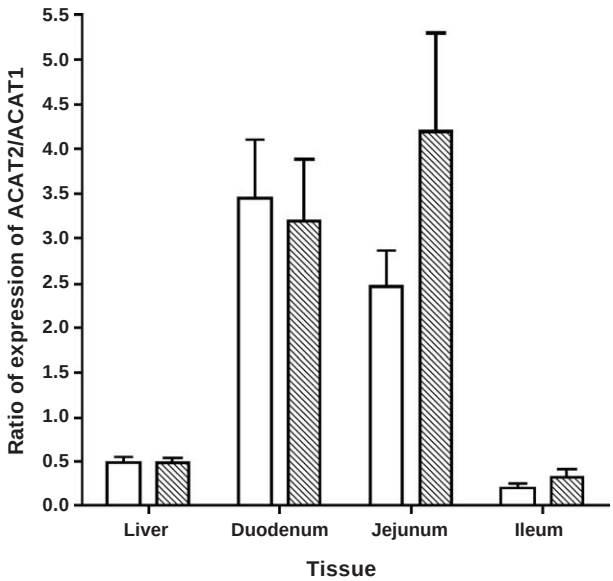

Fig. 4. Ratio of expression of ACAT2/ACAT1 in the liver and intestine of 12 high-responding (open bars) and 12-responding (striped bars) baboons maintained on an HCHF diet. The expression of ACAT1 and ACAT2 was normalized to 18s ribosomal RNA and expressed as (ACAT1 or ACAT2/18s ribosomal RNA)×10^5.

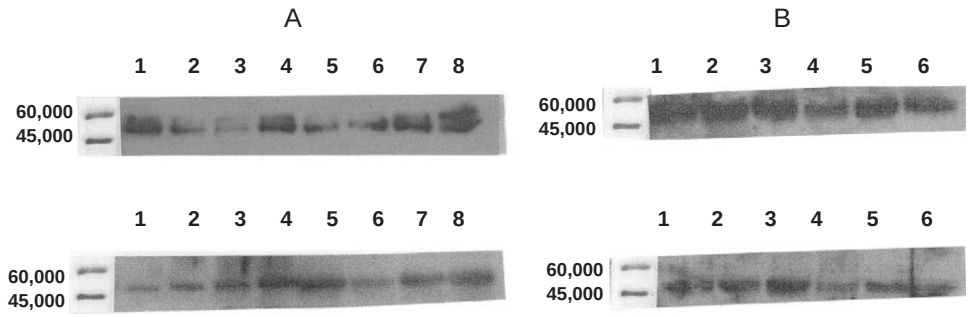

Fig. 5. Western blot analysis showing ACAT1 (top lane) and ACAT2 (bottom lane) protein bands from the liver (A) and duodenum (B) of high-responding (1–4 for liver and 1–3 for duodenum) and low-responding (5–8 for liver and 4–6 for duodenum) baboons. Molecular weight markers are shown in the left-hand side of each blot. There was no difference in the protein mass of ACAT1 and ACAT2 in the livers between the high- and low-responding baboons. Similarly, there was no difference in the mass of ACAT2 in the duodenum between high- and low-responding baboons. However, there was a significant difference in the protein mass for ACAT1 between high- and low-responding baboons (high/low ratio = 1.44; $P = .032$). The intensity of protein bands was quantified using ChemiImager (Alpha Innotech).

0.481±0.075 in high-responding baboons and 0.488±0.064 in low-responding baboons (Fig. 4).

3.4. Expression of ACAT1 and ACAT2 in the intestine of high- and low-responding baboons

Fig. 2 also presents expression levels of ACAT1 and ACAT2 in three segments of the intestine. There was no difference in the expression levels of ACAT2 in any segment of the intestine between high- and low-responding baboons. Similarly, there was no difference in the expression of ACAT1 in the jejunum and ileum between high- and low-responding baboons. However, the expression of ACAT1 was higher ($P = .022$) in the duodenum of high-responding baboons than in that of low-responding baboons (Fig. 2). The expression of ACAT2 was much higher than that of ACAT1 in the duodenum ($P = .011$) and the jejunum ($P = .005$) of baboons (Fig. 3; pooled data for high and low responders). However, the expression of ACAT1 was higher than that of ACAT2 in the ileum of baboons ($P < .001$) (Fig. 3). The ratio of ACAT2 to ACAT1 was 3.442±0.664 in the duodenum and 2.594±0.400 in the jejunum of high-responding baboons (Fig. 4). The ratio of ACAT2 to ACAT1 was 3.196±0.689 in the duodenum and 4.195±1.154 in the jejunum of low-responding baboons (Fig. 4). The ratio of ACAT2 to ACAT1 in the ileum was 0.194±0.060 in high-responding baboons and 0.326±0.112 in low-responding baboons (Fig. 4).

3.5. Detection of ACAT1 and ACAT2 protein in the liver and intestine of high- and low-responding baboons

Fig. 5 shows the Western blot analysis of ACAT1 and ACAT2 proteins as detected by human antibodies to these proteins in the liver and intestine (duodenum) of high- and low-responding baboons. ACAT2 had a slightly lower molecular weight than ACAT1 (Fig. 5). The intensity of protein bands for ACAT2 did not differ between high- and low-responding baboons in the liver or the intestine (duodenum). Similarly, there was no difference in the intensity of protein bands for ACAT1 between high-and low-responding baboons in the liver. However, the intensity of protein bands for ACAT1 in the duodenum was much higher in the high-responding baboons than in the low-responding baboons (ratio of high- to low-responding baboons, 1.44; $P = .003$).

3.6. Relationship between expression of ACAT1 in the duodenum and plasma cholesterol levels in high- and low-responding baboons

There was a strong positive correlation between the expression level of ACAT1 in the duodenum and total plasma cholesterol ($r = .448$; $P = .032$) in high- and low-responding baboons (Fig. 6). Similarly, there was a positive correlation between the expression level of ACAT1 in the duodenum and LDL cholesterol concentration ($r = .410$; $P = .05$; data not presented) but not HDL cholesterol concentration. However, there was no such correlation between the ACAT1 expression level in the liver or any

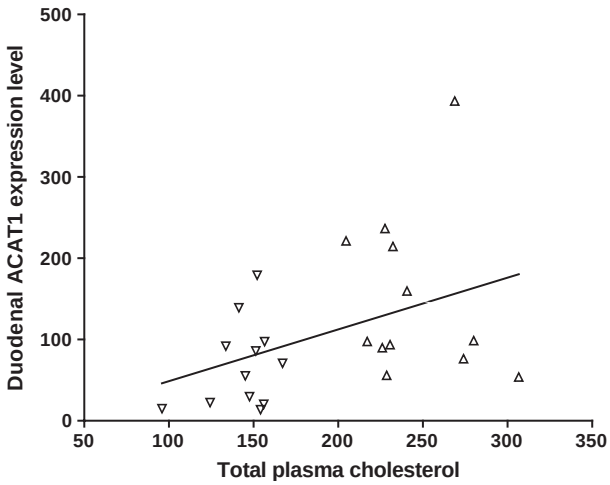

Fig. 6. Correlation between ACAT1 expression level in the duodenum and total plasma cholesterol in high-responding baboons (Δ) and low-responding baboons (∇) ($n = 24$) maintained on an HCHF diet. There was a significant positive association between ACAT1 expression in the duodenum and total plasma cholesterol ($r = .448$; $P = .032$).

segment of the intestine and plasma or lipoprotein cholesterol concentrations. Similarly, there was no correlation between ACAT2 expression and plasma or lipoprotein cholesterol concentrations.

4. Discussion

The main aim of the present study was to quantify expression levels of ACAT1 and ACAT2 genes in the liver and intestine of baboons to assess whether ACAT1 and ACAT2 expression in the liver of baboons is similar to that in humans [6,15,20] or to that in mice [2,16] and monkeys [17]. These studies show that the major isoenzyme of baboon liver is ACAT1 as in humans. However, ACAT2 is expressed in slightly higher levels, and the ratio between ACAT2 and ACAT1 was 0.48 instead of 0.112 as recently reported [19]. We used tissues from baboons that were maintained on an HCHF diet for 18 months and it is possible that ACAT2 was induced because of dietary cholesterol and fat [10] and may have influenced the ACAT2/ACAT1 ratio. However, we do not have results of similar studies on the chow diet and therefore cannot address this possibility. There were no differences in the expression or protein mass of ACAT1 and ACAT2 between the livers of high- and low-responding baboons; thus, ACAT1 or ACAT2 expression or protein mass does not influence dietary responsiveness in baboons.

These studies further demonstrate that, as in humans and other animals, ACAT2 is the major isoenzyme of the duodenum and jejunum, where the bulk of the cholesterol is absorbed. The highest expression of ACAT1 and ACAT2 was in the duodenum, followed by the jejunum, where the ACAT2/ACAT1 ratio ranged from 2.59 to 4.19. These studies also show that the expression of ACAT2 was the lowest in the ileum, where the ACAT2/ACAT1 ratio was similar to that in the liver. The significance of the low expression of ACAT2 in the ileum is not clear from these studies. It is possible that because the ileum does not play a major role in cholesterol absorption, the decreased expression of ACAT2 in the ileum may be related to its lesser ability to absorb dietary cholesterol. There was no difference in the expression or protein mass of ACAT2 in the intestine between high- and low-responding baboons. However, there was an increased expression of ACAT1 in the duodenum of high-responding baboons compared with low-responding baboons and there was a strong positive association of ACAT1 expression in the duodenum and total plasma and LDL cholesterol concentrations. No such association was present in the expression of ACAT1 or ACAT2 in any other segment of the intestine and plasma or lipoprotein cholesterol. Thus, it is likely that ACAT1 expression may modulate plasma cholesterol concentration on the HCHF diet in baboons. Our previous studies showed that high-responding baboons had higher fractional cholesterol absorption than did low-responding baboons on the HCHF diet [18]. Therefore, the difference in the expression of ACAT1 in the duodenum may influence cholesterol absorption and in turn may modulate plasma cholesterol concentrations on the HCHF diet.

One study showed that ACAT1 is regulated by posttranscriptional mechanisms [20]. However, differences in the expression of ACAT1 in the duodenum were consistent with the differences in the protein mass of ACAT1 between high- and low-responding baboons. These observations suggest that the differences in ACAT1 expression in the duodenum between high- and low-responding baboons are regulated at the transcriptional level. The transcriptional regulation of ACAT1 may be caused by a difference in the promoter region. The expression of ACAT1 gene was consistently higher in all the tissues of high-responding baboons (Fig. 2) but significant differences were only observed in the expression levels of the organ where it was expressed maximally. Thus, it is likely that differences in the transcription of ACAT1 gene are caused by a difference in the promoter sequence.

It has been suggested that the activity of ACAT plays a central role in cholesterol absorption [7,13] and that both forms of ACAT are present in the intestinal membrane. Differences in the expression of either isoenzyme of ACAT would affect cholesterol absorption and plasma cholesterol response to dietary lipids. In baboons, the differences in the expression of ACAT1 in the duodenum are important as the expression levels were positively associated with plasma and LDL cholesterol levels. ACAT1 has two promoters, one on Chromosome 1 and another on Chromosome 7 [21]. Preliminary efforts to characterize the differences in the promoter region between high and low responders were not successful because of the differences in the promoter sequences for ACAT1 between humans and baboons. It will be essential to clone the ACAT1 gene in baboons to determine the differences in the promoter region of ACAT1 between high- and low-responding baboons.

In addition to ACAT, Niemann–Pick C1Like 1 (NPC1L1) and ATP-binding cassette (ABC)G5 and ABCG8 play important roles in cholesterol absorption. NPC1L1 is expressed in the brush border membrane of enterocytes and plays a critical role in the absorption of cholesterol and other plant sterols by the intestine [22]. The drug ezetimibe inhibits intestinal cholesterol absorption by inhibiting NPC1L1-dependent cholesterol uptake by enterocytes [23]. ABCG5 and ABCG8 heterodimers also play an important role in cholesterol absorption and secretion into the bile [24–28]. Treatment of mice expressing ABCG5 and ABCG8 with Liver X receptor (LXR)α agonist produced a threefold increase in biliary cholesterol concentration and a decrease in cholesterol absorption [28,29]. However, LXR agonist treatment of transgenic mice expressing no ABCG5 or ABCG8 did not increase biliary cholesterol secretion [28]. In our previous studies, we measured intestinal expression of ABCG5 andABCG8 in high- and low-responding baboons but did not find any difference in the expression of these genes between the two responding phenotypes [30].

We have not measured the expression of the NPC1L1 gene in high- and low-responding baboons. However, inactivation of NPC1L1 causes multiple lipid transport defects and a down-regulation of ABCA1 in mice [31]. We did not observe any other defect or difference in the expression of ABCA1 gene in the intestine of high- and low-responding baboons. Thus, although NPC1L1 and ABCG5 and ABCG8 play important roles in cholesterol absorption, they do not seem to play a role in mediating differences in cholesterol absorption between high- and low-responding baboons.

In summary, the present results suggest that like humans, and unlike mice and other monkeys, ACAT1 is the major isoenzyme of the liver in baboons. However, as in humans, mice and monkeys, ACAT2 is the major isoenzyme of the duodenum and jejunum in baboons. The ileum had the lowest expression of ACAT2 and the ACAT2/ACAT1 ratio in the ileum was similar to that in the liver. ACAT2 and ACAT1 expression levels were consistent with the protein mass of these enzymes in the liver and intestine (duodenum). There were no differences in the hepatic expression levels or the protein levels of ACAT2 between high- and low-responding baboons. However, low-responding baboons had a lower expression and protein mass of ACAT1 in the duodenum and the expression of ACAT1 in the duodenum was positively associated with total plasma and LDL cholesterol. Thus, ACAT1 expression may modulate plasma cholesterol levels in high- and low-responding baboons.

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