Human intestinal monoacylglycerol acyltransferase: differential features in tissue expression and activity

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Lockwood, John F., Jingsong Cao, Paul Burn, and Yuguang Shi. Human intestinal monoacylglycerol acyltransferase: differential features in tissue expression and activity. Am J Physiol Endocrinol Metab 285: E927–E937, 2003. First published June 24, 2003; 10.1152/ajpendo.00179.2003.—Acyl CoA-monooacylglycerol acyltransferase (MGAT) catalyzes the first step in triacylglycerol resynthesis involved in dietary absorption in enterocytes. Despite its potentially important role in dietary fat absorption, a gene encoding a human intestinal MGAT has not been identified. In this study, we report the identification and functional characterization of a human intestinal MGAT (hMGAT2) and its splice variant (hMGAT2V). The hMGAT2 gene encodes a peptide of 334 amino acids with a molecular mass of 38.2 kDa that shares 81 and 47% amino acid identities with the mouse MGAT2 and the human diacylglycerol acyltransferase (DGAT2) enzymes, respectively. The hMGAT2 gene is localized on chromosome 11q13.5, adjacent to the DGAT2 gene, suggesting gene duplication. Transient expression of hMGAT2, but not an alternatively spliced variant, hMGAT2V, in COS-7 cells led to a ninefold increase in the synthesis of DAG. The human and mouse differ significantly in tissue distribution of MGAT2. In addition to a predominant expression in the small intestine in both species, distinct levels were also found in the human liver, contrasting with higher levels in the mouse kidney. In comparison with a single 1.8-kb transcript in mouse, the hMGAT2 gene expressed two transcripts of 3.0 and 6.0 kb in size that encode MGAT2 and an inactive peptide with unknown functions, respectively. Despite a significant level of hMGAT2 mRNA in the human liver, little MGAT activity was detected in liver microsomes when tested against monoacylglycerols with different unsaturated side chains, suggesting possible posttranscriptional regulation.

The ability of eukaryotic cells to absorb, transport, and store the energy-rich molecule triacylglycerol (TAG) is an important evolutionarily conserved function in mammals. TAG represent the most important storage fuel for eukaryotes and also play a significant role in meeting the cellular demands for membrane biogenesis and integrity and lipoprotein assembly for transport (2, 11). Multiple tissues have a variety of specialized roles in lipid metabolism. For example, the absorption of dietary TAG occurs via the enterocytes lining the small intestine, whereas TAG stored as energy depots are found predominantly in muscle and adipose tissue (29). In hepatocytes, glycerolipids are used in the synthesis and secretion of serum lipoproteins for transport (2). The absorption process within the small intestine is very efficient, where >95% fat as TAG is retained (1), and it is stored in tissue depots when available TAG are in excess of energy requirements. Given that the Western diet can be composed of as much as 30% fat as calories, a large portion of available TAG can be obtained through the diet alone (24), which has been a contributing factor in the ongoing epidemic of obesity.

Dietary TAG are subjected to a series of complex biochemical processes before they are able to enter intestinal cells and can be transported in the circulation. Fat digestion is initiated in the stomach, where the TAG are partially broken down to sn-1,2-diacylglycerol and free fatty acids by gastric lipase, which is stable and active at the acid pH of the stomach (5). The process continues in the small intestine, where it is facilitated by the emulsification by bile salts (22) and results in complete hydrolysis by pancreatic lipase into free fatty acids and sn-2-monoglycerides that are absorbed into enterocytes lined with the brush border of the small intestine (27). Once inside the enterocytes, these products are transported to the endoplasmic reticulum (ER) where they undergo consecutive reesterification, first into sn-2,3(1)-diacylglycerols and then into TAG, which are packaged into chylomicrons for secretion into the lymphatic system for transport (24, 30).

There are two main biochemical pathways for TAG synthesis (16). One, a de novo pathway that is present in most tissues, is the glycerol 3-phosphate pathway, which begins with acylation of glycerol 3-phosphate with fatty acyl-CoA producing lysophosphatidic acid, followed sequentially by acylation and dephosphorylation to synthesize sn-1,2-diacylglycerol. A second pathway is the monoacylglycerol pathway, which proceeds with the direct acylation of sn-2-monoglycerides and fatty acyl-CoA catalyzed by monoacylglycerol acyltransferase (MGAT), yielding sn-1,2(2,3)-diacylglycerols (11). The final step in the pathway converting diacylglycerols (DAG) to TAG is shared by both path-

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ways and is mediated by the enzyme diacylglycerol acyltransferase (DGAT; see Ref. 14). The relative activity of each pathway is determined by the abundance of sn-2-monoacylglycerol and free fatty acids. In intestinal mucosa under normal lipid absorption conditions, the monoacylglycerol pathway contributes to ~80% of TAG incorporated into chylomicrons (24, 30), whereas free fatty acids taken up from the plasma are primarily oxidized or incorporated into phospholipids (13, 23).

In contrast to the small intestine, where the monoacylglycerol pathway predominates in dietary fat absorption, hepatic glycerolipid synthesis in adult mammals is believed to occur primarily via the sequential acylation of glycerol 3-phosphate, since very little MGAT activity has been detected in adult rat liver microsomes (31). Additionally, hepatic MGAT activity was shown to be induced >800-fold in suckling rats and demonstrates a strong preference for sn-2-monoacylglycerols over the sn-1-isomers, which is believed to play a pivotal role in selective retention of essential fatty acids during development (31).

Historically, the isolation and characterization of MGAT have been hindered by the fact that the enzyme is an integral membrane protein within intestinal microsomes. There is evidence that MGAT and DGAT along with acyl-CoA ligase may form a “triaclylglycerol synthetase complex,” thus making the purification and characterization of individual enzymes more difficult (25). Nonetheless, intense efforts have resulted in partial purification and characterization of the MGAT enzyme (3, 15, 18, 25). We have recently reported the cloning and characterization of individual enzymes more difficult (25). Nonetheless, intense efforts have resulted in partial purification and characterization of the first mammalian intestinal monoacylglycerol acyltransferase, MGAT2, from mouse (4), which has made it possible to clone the gene from other species. In the present study, we report the identification and characterization of a human gene encoding the intestinal monoacylglycerol acyltransferase (hMGAT2) that demonstrated differential features from the mouse MGAT2 in tissue distribution and MGAT activities with different stereospecific isomers in comparison with those from human small intestine and liver. The abundant expression of hMGAT2 in small intestine, along with features that are unique to the human isoforms, warrant further characterization of hMGAT2 and its potential role in obesity intervention.

MATERIALS AND METHODS

Cloning of full-length MGAT2 cDNA. A cDNA clone was identified in NCBI databases on the basis of sequence homology to the known mouse MGAT2. According to flanking cDNA ends, primer pairs (forward: 5'-CCGCGCAGCATGG-TAGAGTTCGC-3'; reverse: 5'-AAAGGGAACAAAGCTG-GAGCTCC-3') were designed to amplify the full-length coding region of the human MGAT2 gene from Marathon-Ready cDNA prepared from human small intestine (BD Biosciences Clontech, Palo Alto, CA). Amplification was performed by PCR using Pfu DNA polymerase (Strategene, La Jolla, CA), resulting in an ~1.0-kb cDNA product. The PCR product was cloned into the pPCR-script Amp SK(+) vector (Strategene) and sequenced. An inactive isoform of MGAT2 (accession no. AK026297), which we call hMGAT2v, was cloned as above using a different 3-end primer (5'-TCAAAGCCAGCTTTTG-GGATA-3') and sequenced for use along with hMGAT2 in enzyme analysis.

Northern blot analysis. To analyze the tissue distribution pattern of hMGAT2 mRNA, multiple tissue poly(A)+ RNA blots (BD Biosciences Clontech and OriGene Technologies, Rockville, MD) and total RNA blots (Biochain Institute, Hayward, CA) were hybridized with [a-32P]dCTP-labeled probes prepared from PCR-amplified cDNA probes recognizing specifically unique COOH-terminal regions of the active (forward: 5'-GACCCCTGTTGCAATCTTCT- TCT-3'; reverse: 5'-AGCAGTTGAGTCTGTCAGGCC-3'), inactive (forward: 5'-TATCAAGCCTCTGGAGAAGCA-3', reverse: 5'-TCAAGTCGCCAGTTGGGATA-3'), and both (forward: 5'-CAGAGCTCAGAACCTGGGAG-3'; reverse: 5'-TCAATTGCAGCTTGGGATA-3') isoforms by using a Random Primers DNA Labeling System (Invitrogen, Carlsbad, CA). Hybridization was carried out in ULTRAhyb (Ambion, Austin, TX) at 50°C overnight, followed by three washes at 55°C in 2× SSC buffer containing 0.1% SDS and 1 mM EDTA. Blots were then stripped with boiling 1% SDS to remove radiolabeled probe and rehybridized with [a-32P]dCTP-labeled actin cDNA as an internal control. The blots were exposed to Bio-Max MR film or a PhosphorImager screen to visualize the signals.

Expression of MGAT2 in mammalian cells. A mammalian expression plasmid coding full-length hMGAT2 and hMGAT2V was engineered by unidirectional subcloning of the 1-kb cDNA fragment from the pPCR-script Amp SK(+) vector described above into the NotI and EcoRV sites of pcDNA3.1/Hygro(-) (hMGAT2) and NotI and HindIII sites of pcDNA3.1/Hygro(+) (hMGAT2V) mammalian expression vectors (Invitrogen, San Diego, CA). Transient transfection was performed in COS-7 cells with vectors with or without human or mouse MGAT2 plasmids, as described previously (4). After transfection (48 h), cells were harvested in ice-cold PBS, lysed, used immediately in assays or aliquots and frozen at −70°C for later use.

In vitro assays for MGAT activity. Phosphatidylcholine, 1-monolinolenoyl-rac-glycerol (C18.2, [cis,cis]-9,12), 1-monolinolenoyl-rac-glycerol (C18.3, [cis,cis]-9,12,15), and oleoyl-CoA were purchased from Sigma Chemical (St. Louis, MO), and sn-2-oleoyl glycerol was purchased from Doosan Serdary Research Laboratories (Toronto, ON, Canada). Radiolabeled chemicals [14C]oleoyl-CoA (50 mCi/mmol), [14C]rac-1-oleoyl glycerol (50–60 mCi/mmol), and [3H]sn-2-oleoyl glycerol (40– 60 Ci/mmol) were from American Radiolabeled Chemicals (St. Louis, MO). Cell pellets from COS-7 transfected cells were homogenized in 20 mM NaCl with three short 10-s pulses from a Brinkmann Polytron. The resultant homogenates were used to assess the activity of MGAT in transfected mammalian cells. Homogenate protein was determined with a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer’s instructions. Human liver microsomes were purchased from three different vendors: sample 1 was from In Vitro Technologies (Baltimore, MD), sample 2 was from BD Gentest (Woburn, MA), and sample 3 was from Tissue Transformation Technologies (Edison, NJ). Intestinal microsomes were purchased from In Vitro Technologies. MGAT activity was determined at room temperature in a final volume of 100 μl, as previously described (4, 6, 33). Briefly, MGAT activity was determined by measuring the incorporation of [1-14C]oleoyl-CoA into sn-2-oleoyl glycerol. Acyl acceptors (sn-2-oleoyl glycerol, rac-1-linoleoyl glycerol, and rac-1-linolenoyl glycerol) and phosphatidycholine (molar ratio, 1:5) were introduced in the assay as liposomes. The sn-2-linoleoyl glycerol and sn-2-linolenoyl glycerol versions of the acyl acceptors were not commercially available.
available. The reaction mixture contained 100 mM Tris·HCl, pH 7.4, 5 mM MgCl₂, 1 mg/ml fatty acid-free BSA, 200 mM sucrose, 20 μM [¹⁴C]oleoyl-CoA, 200 μM of sn-2-oleoyl glycerol, rac-1-linoleoyl glycerol, or rac-1-linolenoyl glycerol, and 50 μg cellular homogenate or 10 μg microsomes. For radio-labeled acyl acceptor studies, 50 μM cold oleoyl-CoA and 20 μM [¹⁴C]sn-2-oleoyl glycerol or 2 μM [³H]sn-2-oleoyl glycerol were used. After a 20-min incubation period, reactions were terminated with 2 ml of chloroform-methanol (2:1, vol/vol). Samples were centrifuged, 500-μl aliquots were dried under vacuum, and lipids were separated by the Linear-K Preabsorbent TLC Plate (Waterman, Clifton, NJ) with hexane-ethyl ether-acetic acid (80:20:1, vol/vol/vol). Individual lipid moieties were identified by standards with exposure to I₂ vapor. The TLC plates were exposed to a Phosphor screen, and PhosphorImaging signals were visualized using Storm 860 (Molecular Dynamics, Sunnyvale, CA) and quantitated using ImageQuant software.

**DAG lipase assay.** Detection of hepatic DAG lipase activity in microsomes was run as described above for the incorporation of [¹⁴C]oleoyl-CoA into sn-2-oleoyl glycerol with the following exceptions. After the 20-min incubation of the reaction components in the presence of 10 μg of intestinal microsomes, the reaction was heated at 55°C for 10 min. Fresh liver or intestinal microsomes (10 μg) were then added, and the reaction was allowed to continue at room temperature for an additional 20 min before being terminated with chloroform-methanol (2:1), followed by extraction and TLC separation.

**RESULTS**

**Identification of the human MGAT2 gene.** We have recently reported the cloning and characterization of the first mammalian intestinal MGAT, MGAT2 (4). Using the mouse MGAT2 cDNA as a query sequence for BLAST analysis of the public genomic and EST databases, we identified and cloned a human MGAT candidate gene (hMGAT2) and a splice variant (hMGAT2V) by PCR amplification. The hMGAT2 gene encodes a peptide of 334 amino acids with molecular mass of 38.2 kDa that shares 81% identity with the mouse MGAT2. Consistent with the prediction of an ER-associated protein, a 40-amino acid signal peptide (MPWERRLQTLAVLFQVSFLALAEICTVGFIAL LFTRFOWL) is identified from the NH₂ terminus of the hMGAT2. The hMGAT2 gene also shares 47% amino acid identity with DGAT2, and they localize adjacent to one another on chromosome 11q13.5, suggesting gene duplication. In comparison with the full-length hMGAT2, as predicted from the sequence homologies with the mouse MGAT2, the splice variant hMGAT2V predicts a shorter peptide of 284 amino acids, among which the NH₂-terminal 217 amino acids are identical with hMGAT2 (Fig. 1A). The hMGAT2V originates from alternative mRNA splicing between exons 4 and 6, resulting in a long exon that combines the fourth and fifth exons with the intron sequences between the two exons and further extends into the intron sequences beyond the fifth exon (Fig. 1B). The alternative splicing also resulted in different usage of the sequences of the sixth exon as well. Analysis of the NCBI EST database also identified multiple EST transcripts (accession nos. AW810630, BF374729, and AW810166) that match the hMGAT2V, suggesting that the splice variant is not a cloning artifact. However, the alternative splice variant is absent from the mouse MGAT2 gene.

**Analysis of differential tissue distribution of hMGAT2 and its splice variant.** The tissue distribution of the human MGAT2 and hMGAT2V mRNAs was analyzed by Northern blot analysis using differential probes that distinguish the full length from the splice variant. Using a probe that is specific for the full-length hMGAT2 gene, a major transcript of 3.0 kb was detected primarily in small intestine and liver. The transcript was also expressed in adipose tissue and colon, although at much lower levels, but not in any other tissues examined (Fig. 2A). In contrast, the 6.0-kb transcript is the major product of detection when probed with labeled DNA that is specific for hMGAT2V (Fig. 2B). Thus the 3.0-kb and 6.0-kb transcripts represent splice variants that encode the full length and the alternative spliced isoforms of the human MGAT2 gene, respectively. The results contrast a recent report by Yen and Farese (32), who demonstrated that the inactive isoform was exclusively expressed in the stomach as a 1.4-kb transcript.

We next analyzed hMGAT2 and hMGAT2V expression in different parts of the gastrointestinal tract using a probe that detects both splice forms (Fig. 2C, with quantification in Fig. 2D). Different levels of the 3.0-kb and 6.0-kb transcripts were detected in the gastrointestinal tract, with the highest levels being detected in the small intestine, supporting a role of MGAT in dietary fat absorption. Despite variable levels in different sections of the gastrointestinal tract, the relative abundances of the 3.0-kb and 6.0-kb transcripts maintained a constant ratio, suggesting a possible functional role of the hMGAT2V splice variant in those tissues.

**Functional analysis for MGAT activity of hMGAT2 and its splice variant transiently expressed in COS-7 cells compared with microsomes from the human small intestine and liver.** To determine whether the proteins encoded by hMGAT2 and hMGAT2V cDNAs possess MGAT activity, hMGAT2 and hMGAT2V were transiently expressed in COS-7 cells followed by in vitro enzyme assay analyses. Activity was determined by measuring the incorporation of oleoic acid from 20 μM [¹⁴C]oleoyl-CoA into DAG (1,2(2,3)-DAG) with 200 μM sn-2-oleoyl glycerol (2-MAG) as the acyl acceptor using 50 μg of cell homogenate. As a result, MGAT activity in membrane preparations from hMGAT2-transfected cells (Fig. 3A, lane 3) was significantly higher than that observed in mock-transfected cells (Fig. 3A, lane 2) as evidenced by a ninfold increase in the level of radio-labeled DAG (quantified in Fig. 3B). In contrast, the peptide encoded by the hMGAT2V splice variant is devoid of any detectable MGAT activity (Fig. 3C, lane 2) when compared with the mouse MGAT2 (quantified in Fig. 3D, lane 3) as a positive control.

Because the hMGAT2 is also expressed in the human liver with the second highest levels among all the tissues examined, we next compared the hMGAT activity of microsomes isolated from human liver with
that of microsomes isolated from small intestine by use of hMGAT2 transiently expressed in the COS-7 as a positive control. Experiments were performed under experimental conditions as described above using 10 μg of microsomes from human liver and intestine. As indicated in Fig. 3A and quantified in Fig. 3B, more than a 30-fold increase of radiolabeled DAG was detected in the microsomes from small intestine (Fig. 3A, lane 5, and Fig. 3B) compared with mock-transfected COS-7 cells (Fig. 3A, lane 2, and Fig. 3B). In comparison, the level of MGAT activity in the human liver is barely detectable, although a significant increase in radiolabeled TAG was detected (Fig. 3A, lane 4, and Fig. 3B).

Analyses of MGAT specific activity and product stability in the human liver. To investigate whether the weak MGAT activity in the liver is caused by sampling, MGAT activity was examined using liver microsomes from three different sources and compared with that from intestinal microsomes in the presence or absence of MAG under the same assay conditions as described above. As shown in Fig. 4A and quantified in Fig. 4B, low levels of MGAT activity were detected across all the three samples, which exhibited very little variation in MGAT activity among three different pools of liver samples. The DAG products were detected only in the presence of MAG, suggesting MGAT specific activity in the liver microsomes. However, the MGAT activity associated with liver microsomes (Fig. 4A, lanes 2, 4, and 6) was much lower than that detected in the small intestine (Fig. 4A, lane 8). A significant amount of TAG was detected from both liver and intestinal microsomes in the absence of MAG (Fig. 4A, lanes 1, 3, 5, and 7), suggesting the existence of endogenous DAG. Consistent with a predominant role of small intestine in fat absorption, the levels of endogenous DAG and MAG are much higher in the small intestine than those in the liver, as evidenced by the presence of higher levels of radiolabeled DAG and TAG in the absence of exogenous MAG (Fig. 4A, lane 7).

To address the issue of whether the low level of DAG product detected from liver microsomes was caused by rapid hydrolysis of newly synthesized DAG by liver DAG lipase, radiolabeled DAG was subjected to treatment with liver microsomes to observe DAG lipase activity. Radiolabeled DAG was generated by intestinal microsomes under the same conditions as described above and was subjected to heat inactivation. The
heat-inactivated MGAT reaction mixture was incubated for 20 min in the presence or absence of freshly added liver microsomes, and changes in DAG levels were analyzed by TLC. As indicated in Fig. 4C and quantified in Fig. 4D, the level of DAG was not significantly altered in the presence of liver microsomes (Fig. 4C, lane 2) or intestinal microsomes (Fig. 4C, lane 3), suggesting that liver DAG lipase activity was an unlikely cause of the low level of radiolabeled DAG detected from the liver microsomes.

The use of radiolabeled [14C]oleoyl-CoA in the measurement in the MGAT assay does not discriminate in the synthesis of radiolabeled TAG between exogenous and endogenous DAG substrates. To address the issue of whether the lack of radiolabeled DAG is caused by the rapid conversion of radiolabeled DAG into TAG by endogenous DGAT in the liver microsomes, radiolabeled acyl acceptors ([14C]rac-1-oleoyl glycerol or [3H]sn-2-oleoyl glycerol) were used in the assay. The use of radiolabeled MAG eliminates possible formation of radiolabeled TAG catalyzed by DGAT present in the microsomes with endogenous DAG as substrate. Figure 5 shows that hMGAT2 transiently expressed both in COS-7 cells lysates and from small intestine microsomes produced significant amounts of radiolabeled 1,2(2,3)-DAG/1,3-DAG products (quantified in Fig. 5B, lanes 3 and 5, 8.0- and 18.8-fold increases, respectively). In contrast, liver microsomes showed almost no DAG product compared with the mock-transfected COS-7 cells (Fig. 5, lane 4 vs. lane 2). Consistent with the lack of MGAT activity, very little TAG product was detected (Fig. 5, lane 4 vs. lane 5, bands on top) in the liver microsomes. Similar results were also observed when the [3H]sn-2-oleoyl glycerol acyl acceptor was used (Fig. 5C and quantified in Fig. 5D). The data further confirm that the human liver microsomes possess minimal MGAT activity despite a significant amount of mRNA transcripts detected in the tissue.

Comparison of substrate specificity between hMGAT2 expressed in COS-7 cells and that from isolated human
We next tested the hypothesis that the low level of MGAT activity in liver is caused by lack of preferred substrates, i.e., MAG with polyunsaturated fatty acid side chains, as previously reported (31). MGAT activity from hMGAT2-transfected COS-7 cells was measured against MAG with different polyunsaturated fatty acid side chains and was compared with that from the liver and intestinal microsomes. The assay was performed by incubating 200 μM sn-2-oleoyl glycerol (2-MAG) and [14C]oleoyl-CoA with 50 μg lysates from nontransfected COS-7 cells (lane 1), mock-transfected cells (lane 2), hMGAT2-transfected COS-7 cells (lane 3), or 10 μg microsomal lysates from human liver (lane 4) and small intestine (lane 5). B: quantification of product formation as diacylglycerol (DAG; 1,2/2,3-DAG and 1,3-DAG together) and triacylglycerol (TAG) from A expressed as arbitrary units. C: analysis of MGAT activity of hMGAT2V (lane 2) compared with mock-transfected COS-7 cells (lane 1) or mMGAT2-transfected COS-7 cells (lane 3) analyzed by TLC assay. D: quantification of DAG and TAG products from C. FA, free fatty acid.

DISCUSSION

MGAT catalyzes acylation of MAG to DAG, an important step in TAG synthesis involved in dietary fat absorption in the small intestine and energy storage in other tissues. The biochemical aspects of MGAT have been characterized extensively in the rat and hamster.
intestine and in the liver of neonatal rats (3, 8, 9, 16, 17). Although intense efforts were focused on purification and identification of the gene encoding the enzyme (3, 15, 18, 25), like most biosynthetic enzymes involved in TAG synthesis, it proved difficult to clone the gene encoding MGAT due partly to the nature of the enzyme as a microsome-associated protein (18, 26). We have recently reported the cloning and characterization of the first mammalian intestinal MGAT (4), the mouse MGAT2, which has made it possible to clone the homologous genes from other species. Recently, the human homolog of MGAT2 and a second human intestinal MGAT gene (MGAT3) have also been reported (7, 32).

In this study, we report the cloning and characterization of the human intestinal MGAT2, a homolog of the mouse MGAT2. Like the mouse MGAT2, the human MGAT2 enzyme expressed in mammalian COS-7 catalyzes the synthesis of DAG with various MAG and acyl-CoAs as substrates. Consistent with a role in dietary fat absorption, the human MGAT2 gene is abundantly expressed in the midgut, whereas the level of expression is much lower in the upper sections of the gastrointestinal tract. The hMGAT2 gene is localized on chromosome 11q13.5, side by side with the DGAT2 gene, suggesting a gene duplication event in the origin of the MGAT and DGAT gene superfamily.

The human MGAT2 differs significantly from the mouse MGAT2 in a number of aspects. In comparison with the mouse MGAT2 that is predominantly expressed in the small intestine and kidney (4), the human MGAT2 expression in the kidney is below the level of detection. The human MGAT2 is also expressed in liver, although at lower levels, but is absent from the mouse liver (4, 32). Furthermore, in comparison with the mouse MGAT2 that expresses a single 1.8-kb transcript, the human MGAT2 expresses two transcripts of 3.0 and 6.0 kb in size. Differential analyses indicate that the 3.0-kb transcript encodes a fully active MGAT enzyme, whereas the 6.0-kb transcript encodes a splice variant that is devoid of MGAT activity. The expression level of the 6.0-kb transcript is quite abundant, as evidenced by Northern blot analyses and multiple EST transcripts in the database (accession nos. AW810630, AW810631).
BF374729, and AW810166) and maintained a constant ratio to the 3.0 transcript in all the tissues that expressed the gene, suggesting a functional significance. The two isoforms share identical NH$_2$-terminal sequences of 217 amino acids, indicating that the COOH-terminal 117 amino acids are essential for MGAT enzyme activity. BLAST analyses of genomic and EST databases failed to identify any peptides that share significant sequence homology with the COOH-terminal portion of the hMGAT2V peptide, suggesting that its biological function is unique.

The human liver is a tissue that expressed the second highest level of hMGAT2 mRNA next to the level observed in small intestine. However, only a very low level of enzyme activity was identified from liver microsomes compared with that from microsomes of the small intestine. This is not likely caused by the quality of the liver microsomes, since ample DGAT and other enzyme activities were observed, as evidenced by the abundant production of radiolabeled TAG and free fatty acids, nor was it likely caused by sampling variance, since little variation in MGAT activity was detected from microsomes from three different batches, each of which was isolated from a pool of several individuals. We also excluded the possibilities that a lack of visible radiolabeled DAG product was caused by the rapid hydrolysis by liver DAG lipase or by rapid conversion of radiolabeled DAG into TAG by endogenous DGAT. Hence, treatment of radiolabeled DAG with liver microsomes did not result in major changes of DAG levels under the same conditions used in the MGAT assay. Likewise, no TAG formation was observed when the MGAT assay was carried out using radiolabeled MAG as acyl acceptors, which precludes possible formation of radiolabeled TAG catalyzed by microsomal DGAT(s) with endogenous DAGs as substrates.

We further compared the MGAT activity from liver microsomes with that from microsomes isolated from small intestine and MGAT transiently expressed in COS-7 cells for substrate specificities to test the hypothesis that the poor MGAT activity in human liver is caused by the lack of preferred substrates. MGAT activity in the neonatal rat liver has been demonstrated, with strong preference for sn-2-MAG over sn-1(3)-MAG (8). Additionally, liver MGAT enzymes were shown to acylate polyunsaturated MAG more efficiently as a means of selective retention of essential...
fatty acids in neonatal rodents (12, 21, 31). Again, the use of three monoacylglycerols with different unsaturated side chains failed to revive any MGAT activity from the liver microsomes, even though these substrates were efficiently used by MGAT enzymes associated with microsomes from small intestine and MGAT2 expressed in COS-7 cells. In contrast to the strong preference of MGAT from neonatal rat liver to sn-2-MAG over sn-1(3)-MAG (8), rac-1-MAG was used efficiently by MGAT from intestinal microsomes and by MGAT expressed in COS-7 cells, although a caveat exists that our current assay conditions do not discriminate specific use of MAG isomers originating from acyl migration, a biochemical event believed to be limited. Furthermore, an increase in unsaturation of the sn-2-isomers did not enhance MGAT activity when compared between two sn-2(3)-MAG substrates.

Our current report on low MGAT activity in human liver is consistent with previous reports that hepatic MGAT specific activity is very low in adult rat, chicken, and guinea pig (8, 31) compared with neonatal rat, fetal guinea pig, and chick embryo (8, 10, 28). Thus hepatic glycerolipid synthesis in adult mammals is thought to occur primarily via the sequential acylation of glycerol 3-phosphate. Because of the lack of a cloned MGAT gene, little has been studied about how the MGAT activity is regulated in the liver of adult mammals. In comparison, hepatic MGAT activity in neonatal rodents was demonstrated to be developmentally regulated, increasing >800-fold in suckling rats (8). Rat hepatic MGAT activity peaks between days 5 and 12 and decreases by 98% at weaning age (8). Additionally, MGAT activity was known to be regulated by starvation, hibernation, and disease states such as diabetes (8, 19, 20). The cloning of the human MGAT2 gene has made it possible for future studies on regulation of MGAT gene transcription and its enzyme activities by environmental and nutritional factors. For example, it will be informative to investigate whether hMGAT2 transcription and activity are augmented by lactation and by factors that affect the onset of human obesity, such as a high-fat diet and exercise.

Yen and Farese (32) recently reported the cloning of a human intestinal MGAT gene and a splice variant (named MGAT2trunc) that shares identical sequences with hMGAT2 and hMGAT2V in this study. However,
it is interesting to note that a number of differences exist between the two studies. The hMGAT2 in their studies demonstrated the highest expression in liver, followed by wide distribution in various tissues, including stomach and kidney. In contrast, the present study showed that hMGAT2 is most abundantly expressed in small intestine, a tissue demonstrating the highest MGAT activity both in human and mouse (unpublished data and Ref. 32), which is similar to our previous report on the mouse MGAT2 gene (4). They also detected significant MGAT activity from human liver, although at a level that is far below that predicted by the abundance of mRNA in this tissue. Interestingly, only a small portion of the radiolabeled product was TAG, contrasting with our analysis that the main product from the liver microsomes was TAG catalyzed by microsomal DGAT(s) with endogenous DAG. The two studies further differ in the alternative splicing and tissue distribution of the hMGAT2 splice variant. They reported that MGAT2<sup>trunc</sup> originated from alternative splicing of the fourth exon, whereas our analysis showed that hMGAT2V resulted from a totally different splicing of the last three exons from the full-length hMGAT2 transcript. Furthermore, the MGAT2<sup>trunc</sup> differs from hMGAT2V in tissue distribution. In contrast to the wide tissue distribution of the 6.0-kb transcript that encodes the hMGAT2V peptide, the MGAT2<sup>trunc</sup> was exclusively expressed in stomach, as evidenced by hybridization to a 1.4-kb transcript that was absent in other tissues. It is also intriguing to observe that the 1.4-kb band is much smaller than the cloned 2.0-kb hMGAT2V isoform that is identical to MGAT2<sup>trunc</sup> and a cDNA sequence reported in the databank (accession no. AK026297). Although these differences remain to be resolved, they could be partially caused by different tissue sources used in the two studies, further reflecting the complicated nature of hMGAT2 expression in human tissues.

The importance of developing intervention strategies for addressing the growing epidemic of obesity in developed countries cannot be overstated. With Western diets rich in dietary fat and the efficient ability of the mammalian system to absorb, transport, and store the high-energy TAG, more efforts need to be focused on the biochemical pathways used in this area of metabolism. The cloning and characterization of the human MGAT2 gene have made it possible to test the hypothesis that development of hMGAT2 inhibitors could be used as an alternative means in treating or even preventing human obesity.

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