A Predominant Role of Acyl-CoA:monoacylglycerol Acyltransferase-2 in Dietary Fat Absorption Implicated by Tissue Distribution, Subcellular Localization, and Up-regulation by High Fat Diet*

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Acyl-CoA:monoacylglycerol acyltransferase-2 (MGAT2) catalyzes the synthesis of diacylglycerol and differs from the MGAT1 and MGAT3 in tissue distribution at the mRNA level. In addition to the small intestine, MGAT2 mRNA is also expressed at high levels in human liver, the lower gastrointestinal tract, and the mouse kidney, but the physiological significance of such expression has not yet been studied. Using an affinity-purified antibody, the present study investigated the expression of murine MGAT2 protein along the intestinal tract, determined its subcellular localization, and studied its regulation by diet and in ddb/db mouse. Results demonstrate a high level of MGAT2 expression in the small intestine in a proximal-to-distal gradient that correlated well with both MGAT enzyme activity and fat absorption pattern. In contrast, MGAT2 protein was not detectable in other sections of the digestive tract, including stomach, cecum, colon, and rectum, or other mouse tissues such as kidney, liver, and adipocytes. Immunohistological studies provided direct evidence that the enzyme is expressed not only in the villi, but also in the crypt regions of the small intestine, which suggests that MGAT2 expression occurs prior to the maturation of enterocytes. MGAT2 is localized in the endoplasmic reticulum (ER) in both MGAT2-transfected COS-7 and Caco-2 cells, indicating that the ER is the primary site for dietary fat re-synthesis. MGAT2 expression appeared not to be affected by diabetes in the ddb/db mouse, however, the total intestinal MGAT activity was significantly enhanced. Finally, an up-regulation of both MGAT2 protein expression and MGAT activity was observed in mice fed a high fat diet, implicating a role of MGAT2 in diet-induced obesity. Taken together, our data suggest a predominant role of MGAT2 in dietary fat absorption.

In mammals, the small intestine plays a predominant role in the absorption of dietary lipids (mainly triacylglycerol). The absorption of triacylglycerols by the intestinal tract is a complex process that requires several steps, including the hydrolysis of triacylglycerols by pancreatic lipase in the intestinal lumen, the uptake of released 2-monoacylglycerol and fatty acids into enterocytes, and the reconstitution of triacylglycerol in the enterocytes by the monoacylglycerol pathway where 2-monoacylglycerol and fatty acids are utilized to sequentially re-synthesize diacylglycerol and triacylglycerol by acyl-CoA:monoacylglycerol acyltransferase (MGAT) and acyl-CoA:diacylglycerol acyltransferase (DGAT), respectively. Another pathway involved in the triacylglycerol synthesis is the glycerol 3-phosphate pathway, a de novo pathway that is present in most tissues, including small intestine. The pathway begins with acylation of glycerol 3-phosphate with fatty acyl-CoA producing lysophosphatidic acid, followed sequentially by further acylation and dephosphorylation to yield diacylglycerol. Both pathways share the final step converting diacylglycerol to triacylglycerol by DGAT. In the small intestinal mucosa the monoacylglycerol pathway contributes to ~80% of triacylglycerol because of the large amount of 2-monoacylglycerol released from dietary lipids (2, 3). The newly formed triacylglycerol is then packaged with other lipids such as cholesterol ester, phospholipids, and protein to form chylomicrons, which are quickly transported to other tissues through the lymphatic system and blood stream (3–5). The enzymatic reaction catalyzed by MGAT is an essential step for fat absorption in the small intestine, because triacylglycerol biosynthesis is believed to take place mainly through the monoacylglycerol pathway. High levels of MGAT activity were also found in mammalian white adipose tissue where MGAT is believed to play a role in the storage of triacylglycerol in adipocytes (6), where excess storage of triacylglycerol results in obesity. In addition, unlike DGAT, MGAT is not involved in the glycerol 3-phosphate pathway that is present in most tissues. These differential features suggest that MGAT may present a better target for intervening the dietary absorption as a means of treating obesity.

Because of the importance of MGAT in fat absorption, considerable studies have been conducted to elucidate its molecular and enzymatic features (7–9). Facilitated by the recent advances in genomics and bioinformatics, three mammalian MGAT enzymes, designated MGAT1, MGAT2, and MGAT3, have been identified recently (10–13). MGAT1 transcripts were detected mainly in the stomach and kidney and at lower levels in adipose tissue and liver, but were absent in the small intestine where the highest level of MGAT activity was detected (13). The subsequently identified MGAT2 and MGAT3 are abundantly expressed in small intestine and could account for...
the high MGAT activity in this tissue (10, 11). Properties of mouse MGAT2 expressed in mammalian cell lines and bacterial system were further characterized with respect to substrate specificity, requirement for lipid cofactors, intrinsic DGAT activity, activators, and inhibitors (14, 15). Although the biochemical properties of MGAT in small intestine were previously intensively investigated (7, 16–18), the in vivo characterization of the intestinal MGAT per se has not been described because of the lack of identified genes, decoded peptide sequences, or antibodies.

MGAT activity has been reported to be regulated by a number of environmental and physiological factors, including lactation, starvation, and diabetes (19–22). Specifically, diabetes was shown to increase MGAT activity in streptozotocin-induced diabetic rats and in Otsuka Long-Evans Tokushima Fatty (OLETF) rats (22). However, due to the lack of a cloned MGAT gene at the time of those studies, it remains unknown whether the observed changes in MGAT activities were due to alterations in both mRNA and protein levels.

In the present studies, we examined the expression and localization patterns of the mouse MGAT2 in the intestinal tract and other tissues by using an affinity-purified peptide antibody specific to mMGAT2. The data indicated that both MGAT2 mRNA and protein were expressed along the entire length of the small intestine with the highest levels in jejunum and lowest levels in the distal region of ileum (ileo-cecal valve), which correlated well with the MGAT enzyme activity. Immunochemical and immunocytochemical analysis showed a MGAT2-specific staining surrounds the villus and crypts of the small intestine, and the enzyme was localized in the ER in both COS-7 cells and Caco-2 cells transiently transfected with MGAT2. We further investigated MGAT expression and enzyme activities in normal mice fed a high fat diet and in db/db mouse, a genetic model of diabetes caused by mutation of leptin receptor gene, and compared those with the wild-type mice.

**EXPERIMENTAL PROCEDURES**

**Materials**—Phosphatidycholine, sn-2-monooleoylglycerol, sn-1,2-dioleoylglycerol, sn-1,3-dioleoylglycerol, 1,2,3-trioleoylglycerol, oleic acid, and oleoyl-CoA were purchased from Doosan Serdary Research Laboratories (Toronto, Canada). [(14C)]Oleoyl-CoA (50 mCi/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). All other chemicals and solvents used in this study were obtained from Sigma-Aldrich (St. Louis, MO).

**Antibodies**—Rabbit polyclonal antibody against a peptide corresponding to position 293–311 of mouse MGAT2 (C-TFQPSREEVRDL-HQRYKE) was raised commercially (ProSci Inc., Poway, CA). A terminal cysteine was added to the peptide to aid in coupling to keyhole limpet hemocyanin as a carrier protein. An affinity purification of immune serum containing anti-MGAT2 IgG was further undertaken using an immunofinity column prepared by cross-linking the above peptide to CNBr activated Sepharose 4B. Aliquots of the purified antibody were stored at −80 °C, or kept at 4 °C for short-term storage.

**Animals and Specimen Collection**—Normal or db/db diabetic male C57BL/6 mice, 6–8 weeks, were purchased from Harlan Laboratories (Indianapolis, IN). All animals were maintained in an environmentally controlled facility with diurnal light cycle and free access to food and water for at least 1 week before use. Ten normal male C57BL/6 mice were divided into two groups (5 each): one group (Control) was fed standard chow food (PMI 5001, protein 28.4%, fat 12.3%, and carbohydrate 41.9%). The mice were fed the different diets for 4 weeks. Animals were sacrificed with CO₂ then immediately perfused with 10 ml of ice-cold PBS via transcardiac puncture. The whole small intestine was rinsed with ice-cold PBS, divided into five equal segments (6–7 cm each), weighed, immediately frozen on dry ice, and stored at −80 °C for later use for RNA isolation or total protein extraction. Eight 10% homogenates were prepared from small intestinal samples. The homogenate, starting from the pylorus, was designated as the number 1, whereas the most distal segment close to the ileocecal valve was assigned number 5. Sections approximately represent the following parts of the small intestine: 1, duodenum and proximal jejunum; 2, jejunum; 3, distal jejunum and proximal ileum; 4 and 5, ileum. Cecum, colon, rectum, liver, kidney, and epididymal and inguinal adipose were also isolated for detection of MGAT2. All animals used in this study were in compliance with approved institutional animal care and use protocols according to National Institutes of Health guidelines (56).

**Tissue Homogenate Preparation**—Frozen mouse tissues were homogenized in 10 vol. of PBS (w/v) containing protease inhibitor mixture (Complete EDTA-free, Roche Applied Science, Mannheim, Germany) on ice with a motor-driven Dounce homogenizer (Heidelberg, Germany). Aliquots of the crude homogenate were stored in liquid nitrogen or immediately processed for Western analysis or enzymatic assay. The protein concentrations in homogenates were determined by a BCA Protein Assay Kit (Pierce Biotechnology, Inc., Rockford, IL) according to the manufacturer’s instructions.

**Expression of MGAT2 in Mammalian Cells**—A mammalian expression plasmid coding a full-length mouse MGAT2 was engineered as described previously (10, 15). For subcellular colocalization study, human MGAT2 was tagged with a C-terminal FLAG epitope (DYKD-DDDK). COS-7 and Caco-2 cells were maintained under the conditions recommended by American Tissue Culture Collection (ATCC, Manassas, VA) and transiently transfected with vectors with or without an MGAT2 cDNA as described previously (10). Forty-eight hours after transfection, cells were fixed with 4% paraformaldehyde in PBS for immunocytohistochemical studies or harvested in ice-cold phosphate-buffered saline (PBS), pelleted by centrifugation, homogenized, and immediately proceeded to Western analysis and enzymatic assays, or frozen in liquid N₂ for later use.

**In Vitro Assays for MGAT Activity**—MGAT activity was determined by measuring the incorporation of [14C]oleoyl moieties into diacylglycerol with [14C]oleoyl-CoA and monooleoylglycerols as previously described (10, 15). Unless indicated elsewhere, the reaction mixture consisted of 100 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1 mg/ml bovine serum albumin (fatty acids-free), 200 mM sucrose, 25 mM [14C]Oleoyl-CoA, 200 μM of monooleoylglycerol in liposomes prepared using phosphatidylcholine, and 50 μg protein from small intestine crude homogenate. The reaction was conducted at room temperature for 10 min in a total volume of 100 μl. Lipids were extracted, separated, and quantitated essentially as described (10, 15).

**Northern Blot Analysis**—Total RNA was prepared from frozen tissues by using TRIzol reagent according to the manufacturer’s instruction (Invitrogen, Carlsbad, CA). Twenty micrograms of total RNA was separated in a 1% agarose-formaldehyde 3:1-N-morpholino propane-sulphonic acid gel and transferred to a Nylon membrane blot. The membrane was hybridized with [α-32P]dCTP (3000 Ci/mmol, ICN Radiochemicals) labeled probes prepared from full-length cDNA of mouse MGAT2 using a Random Primers DNA Labeling System (Invitrogen). Hybridization was carried out in ULTRAbhy (Ambion, Austin, TX) at 50 °C overnight, followed by three washes at 55 °C in 2 × SSC buffer containing 0.1% SDS and 1 μl EDTA. Blots were stripped with boiling 1% SDS 2× MOPS/0.1% SDS and immediately probed with glyceroldehyde-3-phosphate dehydrogenase (GAPDH) cDNA as an internal control. The blots were exposed to a PhosphorImager screen to visualize the signals using a Molecular Dynamics STORM 860 scanner (Sunnyvale, CA), which were quantitated using ImageQuant software (Molecular Dynamics Inc.).

**Western Blot Analysis**—For Western analysis, the freshly made crude homogenate from tissues or COS-7 cells was immediately denatured by boiling in SDS-loading buffer containing 2.5% 2-mercaptoethanol, 20–25 μg of the denatured protein was resolved on 8–16% or 4–20% Novex Tris-glycine gels by denaturing SDS-PAGE and transferred to nitrocellulose membrane. The membrane was incubated for 1 h in blocking buffer (0.9% NaCl, pH 7.5, 0.1% Tween 20) containing 5% nonfat milk to block nonspecific binding. The blots were then incubated with peptide-specific primary antibody (affinity-purified rabbit polyclonal anti-MGAT2 antibody, 0.5 μg/ml) dissolved in the same buffer at 4 °C overnight. After four washes (5 min each), the membrane was incubated with anti-rabbit IgG conjugated with horseradish peroxidase (1:4000, Amersham Biosciences UK Limited, Little Chalfont, Buckinghamshire, UK) for 1 h at room temperature. After another four 5-min washes, the blots were visualized using ECL Plus (Amersham Biosciences UK Limited) for 5 min and exposed to Kodak BioMax MR film for 10 to 5 min, or scanned using a Molecular Dynamics STORM 860 scanner for quantification using ImageQuant software.

**Immunohistochemistry**—Sacrificed mice were immediately fixed by cardiac perfusion with 4% paraformaldehyde in PBS. After fixation, the small intestine was removed, and segments corresponding approximately to duodenum, jejunum, and ileum were kept overnight at 4 °C in...
the above fixation buffer containing 30% sucrose. Tissue sections (10 µm) were prepared with a microtome (Leica CM3050 S, Dearfield, IL), incubated again with the above fixation buffer for 10 min, washed with PBS, air-dried, and stored at −20 °C until use. Prior to immunostaining, the sections were treated with 0.01 M sodium citrate (pH 6.0) with boiling for 10 min to facilitate the exposure of the antigen domain. The sections were then treated with 1% hydrogen peroxide diluted in methanol for 5 min to quench the endogenous peroxidase activity. The in situ immunodetection of mMGAT2 was performed using an ImmunoCruz staining system (Santa Cruz Biotechnology, Santa Cruz, CA). After being rinsed with PBS twice for 5 min each, the sections were incubated with 1.5% goat serum in PBS for 2 h to block nonspecific sites. Sections were incubated with the primary antibody (affinity-purified rabbit polyclonal anti-mMGAT2 IgG, 5 µg/ml) overnight at 4 °C. After being washed with PBS twice for 5 min each, the sections were incubated with biotinylated secondary antibody for 1 h, washed three times with PBS as above, and incubated with horseradish peroxidase-streptavidin for another 30 min. After another three extensive washes with PBS, sections were stained with a horseradish peroxidase mixture containing 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide for 30 s to 10 min until the desired stain intensity (brown) develops. The sections were then rinsed with water, dehydrated sequentially by using 95% ethanol, 100% ethanol, and xylene, immediately mounted in a permanent mounting medium, and observed by light microscopy. A negative control staining was conducted essentially as described above except for substitution of primary antibody with normal rabbit IgG. To further distinguish the specific staining against MGAT2 from nonspecific staining, a peptide neutralization control was performed by incubating the affinity-purified rabbit anti-MGAT2 antibody with the blocking peptide used to raise the antibody (1:1, w/w) prior to proceeding to the staining.

**Immunocytochemistry**—Cells were grown and transfected on a 6-well plate (Costar 3506). Forty-eight hours after transfection, cells were first incubated in the growth medium with 100 µM MitoTracker Red CMXRos for 30 min at 37 °C to achieve the specific staining on mitochondria. Then, cells were fixed with freshly prepared, prewarmed growth medium containing 4.0% paraformaldehyde and permeabilized with 0.2% Triton X-100 in PBS. After being rinsed with PBS twice for 5 min each, the samples were incubated with 5% normal donkey serum in PBS for 1 h to block nonspecific sites. Samples were incubated with mouse monoclonal anti-FLAG M2 antibody (5.0 µg/ml, Sigma) or rabbit anti-calnexin amino-terminal polyclonal antibody (1.0 µg/ml, StressGen Biotechnologies Corp., Victoria, Canada) for 2 h at room temperature. After being washed with PBS three times for 5 min each, the plates were incubated with Cy3-conjugated donkey anti-mouse IgG and Cy3-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for 1 h, washed four times with PBS, and analyzed in a fluorescence microscope using the appropriate filter combinations for excitation of the secondary antibodies. The cells were also counterstained with DAPI (Molecular Probes, Eugene, OR) to visualize nuclei.

**Statistical Analysis**—Data are given as mean ± S.E. The differences between two groups were analyzed by Student’s t test.

**RESULTS**

Expression of MGAT2 mRNA Along the Intestinal Tract in Mouse—The intestinal distribution of mouse MGAT2 mRNA was analyzed by Northern blot with GAPDH as internal control (Fig. 1). As shown in a representative Northern blot from one mouse (Fig. 1A) and the quantitative analysis derived from 3 mice (Fig. 1B), MGAT2 mRNA was expressed in all segments of the small intestine. The expression in section 1 (approximately equivalent to duodenum) was lower than that in sections 2 and 3 (equivalent to Jejunum), whereas the lowest level of expression was found in the distal region of ileum (Fig. 1). Thus, a clear proximal-to-distal gradient starting from proximal jejunum was detected in MGAT2 mRNA expression along the small intestine (Fig. 1). It appears that the expression of MGAT2 mRNA was restricted only to the small intestine within the intestinal tract, because the transcripts were barely detected in cecum, colon, and rectum (Fig. 1).

**Tissue Distribution Pattern of MGAT2 Protein in Mouse**—To further characterize the expression and localization of MGAT2 in vivo, we selected several peptides present in mMGAT2 to raise specific rabbit antibodies against the enzyme. Immunization with one peptide (TPQPSREEVDRLLHQRYYIKE) that corresponds to the region of 293–311 of mMGAT2 protein generated a high titer mMGAT2-specific antibody. The peptide antibody was verified for mMGAT2 specificity by immunoblot analysis of recombinant mMGAT2 in COS-7 cells transiently transfected with MGAT2 cDNA. Cells transfected with empty vector served as a negative control. Forty-eight hours after transfection, cell lysates were separated by SDS-PAGE under denatured conditions and analyzed by Western blot. The antibody exclusively recognized a band migrating at apparent molecular mass of 38 kDa from mMGAT2-transfected cells, which was absent in vector-transfected cells and was not detected by pre-immune serum from the same rabbit (Fig. 2A). The 38-kDa peptide detected by the antibody also agreed well with the molecular mass of 38.6 kDa predicted from the open reading frame of the mouse MGAT2 gene, as well as the recombinant mMGAT2 protein previously produced in Escherichia coli (15), suggesting a lack of major post-translational modifications, such as glycosylation. These features validated the specificity of the antibody, which was further purified by an immunoadfinity column (see “Experimental Procedure”) and used throughout the studies.

The expression of mMGAT2 along the small intestinal tract was examined by Western blot using the above affinity-purified specific peptide antibody against mMGAT2. As shown in Fig. 2B, a representative immunoblot, the antibody recognized a peptide band with a molecular size of 38 kDa, which is consistent with what was observed in MGAT2-transfected COS-7 cells.

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A quantitative analysis of MGAT2 protein levels in different segments from three mice is shown in Fig. 2C. As was the case in the mRNA expression pattern, MGAT2 protein was expressed throughout the small intestine. A gradient from the proximal regions to the distal regions was clearly observed, with the highest levels in section 2 (jejunum). The expression levels of MGAT2 in the most distal region of the small intestine were at least 50% lower than proximal jejunum (Fig. 2C).

Further study was undertaken to examine the protein expression of MGAT2 in other digestive tissues or in tissues where MGAT2 transcripts were detected (10). As shown in Fig. 2D (upper panel), the protein expression of MGAT2 in liver, kidney, stomach, epididymal fat, inguinal fat, cecum, colon, and rectum was not detected under our experimental conditions in comparison with high expression of MGAT2 in small intestine and MGAT2-transfected COS-7 cells. As a control, β-actin was detected in all tissues tested (Fig. 2D, lower panel). This finding implied that MGAT2 protein levels in these tissues were dramatically lower than that detected in the small intestine, even though high levels of MGAT2 transcripts were observed in some of these tissues such as kidney and adipose (10).

Correlative Analysis of mRNA, Protein, and Enzyme Activity Levels of MGAT2 Along the Small Intestine—To establish a correlation between MGAT2 expression and MGAT activity along the small intestine, we next measured MGAT activity in homogenates from intestinal segments of wild-type mice. As shown in Fig. 3A, a representative image of TLC plate displaying the formation of products from a single mouse, and Fig. 3B, the quantitative TLC data collected from three different mice, the highest MGAT activity was observed in jejunum (segment 2). From jejunum to the distal ileum, the activity was progressively lower, which showed a clear proximal to distal gradient. Such a distribution of MGAT activity along the small intestine is consistent with the expression pattern of both MGAT2 mRNA and protein as demonstrated in Figs. 1 and 2. A combined analysis of the levels of mRNA, protein, and enzyme activity extrapolated a close correlation among the three indicators for MGAT2 along the small intestinal tract as shown in Fig. 3C, suggesting a pivotal role for MGAT2 in dietary fat absorption.

In Situ Localization of MGAT2 by Immunohistochemistry—Immunohistochemical analyses were carried out to investigate the tissue distribution patterns of MGAT2 in the small intestine. Immunohistochemical studies were performed on slices prepared from different segments of wild-type mouse small intestine by using an immunoperoxidase staining system, which generates a brown-colored deposit upon specific immunoreactivity with mMGAT2. An intense brown staining was clearly detected in a layer surrounding the upper villi and vilus tips (Fig. 4, A and B, arrows) where the mature, absorptive enterocytes are located. A striking mMGAT2 staining was also found in the lower crypt regions (Fig. 4, A and B, arrowheads) where younger and secretory epithelial cells are believed to populate. The staining patterns were preserved in different segments of the small intestine. The staining in segments corresponding to jejunum (Fig. 4A) and proximal ileum (Fig. 4B) is shown. Intense brown staining was found inside the villi, rather than at an external site or on the surface, indicating MGAT2 resided inside of the enterocytes. As a negative control of the immunostaining process, normal rabbit IgG did not pose any noticeable staining (Fig. 4C). The specificity of the staining toward mMGAT2 was further confirmed by the disappearance of the signal in a peptide neutralization control, where the primary antibody was incubated with an equal amount (w/w) of
the peptide used to immunize the rabbit (Fig. 4D). These findings provided direct evidence that MGAT2 is localized inside of enterocytes along both the villus and the crypt regions in the small intestine.

Subcellular Localization of MGAT2—To define the subcellular localization of MGAT2, we next performed immunocytohistology studies in COS-7 cells as well as in Caco-2 cells transiently transfected with a C-terminally FLAG-tagged form of human MGAT2. 48 h after transfection, cells were processed for mitochondria staining with MitoTracker Red CMXRos or indirect immunofluorescence staining with antibodies specific for the FLAG peptide and calnexin, a resident ER transmembrane protein that served as an ER marker (23). Cells were also counterstained with DAPI to visualize nuclei (blue). FLAG-MGAT2 expressed in both cell lines displayed a perinuclear, reticular, and punctated pattern (Fig. 5, left panel). FLAG-MGAT2 was not colocalized with MitoTracker Red CMXRos-stained mitochondria (Fig. 5, middle panel of A and C), as demonstrated by the well separated green (FLAG-MGAT2) and red (mitochondria) colors in the merged image (Fig. 5, right panel of A and C). Rather, FLAG-MGAT2 was clearly colocalized with the ER marker, calnexin (Fig. 5, middle panel of B and D), as demonstrated by the significant yellow color in the merged images (Fig. 5, right panel of B and D). The observation that MGAT2 colocalized well with the ER marker calnexin in both COS-7 and Caco-2 cells suggests that the subcellular distribution of MGAT2 to ER was not cell-type specific. Furthermore, the result from Caco-2 cells reflects more closely the in vivo situation, because Caco-2 cells were originally derived from the intestinal tract and the cell line behaves in many ways similar to that of intestinal enterocytes (24), although the expression of the three MGATs is undetectable endogenously by Northern blot analysis in the cell line (data not shown). As negative controls for the immunostaining process, no noticeable staining was observed in cells transfected with blank vector with either anti-FLAG antibody or normal mouse and rabbit IgG-stained cells (data not shown).

Analysis of the Expression of MGAT2 and MGAT Activity in Diabetic db/db Mice, as Compared with Those from the Wild-type Controls—Alterations in physiological or pathophysiological conditions are known to modulate MGAT activity (19–21). Increased MGAT activity was observed in streptozotocin-induced diabetic rats and OLEFT rats, suggesting a change in fat absorption as a consequence of hyperglycemia. To investigate a
possible role of MGAT2 in diabetes and obesity, we analyzed the level of MGAT2 expression and enzyme activity in the small intestine of db/db mice, a genetic model of diabetes and obesity caused by a mutation of the leptin receptor gene. At 2 months of age, the db/db mice were hyperphagic, obese, and diabetic. The body weight, plasma glucose, and triglycerides of the db/db mice used in the present studies were 32.1 ± 0.8 g, 285.1 ± 46.7 mg/dl, and 2.0 ± 0.3 mm, respectively, which are significantly higher than the wild-type mice (21.0 ± 0.4 g, 137.8 ± 6.4 mg/dl, and 0.9 ± 0.1 mm, respectively). The expression of MGAT2 protein and MGAT enzymatic activity in the small intestine of diabetic db/db mice were examined by Western blot analyses and TLC enzyme assays, respectively, and compared with those from wild-type mice. When measured as per unit of protein extract, the levels of MGAT2 protein expression in the homogenate of whole small intestine were 330 ± 35 for wild-type mice and 317 ± 43 for db/db mice (mean ± S.E., n = 3, arbitrary units), respectively. The MGAT activities in these samples were 12.4 ± 1.8 for wild-type mice and 10.0 ± 2.4, for db/db mice (mean ± S.E., n = 3, nmol/min/mg of protein), respectively. Although there is no significant change in either MGAT2 expression or MGAT activities in diabetic db/db mice as compared with that from wild-type mice when measured as per unit of intestinal extract, the total intestinal MGAT activity was still significantly higher in the db/db mice than that from the wild-type controls, due primarily to a 70% enlargement of the small intestine in the db/db mice (measured by weight).

Expression of MGAT2 and MGAT Activity Was Significantly Up-regulated in Mice Fed the High Fat Diet—In view of the important role of MGAT2 in intestinal fat absorption, we next examined whether the expression of MGAT2 and MGAT activities along the small intestine could be regulated by feeding a high fat diet. The mice were supplied with either standard chow diet or high fat diet for 4 weeks as described under “Experimental Procedures.” Fig. 6A shows the expression levels of the MGAT2 protein in each of the five equal segments and the entire small intestine from mice fed standard chow diet or high fat diet. Fig. 6B shows the GAPDH levels from the same blots in panel A, which were stripped and re-probed with anti-GAPDH antibody. MGAT2 signals were normalized with those of corresponding GAPDH bands. The quantitative values of MGAT2 expression were expressed as percent of the mean values of control mice (Fig. 6C). The data clearly show that the expression levels of the intestinal MGAT2 protein was significantly increased in mice fed high fat diet in comparison with controls. MGAT2 expression of high fat feeding mice was increased in all segments tested, with a greater difference observed in the lower intestinal tracts (Fig. 6C). In conjunction with the increased MGAT protein expression, MGAT enzyme activity was also significantly enhanced in mice fed the high fat diet in comparison with the controls (Fig. 6D).
DISCUSSION

Our previous studies reported that MGAT2 mRNA is highly expressed in small intestine both in human and mouse, although the two species differ in tissue and isoform distribution (10, 14). The present studies provide direct evidence that not only mRNA but also MGAT2 protein is most abundantly expressed in small intestine, forming a proximal-to-distal gradient that correlates well with MGAT enzyme activity. This is consistent with previous reports that MGAT activity is most active in jejunum, and less efficient in distal ileum (25, 26). Furthermore, the distribution pattern of MGAT2 mRNA, protein, and activity coincides well with that of dietary fat absorption along the small intestinal tract (3, 27–29). It was previously speculated that poor absorption of dietary fat in the distal small intestinal tract is caused by a lack of phospholipids or alteration of intracellular membrane lipid (27–29), whereas our current data suggest that lower MGAT2 expression and activity in distal regions of the small intestine could also contribute...
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to this observation. In contrast to its high protein expression in small intestine, mMGAT2 protein was not detected in the lower intestinal tract, including cecum, colon, and rectum. The distribution of MGAT is also consistent with that of DGAT1 and DGAT2 mRNA (30), supporting the notion that re-synthesis of triacylglycerol is an obligatory process during dietary fat absorption.

In addition to its predominant expression in the gastrointestinal tract, MGAT2 transcripts were also detected in kidney, liver, and adipocytes (10, 14), suggesting that the enzyme could also be involved in fat storage in these tissues. In an attempt to define a role of MGAT in these tissues, we carried out protein expression analyses of MGAT2 using an mMGAT2-specific antibody. Our results indicate that the level of mMGAT2 expression is dramatically lower in these tissues than that in the small intestine, including mouse kidney where a high level of mMGAT2 transcripts was detected (10), suggesting a post-transcriptional regulation. The results also implicate a predominant role of MGAT2 in dietary fat absorption. The data are consistent with our previous report of a lack of MGAT activity in human liver, a tissue that expresses very high levels of MGAT2 mRNA (14). The mechanisms of the post-transcriptional control of MGAT2 expression remain unknown but could relate to poor translation initiation of MGAT2 in these tissues or a fast degradation of newly synthesized MGAT2 protein. The latter has been evidenced in the expression of apolipoprotein B as well as hydroxymethylglutaryl-CoA reductase (31–35), which are also involved in lipid metabolism. Another intestinal MGAT enzyme, designated MGAT3, was recently identified by Cheng et al. (11). Quantitative PCR analysis revealed that the expression of human MGAT3 is also restricted to the gastrointestinal tract with the highest mRNA level found in the ileum, although the existence of the MGAT3 homologue remains to be confirmed in rodents. Hence, MGAT2 and MGAT3 may play a complementary or synergistic role to compensate or maximize dietary fat absorption. Additionally, the different isoforms of MGAT may be involved in the synthesis of diacylglycerol in non-intestinal tissues, such as adipocytes, kidney, and stomach (10, 12–14).

There are two main biochemical pathways for triacylglycerol synthesis, the glycerol 3-phosphate pathway and the MGAT pathway (1). The glycerol 3-phosphate pathway is believed to be a de novo pathway that is present in most tissues and plays a major role in triacylglycerol storage and membrane integrity. The relative activity of each pathway is determined by the abundance of sn-2-monacylglycerol and free fatty acids. In intestinal mucosa under normal lipid absorption conditions, the monoaoylglycerol pathway contributes to ~80% of triacylglycerols incorporated into chylomicrons (2, 3), whereas hepatic glycerolipid synthesis in adult mammals is believed to occur primarily via the sequential acylation of glycerol 3-phosphate, because very little MGAT activity has been detected in adult rat or human liver microsomes (14, 36). Consistent with the involvement of the DGAT enzymes in both pathways, both DGAT1 and DGAT2 are ubiquitously expressed (37, 38). In contrast, all the three known MGAT enzymes are predominantly expressed in the gastrointestinal tract. A lack of MGAT2 protein expression in tissues other than the small intestine demonstrated in this study, together with limited tissue distribution of the other two MGAT enzymes, further supports the notion that MGAT enzymes are more committed to absorption than storage. Thus, although both enzymes are involved in the re-synthesis of triacylglycerol, MGAT and DGAT may differ significantly in their physiological roles, suggesting that MGAT2 may present a better target for intervening dietary fat absorption as a means to treat obesity. The immunohistochemical study allowed us, for the first time, to visualize the in situ localization of MGAT2 protein in small intestine and provided direct evidence that the enzyme resides inside the enterocytes. Our results show that, in addition to enterocytes along villi and villus tips, the crypt cells also exhibit striking staining. It is commonly believed that the villi is covered predominantly with mature, absorptive enterocytes and is continuously replaced by crypt cells moving upward and maturing into the absorptive cells of the villus (39, 40). In contrast to a claim that MGAT is found to be absent from crypts (41), the immunohistochemical staining of mMGAT2 in crypt regions supports the conclusion by Hoffman and Kukksis (42) that the crypt cells possess intrinsic MGAT activity. Thus, MGAT2 expression and activity appear to be acquired prior to the development into mature enterocytes. MGAT activity was believed to reside in the microsomal fractions of both liver and small intestine (43, 44), which was indirectly supported by staining patterns of MGAT enzymes in transiently transfected cells (11–13). Using the specific markers for ER and mitochondria, the present study provided direct evidence that the MGAT2 enzyme is localized in the ER, but not in the mitochondria. Such a distribution pattern is not cell-type specific, because identical patterns were achieved with MGAT2 transiently expressed in COS-7 cells as well as in Caco-2 cells, a cell line derived from the colon.

Abnormal lipid metabolism resulting in increased levels of chylomicrons and very low density lipoproteins and hypertriglyceridemia is one of the common features associated with diabetes and obesity. In addition, abnormal MGAT activity has been observed in some rodent models of diabetes. In streptozotocin-induced diabetes, hepatic mean MGAT-specific activity increased 11.8-fold (20). In OLEPT rats, MGAT activity in small intestine was 2.7-fold higher than that in control rats (22). However, in obese Zucker rats, the activities of enzymes involved in the monoacylglycerol pathway were preserved, whereas activities of glycerol 3-phosphate pathway enzymes increased significantly in response to obesity (26). In comparison, in this study diabetes in db/db mice affected neither MGAT2 expression levels nor MGAT activity in small intestine when expressed as per gram protein. However, because the small intestines of db/db mice are 70% heavier than that of the wild-type animals, the total MGAT2 enzyme and activity in the small intestines of db/db mice were still significantly elevated. Despite the observed hypertriglyceridemia in db/db mice, there was no detectable liver MGAT2 protein and only minimal MGAT activity observed (data not shown), suggesting that the MGAT pathway in the liver is unlikely involved in the abnormal lipid metabolism observed in db/db mice.

The mechanism regarding the augmentation of MGAT2 by high fat diet remains elusive. Yet, it can be envisaged that activation of PPARα, which is known to be expressed abundantly in small intestine (45), may play a role in the process of up-regulation of MGAT2 in response to high fat diet. PPARα was shown to up-regulate the expression of several genes involved in fatty acid metabolism in small intestine, such as fatty acid translocase and fatty acid transport protein-1 (46). Thus, transcription of the MGAT2 gene may similarly be subject to transactivation by PPARα or other isoforms of PPAR in response to elevated levels of fatty acids in the mucosa to increase the rate of dietary absorption. Future studies are also needed to depict a role of MGAT2 in human obesity, such as a possible linkage of MGAT2 polymorphism with certain types of human obesity as demonstrated with DGAT (47, 48). Furthermore, generation and characterization of mice deficient in MGAT2 expression will shed important light on a role for MGAT2 in dietary fat absorption, as well as in diet-induced obesity.
Consumption of a Western diet is believed to be one of the major contributors to the current obesity epidemic in the industrialized countries. The small intestine is subjected to large daily fluctuations in the amount and composition of dietary fats, and enterocytes must promptly adjust their capacity to guarantee an efficient lipid absorption process. For example, Thomson et al. (49) have previously shown that rats fed a high fat diet demonstrated an increase in the height of the villi and a 30% increase in the enterocyte migration rate without affecting the crypt cell production rate. Exposure to a high fat diet also resulted in significant increases in fat absorptivity (50), the production and secretion of pancreatic lipases, as well as the ability of isolated rabbit enterocytes to secrete chylotrimers (51–53). Furthermore, rats fed a high fat diet showed a diminished satiation and reduced sensitivity to leptin (54, 55).

In this report, we demonstrated for the first time that the intestinal MGAT2 expression and activities were significantly up-regulated in response to a high fat diet, which may in part account for the adaptive changes in the enterocytes to maximize dietary fat absorption. More importantly, the results suggest that MGAT may play a role in the diet-induced obesity. Taken together, our current data on tissue distribution, subcellular localization, and regulation by high fat diet of the MGAT2 protein suggest a predominant role of the enzyme in dietary fat absorption.

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A Predominant Role of Acyl-CoA:monoacylglycerol Acyltransferase-2 in Dietary Fat Absorption Implicated by Tissue Distribution, Subcellular Localization, and Up-regulation by High Fat Diet

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