Intestinal Monoacylglycerol Metabolism

DEVELOPMENTAL AND NUTRITIONAL REGULATION OF MONOACYLGLYCEROL LIPASE AND MONOACYLGLYCEROL ACYLTRANSFERASE

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Intestinal monoacylglycerol (MG) metabolism is well known to involve its anabolic reesterification to triacylglycerol (TG). We recently provided evidence for enterocyte MG hydrolysis and demonstrated expression of the monoacylglycerol lipase (MGL) gene in human intestinal Caco-2 cells and rodent small intestinal mucosa. Despite the large quantities of MG derived from dietary TG, the regulation of MG metabolism in the intestine has not been previously explored. In the present studies, we examined the mRNA expression, protein expression, and activities of the two known MG-metabolizing enzymes, MGL and MGAT2, in C57BL/6 mouse small intestine, as well as liver and adipose tissues, during development and under nutritional modifications. Results demonstrate that MG metabolism undergoes tissue-specific changes during development. Marked induction of small intestinal MGAT2 protein expression and activity were found during suckling. Moreover, while substantial levels of MGL protein and activity were detected in adult intestine, its regulation during ontogeny was complex, suggesting post-transcriptional regulation of expression. In addition, during the suckling period MG hydrolytic activity is likely to derive from carboxyl ester lipase rather than MGL. In contrast to intestinal MGL, liver MGL mRNA, protein, and activity all increased 5–10-fold during development, suggesting that transcriptional regulation is the primary mechanism for hepatic MGL expression. Three weeks of high fat feeding (40% kcal) significantly induced MGL expression and activity in small intestine relative to low fat feeding (10% kcal), but little change was observed upon starvation, suggesting a role for MGL in dietary lipid assimilation following a high fat intake.

sn-2-Monoacylglycerol (MG) is one of the major digestive products of dietary triacylglycerol (TG). Along with fatty acid, it is formed by the action of pancreatic triacylglycerol lipase (PTL) in the intestinal lumen, because PTL preferentially cleaves the sn-1 and 3 positions of TG (1). Both hydrolysis products are absorbed as monomers across the apical membrane of the intestinal epithelial cell (1, 2). The mechanism of sn-2-MG uptake into the enterocyte has been demonstrated to be a saturable function of the monomer concentration of sn-2-MG at both apical and basal lateral surfaces of the cell, suggesting carrier-mediated uptake (2, 3). At higher concentrations, a diffusion uptake pathway is also apparent (2, 3). After absorption, sn-2-MG is rapidly reincorporated into TG in the endoplasmic reticulum (ER) via the so-called monoacylglycerol acyltransferase (MGAT) pathway, which is catalyzed by two enzymes, MGAT2 and diacylglycerol acyltransferase (DGAT). Two DGAT isoforms (DGAT1 and 2) have been identified, and both are expressed in small intestine (4, 5). In addition to the MG pathway, the intestine can also synthesize TG via the glycerol-3-phosphate (G3P) pathway, which is the dominant TG synthetic pathway in other tissues such as adipose and liver (1). In the intestine, however, more than 75% of postprandial TG resynthesis is catalyzed by the MGAT pathway (6, 7). Reesterified TG and apolipoproteins are assembled into chylomicron particles, which are then secreted into the lymphatic circulation.

Intestinal MG metabolism has generally been thought to involve only an anabolic pathway, the reesterification to TG via the MGAT pathway. Nevertheless, the presence of a MG hydrolytic activity in small intestine was noted several decades ago (8) and the partial purification of MG lipase (MGL; EC 3.1.1.23) activity from rat intestinal mucosa was reported (9). Recently, additional insight into intestinal MG metabolism was obtained. After incubation of Caco-2 cells with sn-2-[^3]H]MG at either the apical or basal lateral surface, a substantial amount of radioactivity was recovered in the unesterified fatty acid fraction (10). In addition, human MG lipase mRNA expression was detected in Caco-2 cells, and the murine MG lipase gene (11) was also shown to be expressed in rodent small intestine (10). These results suggested that catabolic processing of sn-2-MG may occur in intestinal mucosa, in addition to the well known anabolic processing. The regulation and function of this MG hydrolytic activity in the enterocyte are at present entirely unknown.

On a daily basis, the human small intestine metabolizes an estimated average of 100 g of dietary fat which is composed of more than 90% TG (1). Therefore, the mechanism by which the enterocyte metabolizes sn-2-MG, which is one of the major
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products of luminal TG hydrolysis and a backbone for TG reesterification, is of great importance for dietary lipid assimilation. Despite this physiological significance, surprisingly little is known about the regulation of intestinal MG metabolism. In the liver, MG metabolism has been shown to be developmentally regulated, with hepatic MGAT activity dramatically higher in the suckling period than in adult rat liver (12). At present, nothing is known about the developmental expression of hepatic MGL, nor about the developmental expression of either MGL or MGAT2 in the intestine. Therefore, in the present studies, we examined the expression and activity of the two MG metabolizing enzymes, MGAT and MGL, in intestine as well as liver during ontogeny.

Cao et al. (13) reported that a high fat diet induced MGAT2 expression and activity in small intestine, indicating a functional role for this enzyme in dietary fat absorption. It is known that the expression of other genes involved in intestinal lipid metabolism are also altered upon a change in nutritional status. For example, the induction of small intestinal liver fatty acid-binding protein (LFABP) expression by high fat feeding has been reported (14, 15), as has the expression of microsomal triacylglycerol transfer protein (MTP), an essential protein for chylomicron assembly (14). Furthermore, it is well known that a standard fasting and refeeding regime markedly alters hepatic lipid metabolism (16, 17), but the influence on intestinal lipid metabolism is less clear. Thus, the effects of nutritional status on intestinal MG metabolism, in comparison with other tissues, were also investigated in the present studies.

The results show that MG metabolism is dramatically altered in a tissue-specific manner in both intestine and liver during development and following a high fat diet. Intestinal MGL expression and activity are increased by high fat feeding whereas little or no changes are found in the fasted state, suggesting a potential role for MGL in dietary lipid assimilation.

EXPERIMENTAL PROCEDURES

Materials—sn-2-[14C]Monoolein (oleoyl-1-[14C], 55 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. [14C]Oleoyl-CoA (oleoyl-1-[14C], 57 mCi/mmol) was purchased from PerkinElmer Life Sciences. Unlabeled sn-2-monoolein was obtained from Doosan Serdary Research Laboratories (Toronto, Canada). The 3% borate impregnated thin layer chromatography (TLC) plates were purchased from Analtech (Newark, DE). Silica gel G TLC plates were obtained from Sigma. Mouse MGL cDNA was a generous gift from Dr. Cecilia Holm (Lund University), and mouse MGAT1 and 2 cDNAs were generously provided by Dr. Robert Farese (UCSF). Antibodies against mouse MGL and MGAT2 sequences were generous gifts from Dr. Daniele Piomelli (UC Irvine) and Dr. Yuguang Shi (Lilly Research Laboratory), respectively. The anti-CEL antibodies were generously provided by Dr. David Hui (University of Cincinnati). β-Actin antibody was purchased from Sigma, and mouse cyclophilin A antibody and cDNA were obtained from Ambion (Austin, TX).

Animals, Diets, and Tissue Collection—For each developmental regulation study, wild-type mice (C57BL/6, n = 3 at each age) from ages 6 days before birth to 3-months old were reared in the animal facility at Rutgers University. Prenatal mice were obtained from pregnant dams. The day when the vaginal plug was seen was considered as gestational day 0, with embryonic days counted thereafter. Litter size was consistent at 7–10. Animals were maintained on a 12 h light and dark cycle and fed regular chow diet (Purina Mouse Chow 5015, Purina Co., St. Louis, MO) ad libitum after weaning. For the high fat feeding studies, 3-month-old male mice (C57BL/6) were divided into two groups (n = 8 per group). Purified rodent diet (10% fat by calories, from soybean oil) was given to the control group and the high fat group was fed a 40% kcal fat diet containing the additional 30% kcal from coconut oil, rich in short chain saturated fatty acids (D12327 and D12325, respectively, Research Diets, New Brunswick, NJ) for 3 weeks ad libitum. In a second high fat feeding protocol, female mice were divided into three groups (n = 8) and fed with either a 10% kcal fat diet, or high fat diets rich in saturated fat from lard (45 or 60% kcal) for 3 months. (D12450B, D12451, and D12492, respectively, Research Diets). For the starvation and refeeding trial, 3-month-old male mice were divided into 4 groups (n = 7 per group) as follows: fed (Purina Mouse Chow 5015, Purina Co.), starvation for 12 h, starvation for 24 h, and refeeding with a high sucrose diet (D11725, Research Diet) after a 24-h starvation (18, 19). Animals were sacrificed using CO2, and the entire small intestine from pylorus to cecum was immediately excised. For the nutritional regulation studies, the intestine was rinsed twice with saline and then the mucosa was harvested by scraping. For developmental studies, whole intestine was collected followed by rinsing. Samples were immediately frozen on dry ice and kept at −70 °C. Liver and adipose tissue (peri-renal and epididymal fat) were also collected, snap frozen on dry ice, and stored at −70 °C.

Northern Blot Analysis of MGL and MGAT1 and 2 mRNA Expression—Tissues were homogenized in Solution D (4 mM guanidinium thiocyanate, 25 mM sodium citrate, 0.1 M 2-mercaptoethanol) using several strokes of a Polytron. Total RNA was further purified by phenol extraction. For detecting MGAT2 transcript in liver, poly(A)+ RNA was prepared using a Qiagen mRNA extraction kit. 20–40 μg of total RNA or 2 μg of poly(A)+ RNA were loaded onto 1% agarose gels, separated by electrophoresis, and transferred onto nylon membranes (PerkinElmer Life Sciences). Full-length coding regions of MGL, MGAT1, and MGAT2 cDNA were labeled with 32P (PerkinElmer Life Sciences) using the Random Prime labeling system (GE Healthcare, Piscataway, NJ). Membranes were prehybridized for 1 h and hybridized for 2–3 h at 68 °C using Quiik hybridization solution (Stratagene, La Jolla, CA). Blots were washed twice at room temperature with 2× SSC, 0.1% SDS for 15 min. An additional high temperature (65 °C) wash with 0.1× SSC, 0.1% SDS for 30 min was completed before exposing the blots to a PhosphorImager screen. Quantification was done using the Molecular Dynamics STORM scanner and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Blots were stripped and reprobed with 18S rRNA cDNA or mouse cyclophilin A cDNA for internal loading controls.

Quantitative RT-PCR for Intestinal MGL mRNA Expression—Relative MGL mRNA expression in small intestine was analyzed by quantitative RT-PCR (SYBR Green method). Total RNA was extracted as described above and further purified...
using the RNeasy clean up kit (Qiagen, Valencia, CA) along with DNase 1 treatment to minimize genomic DNA contamination. Reverse transcription was performed using 1 µg of total RNA, random primer, RNase inhibitor and AMV reverse transcriptase (Promega Madison, WI) in a total volume of 25 µl. Primer sequences for MGL and β-actin (endogenous control) were retrieved from Primer Bank (Harvard Medical School QPCR primer data base), as follows: MGL: forward 5′-CAGAGGGCACAACCTACTTTTCT-3′, reverse 5′-ATGCCGCCCAGGTCTATT-3′; β-actin: forward 5′-GGGCTTATTCCCTCCATCG-3′, reverse 5′-CCAGTGTTGAAC-AATGCCATGT-3′. Efficiencies of PCR amplification for both primers were tested during preliminary experiments and similar PCR efficiencies were confirmed. Real time PCR reactions were performed in triplicate using an Applied Biosystems 7300 instrument. Each reaction contained 80 ng of cDNA, 250 nM of primers and 2 mM of dNTPs. PCR efficiencies were confirmed. Real time PCR reactions were performed in triplicate using an Applied Biosystems 7300 instrument. Each reaction contained 80 ng of cDNA, 250 nM of primers and 2 mM of dNTPs. PCR efficiencies were confirmed during preliminary experiments to ensure linearity with time and protein concentration. 2.5 mM sn-2-monoolein was used for liver and adipose tissue MGL assays, and 25 µM was used for intestine samples. The reaction was initiated by adding tissue homogenate. Liver and adipose tissue samples were incubated for 5 min and intestinal samples for 10 min at 23 °C. Lipids were extracted using chloroform/methanol (2:1, v/v), and the organic phase was subjected to TLC analysis. To monitor isomerization of substrate, 3% borate-impregnated TLC plates (Anatech, Newark, DE) were used for separation of lipids. Spontaneous isomerization to the sn-1 isomer (20–30%) was always accounted for when determining the enzyme activity. All reaction times and temperatures were optimized to minimize spontaneous isomerization during the assay. Quantification of the specific activity of 14C-labeled end products separated by TLC was done using the Molecular Dynamics STORM scanner and ImageQuaNT software. On each plate, known amounts of specific radioactivity of [14C]oleate (PerkinElmer Life Sciences) were spotted and used for formulating standard curves to calculate enzyme activities for each sample.

In Vitro MGAT assay—The MGAT assay followed a well established protocol based on the method of Coleman and Haynes (12), with slight modifications. Activity was measured as the incorporation of [14C]oleoyl-CoA into diacylglycerol (DG). 25 µM oleoyl-CoA and 250 µM sn-2-monoolein (Doosan Serdary Research) were used as substrates. [14C]Oleoyl-CoA was purchased from PerkinElmer Life Sciences (57 mCi/mmol) and cold oleoyl-CoA was purchased from Sigma. The assay buffer contained 100 mM Tris-HCl, 4 mM MgCl₂, 1 mg/ml bovine serum albumin, and 100 µM each of phosphatidylcholine and phosphatidylserine. 5 or 10 µg of membrane fraction protein, prepared by ultracentrifugation at 100,000 × g for 1 h at 4 °C, were used as the enzyme source. The enzyme assay was initiated by adding [14C]oleoyl-CoA, and incubation was for 5 min at 25 °C. Lipids were extracted using chloroform/methanol (2:1, v/v) and the organic phase was subjected to TLC analysis using standard silica gel plates (Sigma) and a solvent system of hexane/ethyl ether/acidic acid, 70:30:1, v/v. The plates were exposed to a PhosphorImager screen to visualize incorporation of [14C]oleoyl-CoA into neutral lipids. Specific activities found in the DG fraction plus half of the TG fractions were considered as MGAT activity (12, 22). Quantification of each lipid fraction was analyzed using ImageQuaNT software.

RESULTS

Developmental Regulation of Intestinal MGL and MGAT2 — Changes in mRNA and protein expression of the two MG-metabolizing enzymes, as well as both enzyme activities were determined over the course of intestinal ontogeny. The results showed a relatively abundant expression of MGL mRNA at early developmental stages, declining thereafter to a lower but detecta-
The expression pattern for MGL protein over the same time period was not consistent with the changes in its mRNA expression. MGL protein was detected in prenatal and adult intestine but not in the suckling period (Fig. 1B). MGL activity was measured by the release of radiolabeled fatty acid from sn-2-[14C]monoolein, and it was found that activity increased throughout intestinal development, plateauing at a relatively high level after day 6 (Fig. 1C). Thus, MGL activity shows discrepancies with the expression of both mRNA and protein levels (Fig. 1D), particularly during the suckling period.

We hypothesized that carboxyl ester lipase (CEL; also known as bile salt-stimulated lipase), another enzyme with known sn-2-monoyglycerol hydrolytic activity, might be present in suckling intestine. The results in Fig. 1E show that CEL protein was detected in neonatal intestine at approximately equivalent levels to those in adult intestine.
For intestinal MGAT2, mRNA levels were very low at day +6 but then rose and remained relatively constant from day +3 to day +90 (Fig. 2A). Protein and activity levels were also very low at day +6 and increased thereafter; both appeared to be up-regulated during the early suckling period, and declined thereafter (Fig. 2, B and C).

Developmental Regulation of Hepatic MGL and MGAT2 — In marked contrast to intestinal MGL, similar patterns were observed for liver MGL mRNA, protein, and activity levels. All were increased 5–10-fold during development (Fig. 3D), suggesting that transcriptional regulation is the primary mechanism of hepatic MGL expression (Fig. 3, A, B, and C).

It has been reported that the MGAT1 transcript was detected in adult mouse liver by Northern analysis (22); however, we were unable to detect MGAT1 in the present studies. The reason for the discrepancy is unknown. As described below, the MGAT1 transcript was detected in adipose tissue, in agreement with Yen et al. (22). The MGAT2 transcript was readily detected in 2 μg of liver mRNA, consistent with the report of Cao et al. (23). Thus, the present results for mRNA regulation in liver represent changes in the MGAT2 transcript during ontogeny. In contrast to the developmental pattern found for hepatic MGL, hepatic MGAT2 mRNA was detected during the pre- and post-natal stages but declined after day 6 (Fig. 4A). MGAT activity levels were generally consistent with this pattern of mRNA expression (Fig. 4B), suggesting that, as for hepatic MGL, transcription is the predominant mechanism of MGAT regulation in the liver during development. Overall, an inverse regulation of MGAT and MGL during ontogeny was found in liver (Figs. 3 and 4); however such reciprocal regulation of the two MG-metabolizing enzymes was not found in small intestine (Figs. 1 and 2).

**FIGURE 2. Developmental regulation of intestinal MGAT2.** A, relative mRNA expression was estimated by Northern blot analysis. 20 μg of total RNA was loaded in each lane and probed with m MGAT2 or 18S ribosomal RNA sequence as a loading control. B, relative MGAT2 protein expression was estimated by Western blotting. The total membrane fraction was prepared as described under “Experimental Procedures.” 10 μg of membrane protein were loaded in each lane and probed with mMGAT2 antibody. C, MGAT activity was determined as described under “Experimental Procedures.” D, comparison of the relative MGAT2 mRNA (solid line), protein (dotted line), and activity levels (hyphenated line). Relative expression at each time point was calculated based on the highest value being 100. Day 0 designates day of birth. One representative series is shown out of three independent series, with n = 3 separate samples for each time point.
Because liver TG content has been reported to decline to low adult levels during development in the rat (24, 25), we wanted to determine whether a similar decline is found in mouse liver. The results in Fig. 4 show that TG levels in adult mouse liver are 5-fold lower than those found in day 0 liver.

**Nutritional Control of Intestinal MGL and MGAT2**—To examine the effect of an increased substrate supply on intestinal MGL, 3-month-old C57BL/6 male mice were fed either a high fat (40% kcal) or standard fat diet (10% kcal) for 3 weeks. Body weight was significantly elevated in the high fat fed group, along with a marked increase in total fat pad weight, nearly 4-fold greater than control (Fig. 5, A and B). Interestingly, we found that intestinal MGL protein expression increased 2–3-fold, and significant elevations in MG hydrolysis in small intestine were observed following high fat feeding (Fig. 5C). Relative mRNA levels of MGL were determined by quantitative RT-PCR, and also showed a consistent induction although it did not reach statistical significance. A similar result was observed in a relatively long term, 3-month high fat feeding study (10, 45, or 60% kcal), though the response was somewhat blunted compared with results from the short term feeding study (results not shown). In contrast to this induction by a high fat challenge, starvation up to 24 h did not significantly alter MGL expression and activity (Fig. 5E). These results show that upon increased lipid flux to the enterocyte, MG catabolism was stimulated, but fasting did not affect intestinal MGL expression or activity.

MGAT2 protein expression in small intestine was increased 3-fold following a 3-week high fat diet without altering its mRNA or activity (Fig. 5D). The starvation-refeeding regimen revealed little or no change in either MGL or MGAT levels (Fig. 5, E and F).

**Nutritional Control of Hepatic MGL and MGAT**—The 3-week high fat diet stimulated liver MGL activity, along with a 2-fold elevation in the amount of MGL protein (Fig. 6A). In

![Developmental regulation of hepatic MGL](image)
contrast, starvation up to 24 h, or refeeding, did not affect MGL activity despite a significant induction of mRNA expression during fasting (Fig. 6C).

Hepatic MGAT activity was not changed after the high fat diet (Fig. 6B), but refeeding with a high carbohydrate diet following starvation resulted in a modest but significant decrease (~35%) in MGAT activity compared with any other group (Fig. 6D).

**Nutritional Control of Adipose Tissue MGL and MGAT1**—In general, MG metabolism in adipose tissue was not substantially changed by high fat feeding, despite the large increase in adipose mass (Figs. 5B and 7A and B). The MGAT1 transcript was significantly down-regulated by high fat feeding; however MGAT activity was not changed (Fig. 7B). MGL protein and activity did not change as well, and an increase in MGL mRNA levels (Fig. 7A) did not reach statistical significance. Starvation and refeeding also did not alter MG metabolic enzyme levels (Fig. 7C and D).

**DISCUSSION**

It is well known that \textit{sn}-2-MG, a major digestive product of dietary TG, is reesterified to TG in the enterocyte via the MGAT pathway. Recently, we presented evidence of MG hydrolysis by MGL in intestinal epithelium and the human intestinal Caco-2 cell line (10). In the present study, the regulation of two MG metabolizing enzymes, MGAT and MGL, in intestine as well as other tissues, has been explored during ontogeny and during alterations in nutritional status.

For both MGL and MGAT, a dynamic and complex pattern of changes was observed during mouse intestinal development. Discordances between mRNA, protein, and activity levels suggest that post-transcriptional and/or post-translational regula-
At present, the mechanisms underlying the complex regulation of intestinal MGL and MGAT are unknown. One possibility is that enzyme activities may be altered by phosphorylation state, well known to regulate activities of many lipid metabolic enzymes. For example in the liver, it has been shown that the activities of key enzymes catalyzing fatty acid oxidation and glycerolipid biosynthesis are regulated reciprocally by their phosphorylation via casein kinase 2 and AMP-activated kinase (26–28). Post-translational regulation of lipid metabolic enzymes in intestine is entirely unexplored at present. A ProSite consensus sequence search (29) indicates that MGL and MGAT have multiple putative phosphorylation sites by protein kinase C, tyrosine kinase, and/or casein kinase 2. Elucidation of the molecular mechanism of regulation of intestinal enzymes involved in lipid metabolism will provide a more complete understanding of lipid assimilation in the enterocyte.

Levels of MGAT2 protein and activity are greatly elevated during the suckling period, possibly contributing to the efficient absorption of milk fat during development. In contrast, during lactation, the intestinal MGL protein was not detectable despite an increase in MG hydrolytic activity over the same time course (Fig. 1D). We found that a substantial amount of carboxyl ester lipase is present in small intestine during the suckling period. CEL has broad catalytic activity toward neutral lipids, including sn-2-position fatty acyl chains (30, 31). It is expressed in pancreas and mammary gland, and is present in maternal milk (30–32). Further, internalization of CEL present in the intestinal lumen into enterocytes has been demonstrated (33, 34). Thus, increased MG hydrolysis without MGL protein during suckling might be caused by the action of CEL.

In contrast to intestine, the activities of hepatic MG-metabolizing enzymes during development are quite consistent with their levels of mRNA and protein expression, suggesting that
transcription is the major mechanism controlling hepatic MG metabolism during ontogeny. The 5–10-fold increases in MGL mRNA, protein, and activity during development (Fig. 3) parallel the known elevation of hepatic TG hydrolase expression, which is believed to mobilize TG in the liver (35, 36). Indeed, hepatic TG content declines during development in both rat (24, 25) and mouse (Fig. 4D). Thus, the profile for hepatic MGL induction during development suggests that it is likely to play a role in the mobilization of liver TG stores. The elevated hepatic MGAT2 mRNA and activity in early developmental stages suggests an important role for MGAT in hepatic TG synthesis, both in the absence of glycerol-3-phosphate acyltransferase (GPAT) activity in the prenatal period, or along with GPAT activity during suckling (37).

The regulation of MG-metabolizing enzymes is tissue-specific, as indicated by several points: (a) the apparent transcriptional regulation of liver but not intestinal MGL and MGAT; (b) the reciprocal regulation of MG esterification and lipolytic activities observed in the liver (Figs. 3 and 4) is not found in the intestine (Figs. 1 and 2); and (c) the activities of hepatic MGAT per mg of liver protein appear to be much lower than the levels of MGL, while the opposite is true of intestinal activities. (It is worth noting that these values are not absolute activities, since tissue samples and not purified enzymes were used.) Taken together, the tissue-specific differences suggest distinct roles for the MG substrate in intestine and liver.

The present studies demonstrate that nutritional manipulation alters intestinal MG metabolism. Intestinal MGL expres-
sion and activity were induced by a high fat diet, but not by starvation (Fig. 5, C and E), suggesting that upon increased lipid flux to the enterocyte, MG catabolism was stimulated. The physiological reason for increased MG catabolism upon high fat feeding is unknown, although it may represent a controlling mechanism to regulate lipid assimilation when excessive lipid has been ingested. However, the levels of intestinal MGL activity per mg of protein are ~10 times lower than intestinal MGAT activity, suggesting an alternate function, perhaps related to cellular signaling as discussed below.

After 3 weeks of high fat feeding a 3-fold induction of MGAT protein was observed, with minimal change in activity (Fig. 5 D). These results are in agreement with those of Cao et al. (13) who found that 4 weeks of high fat feeding (40% kcal) increased MGAT2 protein expression in the small intestine 2-fold, with only a minor (20%) increase in activity. It was also consistently found that intestinal MGAT2 mRNA was altered neither during ontogeny nor by nutritional challenge, despite changes in MGAT2 protein and activity. Together with the developmental expression studies, these results support the post-transcriptional and/or post-translational modification of MGAT2 in the intestine.

The high fat diet stimulates liver MGL activity and protein (Fig. 6 A). Because high fat feeding has been shown to increase hepatic VLDL secretion (38), the induction of MGL might reflect a role in the mobilization of intracellular TG for lipoprotein secretion. Starvation up to 24 h, or refeeding, did not alter MGL activity, despite a significant induction in mRNA expression during fasting (Fig. 6 C). A 48-h fast decreases MGL protein and activity (not shown). The two nutritional control studies thus suggest that hepatic MGL is likely participating in intracellular TG mobilization directed toward secretion in the fed
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state rather than providing substrate for β-oxidation. Neither high fat feeding nor a starvation-refeeding protocol substantially affected MGAT activity. The clear contrast with the known marked induction by starvation/refeeding of lipogenic genes such as fatty acid synthase and GPAT (16, 17), suggests that hepatic MGAT may have a distinct role relative to other lipogenic enzymes. It has been suggested that the function of hepatic MGAT may be associated with the retention of essential fatty acids by reacylation of sn-2-MG during lipolysis (39). The present results are in keeping with this proposal. The high fat diet used in the present studies contained primarily short chain saturated fatty acids, thus it will be of interest to determine whether a diet high in essential fatty acids causes an increase in hepatic MGAT levels.

The metabolism of MG in adipose tissue was not substantially affected by nutritional modification. This suggests that the physiological role of MGAT in this tissue may not be directly related to TG synthesis, or, more likely, that the total level of MGAT present is sufficient to effectively esterify the levels of MG substrate present. That starvation did not result in significant changes in MGL levels is perhaps surprising, because an elevation in MG catabolism might have been expected, as MGL is believed to participate in the complete hydrolysis of TG in adipose tissue (40). Interestingly, however, the absence of changes is consistent with what has been found for hormone-sensitive lipase (HSL) mRNA and protein expression and activity, which were also not stimulated by 12 h or 24 h of fasting. Indeed, no HSL stimulation was found until 3 days of starvation in the rat, although the serum-free fatty acid level doubled within the first day of fasting (41). The mechanisms contributing to the starvation-induced elevation of circulating free fatty acid in the absence of induction of HSL and MGL need to be elucidated. It is possible that the recently identified adipose tissue lipase TG (ATGL, or desnutrin) (42–44) may participate tissue TG lipase (ATGL, or desnutrin) (42–44) may underlie this fatty acid elevation, as Sul and co-workers (42) have shown that desnutrin gene expression is induced during starvation.

The function of intestinal MGL appears to oppose the typical metabolic fate of sn-2-MG, esterification to DG and ultimately TG. As discussed above, tissue-specific functions of MGL are likely. In adipose tissue, the physiological function of MGL in lipid metabolism is thought to be the complete hydrolysis of TG following the action of HSL (40). For liver MGL, the present studies suggest involvement in TG mobilization for secretion. Another intriguing functional aspect of MGL has been found recently in the central nervous system. MGL has been proposed to be a key enzyme modulating endocannabinoid signaling via hydrolysis of sn-2-arachidonoyl glycerol, an endogenous agonist of the cannabinoid receptors, CB1 and CB2 (21, 45). It has been reported that CB1 is present in the enteric plexus of the gastrointestinal tract (46, 47), and endocannabinoid signaling has been proposed to function in gastroprotection against inflammation, control of intestinal motility, and secretory processes (48, 49). Thus, a potential signaling function for intestinal MGL should be considered.

The physiological significance of sn-2-MG metabolism in the small intestine has been indirectly demonstrated by the recently reported anti-obesity effect of 1,3-DG feeding (50–53). Although the mechanisms underlying these effects are not known, we hypothesize that reduced sn-2-MG supply in the small intestinal epithelium, resulting from the lack of sn-2-acylated substrate, may lead to the altered lipid metabolism observed in 1,3-DG-fed mice. It will be of interest to examine how manipulation of intestinal MG-metabolizing enzymes will affect TG reesterification, chylomicron secretion, and, perhaps, whole body energy homeostasis.

In summary, MG metabolism undergoes dramatic changes in both intestine and liver during ontogeny, and is modulated by high fat feeding. Intestinal MGL in particular is significantly elevated by a high fat diet, suggesting a potential function for MGL in dietary lipid assimilation at the substrate and/or regulatory level.

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