Monoacylglycerol Metabolism in Human Intestinal Caco-2 Cells

EVIDENCE FOR METABOLIC COMPARTMENTATION AND HYDROLYSIS*

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Free fatty acids (FFA) and sn-2-monoacylglycerol (MG), the two major hydrolysis products of dietary triacylglycerol (TG), are absorbed from the lumen into polarized enterocytes that line the small intestine. Intensive studies regarding FFA metabolism in the intestine have been published; however, little is known regarding the metabolism of MG. In these studies, we examined the metabolism of sn-2-monoolein (sn-2-18:1) by human intestinal Caco-2 cells. To mimic the physiological presentation of MG to the enterocyte, the metabolism of [3H]sn-2-monoolein was examined by adding taurocholate-mixed sn-2–18:1 and albumin-bound sn-2–18:1 at the apical (AP) and basolateral (BL) surfaces of the Caco-2 cell, respectively. The results demonstrate that more sn-2–18:1 was incorporated into TG from AP taurocholate-mixed sn-2–18:1, whereas more phospholipid was synthesized from BL albumin-bound sn-2–18:1. The TG:phospholipid ratio was 5-fold higher for AP relative to BL MG incubation. Qualitatively similar results were observed for bovine serum albumin-bound MG added at the apical surface. It was also found that substantial sn-2–18:1 radioactivity was recovered in the FFA fraction, suggesting that sn-2–18:1 may be directly hydrolyzed within the Caco-2. We therefore used reverse transcription-PCR with primers designed from the murine MG lipase (MGL) gene, and detected the presence of MG lipase mRNA in Caco-2. The human MGL gene was cloned and found to be 83% identical to the murine MGL, and identical to a previously described lysophospholipase-like protein. Northern blot analysis showed the expression of MGL throughout Caco-2 differentiation. Thus, MG metabolism in Caco-2 cells may include not only well established anabolic processes, but also catabolic processes. Further, the observed polarity of MG metabolism suggests that, as for fatty acids, separate precursor and/or product pools of lipid may exist in the intestinal enterocyte.

sn-2-Monoacylglycerol (sn-2-MG)† and fatty acids (FFA) are the products of pancreatic lipase hydrolysis of dietary triacylglycerol (TG). They are absorbed from the lumen into polarized enterocytes that line the small intestine (1). Following absorption, across the apical (AP) surface of the enterocyte, sn-2-MG and FFA are reincorporated into TG, which are subsequently secreted as the major component of chylomicrons (CM) into the lymphatic system (1). FFA are also taken up across the basolateral (BL) surface of the enterocyte (2), and we have shown recently that MG is taken up across the BL surface of Caco-2 cells as well (3). CM and other TG-rich lipoproteins are hydrolized by lipoprotein lipase (LPL) that extends into the vascular space from the capillary endothelial cells of extrahepatic tissues. LPL catalyzes the release of FFA from TG, and circulating TG-rich lipoproteins have been reported to accumulate MG after LPL hydrolysis (4, 5). Further, serum albumin was found to bind sn-2-monoolein (sn-2–18:1) with an apparent dissociation constant ($K_d$) of $0.2$ μM (6), and it was shown that albumin-bound MG was not hydrolyzed by milk LPL (5). Therefore, it is likely that sn-2-MG is circulating in the plasma either bound to albumin or as a component of lipoprotein remnants, and is taken up into the enterocyte across the basolateral membrane. However, no studies have addressed the metabolic fate of plasma MG taken up by the enterocyte.

The small intestine is capable of synthesizing TG via both the glycerol 3-phosphate (G3P) and the CoA-dependent MG pathways of acylglycerol synthesis, which under normal conditions contribute about 20 and 80%, respectively, to total TG levels in the CM (7). The CoA-dependent MG pathway proceeds by direct acylation of the sn-2-MG with fatty acyl CoA, yielding the sn-1,2-diacylglycerol (sn-1,2-DG) intermediate (8). The MG pathway is achieved by the enzyme complex “TG synthetase” that has been localized to the cytosolic surface of the endoplasmic reticulum (ER) (9). When the FFA and sn-2-MG levels are low, the G3P pathway becomes a major route for synthesis of TG in the enterocyte (7). This pathway involves the acylation of G3P with fatty acyl CoA to yield lysophosphaticid acid, and subsequent acylation and dephosphorylation of lysophosphaticid acid to obtain DG. The final reaction in both the MG and G3P pathways for TG synthesis is the conversion of DG to TG via diacylglycerol acyltransferase (DGAT) (7). Cases et al. (10) have shown that two DGAT genes are expressed in the small intestine. It is not yet clear whether the DGAT activities in the MG pathway and G3P pathway have distinct localizations.

Luminally derived and plasma-derived FFA were found to

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† The abbreviations used are: MG, monoacylglycerol; FFA, unesterified free fatty acid; DG, diacylglycerol; TG, triacylglycerol; PL, phospholipid; TC, taurocholate; BSA, bovine serum albumin; G3P, glycerol 3-phosphate; MGAT, monoacylglycerol acyltransferase; DGAT, diacylglycerol acyltransferase; AP, apical; BL, basolateral; sn-2–18:1, sn-2-monoolein; 18:1, oleic acid; 16:0, palmitic acid; L-FABP, liver fatty acid-binding protein; I-FABP, intestinal fatty acid-binding protein; ACS, acyl-coenzyme A synthetase; MGL, monoacylglycerol lipase; CM, chylomicron; PTL, pancreatic triacylglycerol lipase; ER, endoplasmic reticulum; LPL, lipoprotein lipase; DMM, Dubecco’s modified Eagle’s medium; LCFA, long-chain fatty acid; TER, transepithelial resistance; TLC, thin layer chromatography; RT, reverse transcriptase; PVDF, polyvinylidene difluoride; AMV, avian myeloblastosis virus; PBS, phosphate-buffered saline.

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have different metabolic fates in the enterocyte. Plasma FFA were primarily oxidized or incorporated into phospholipids in rats and humans (2, 11), whereas FFA absorbed from the intestinal tract were mainly incorporated into TG (2). Studies in Caco-2 cells also showed an increase in the ratio of TG/PL for apically, compared with basolaterally, administered FFA, further suggesting polarity of lipid metabolism at the level of the intestinal cell (12). No studies have addressed the metabolic fate of plasma-derived MG taken up by enterocytes; thus, it is not known whether a similar compartmentation of MG metabolism occurs.

The Caco-2 cell line was used to begin to examine enterocyte MG metabolism. When grown in culture, Caco-2 cells spontaneously develop many functions characteristic of mature villus cells of the small intestinal epithelium, including tight junction formation, lipoprotein secretion across the BL membrane, and the expression of l- and I-FABP (13). Caco-2 cells also have a number of traits that differ from those of native enterocytes, including the synthesis of apoB-100 rather than apoB-48 (14), and lower levels of I-FABP expression (15). In addition, we have shown previously that, although Caco-2 cells have both the G3P and MG pathways of TG synthesis, the levels of monoacylglycerol acyltransferase (MGAT) activity are ~10% of those found in the mature villus cell (16). Despite their limitations, Caco-2 cells allow the study of a polarized enterocyte monolayer, provide separate access to AP and BL surfaces, and are a commonly used model system for studies of intestinal lipid absorption and metabolism.

In the present studies, the metabolic fate of sn-2-MG in the Caco-2 enterocyte was examined using taurocholate (TC)-mixed MG micelles at the AP surface, to mimic the postprandial luminal environment, and albumin-bound MG at the BL surface, to mimic the plasma situation. In addition, the metabolic fates of palmitate and oleate, two main dietary FFA that are the predominant FFA species in serum, were studied, to directly compare the metabolic fates of FFA and MG.

**EXPERIMENTAL PROCEDURES**

**Materials—** Polycarbonate Transwell filter inserts (6.5-mm diameter, 0.4-μm pore) were purchased from Costar Corp. (Cambridge, MA). Tritium-labeled [9,10-3H]triolein (28 Ci/mmol), 9,10-3H-labeled oleic acid (14 Ci/mmol), and 9,10-3H-labeled palmitic acid (56 Ci/mmol) were from PerkinElmer Life Sciences. Unlabeled sn-2-monoolein and sn-1-monoolein were obtained from Sigma (St. Louis, MO). The PVDF Immobilon-P blotting membranes were from Millipore Corp. (Bedford, MA). Dulbecco’s modified Eagle’s medium (DMEM), nonessential amino acids, fetal bovine serum, penicillin, streptomycin, trypsin-EDTA, and poly(T) primers were from Invitrogen. Lipid standards were obtained from Avanti Polar Lipids (Birmingham, AL). Chloroform, methanol, acetic acid, diethyl ether, and hexane were obtained from Fisher Scientific (Pittsburgh, PA). The SV total RNA isolation kit and AMV reverse transcriptase were obtained from Promega (Madison, WI). Oligonucleotide primers were made by Genosys (Woodlands, TX). Vent polymerase was purchased from New England Biolabs Inc. (Beverly, MA). QIAquick Gel extraction kits and QIAprep Spin Miniprep kits were obtained from Qiagen Inc. (Santa Clarita, CA). Zero Blunt II-TOPO PCR cloning vector was from Invitrogen (Carlsbad, CA). Purified human glyceral TG lipase (PTL) and polyclonal antibodies against human pancreatic TG lipase were generous gifts of Dr. M. Lowe (Washington University, St. Louis, MO). Polyclonal antibodies against human l- and I-FABP were kindly provided by Dr. J. Veerkamp (University of Nijmegen, Nijmegen, The Netherlands).

**Cell Culture—** Caco-2 cell cultures were grown in DMEM with 4.5 g/liter glucose, 4 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 1% nonessential amino acids, and 20% fetal bovine serum in a 95% air, 5% CO2 atmosphere at 37 °C. The medium was changed every other day. Cells were plated at a density ≥ 105/cm2 in 75-cm2 flasks, and split with 0.25% trypsin, 1 mM EDTA when they reached 70–90% confluence (12, 17). For experiments, cells were plated at a density of 105/cm2 onto 24-mm polycarbonate Transwell filter inserts with 0.4-μm pores (Costar Corp.) (cells) with 0.4-μm pores (Costar Corp.) for 18 days post-confluence. Transepithelial resistance (TER) measurements were made to ensure tight junction formation, and the monolayers were used for the experiments only when TER was >250 Ω cm2.

*Preparation of Radiolabeled sn-2-Monoolein (sn-2-18:1)—* Because there is no commercially available radiolabeled sn-2-MG, and the isomeric ratios of monoacylglycerols between sn-1 and sn-2 positions is likely to occur by acid, alkali, or heat (18), H-labeled sn-2-monoolein was freshly prepared before each experiment. H-Labeled triolein (specific activity 28 Ci/mmol) that has H-label on the double bond of all acyl chains was used as substrate. [3H]sn-2-Monoolein was prepared by digestion of radiolabeled triolein with pancreatic lipase (type VI) (Sigma), followed by 3% sodium borate coated preparative TLC separation and elution into hexane (18). Recovery was typically 20% (maximum recovery is, theoretically, 33%). As determined by borate-TLC separation, the prepared [3H]sn-2-MG in hexane was stable as sn-2-MG over a 1-month period when stored in brown vials filled with N2 at −20 °C.

*Preparation of Radiolabeled FFA and MG Media—* For apical incubation, radiolabeled palmitic acid (18:0), oleic acid (18:1), and sn-2–18:1 (2) were a 1:1:1 molar concentration of [1-14C]oleic acid (14 Ci/mmol) for each lipid, dried under N2. The dried lipids were then dissolved in ethanol (0.5% volume of the final volume of the solution), and subsequently dispersed in 10 mM TC (typical luminal bile salt level) (19) in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 8 mM NaHPO4, pH 7.4) and incubated for 1 h at 37 °C with 90 rpm shaking. Solutions of 30 μM H-labeled 16:0, 18:1, and sn–2–18:1 complexed to 100 μM BSA (essentially fatty acid-free; Sigma) were made by addition of ethanol (0.5% volume to the final volume of solution) to dried H-lipids, followed by addition of 100 μM BSA (typical plasma albumin level) (20) in PBS, and incubated for 1 h at 37 °C before use. The unbound concentrations of 16:0 and 18:1 in TC micelles were determined using the fluorescent probe ADIFAB (Molecular Probes, Inc., Eugene, OR) (21), and the ultrafiltration method described by Schluhness et al. (22), as described previously (3). The unbound concentration of sn–2–18:1 in TC-mixed micelles was determined only by the ultrafiltration method because ADIFAB does not bind MG. The unbound concentrations of LCFA bound to BSA were calculated from the equilibrium binding constants as reported by Spector et al. (23), and the unbound concentration of sn–2–18:1 bound to BSA was calculated from the binding constant determined by Thumser et al. (5). Uptake media containing unbound concentrations of lipids below their critical micellar concentrations were used for the studies.

*Metabolism Studies—* The metabolic fates of radiolabeled sn–2–18:1, 18:1, and 16:0 were determined in Caco-2 cells as follows. Media were replaced with serum-free DMEM for 15 h. The Caco-2 cell monolayers were then incubated with TC-mixed lipids apically, and with BSA-bound lipids basolaterally, for 5 h at 37 °C in TC micelles. Lipid metabolites was analyzed using two-step TLC separation as described previously (12). Briefly, extracts with added carrier lipids (cholesteryl oleate, triolein (TG), oleate, sn-1,2-dioleoyl (DG), monolein (MG), cholesterol, phosphatidylcholine, phosphatidylinerine, phosphatidylethanolamine, phosphatidic acid, and lysophosphatidylcholine) were spotted on thin-layer chromatography (TLC) plates. Total lipids were extracted using the method of Bligh and Dyer (24), and the radioactivity after lipid extraction was found to be ~80% of the radioactivity prior to lipid extraction. After accounting for extraction efficiency, FFA oxidation was estimated by subtraction of the radioactivity recovered in the lipid extract from that in the cell sonicate, and averaged less than 3%. The incorporation of radiolabeled palmitic acid, oleic acid, and sn–2-monoolein into lipid metabolites was analyzed using two-step TLC separation as described previously (12). Briefly, extracts with added carrier lipids (cholesteryl oleate, triolein (TG), oleate, sn-1,2-dioleoyl (DG), monolein (MG), cholesterol, phosphatidylcholine, phosphatidylinerine, phosphatidylethanolamine, phosphatidic acid, and lysophosphatidylcholine) were spotted under nitrogen gas onto 20 × 20-cm TLC plates (Silica gel G; Whatman, Houns, OR). The plates were developed to half the height of the plate with chloroform-methanol-acetic acid-water (25:15:6:2.1 v/v), allowed to dry under the hood, and then developed up the entire height of the plate in hexane-diethyl ether-acetic acid (70:30:1 v/v). Lipids were visualized with iodine vapor. After allowing the iodine to evaporate under the hood, the area corresponding to each lipid was scraped into 3 ml of Ultrafluor (Fisher Scientific) and radioactivities were counted using a scintillation counter.
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Western Blot to Detect Human Pancreatic TG Lipase (PTL)—Caco-2 cells were harvested and lysed by sonication. The lysed cells were centrifuged at 12,000 × g for 10 min at 4 °C. Total protein in the lysate supernatant was measured according to Bradford (25). The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Blots were probed using 10% gels.

PCR was then carried out using primers designed from mouse MG lipase cDNA (26) as follows: 5′-GGTTA ATTCA ATAGC CACG CTACC TCTTTG-3′ and 5′-GGTTTA CTCTCA TGAGG GACG AGTTG GTCAC-3′. Ten µl of the first-strand mix was then added to 100 µl of PCR buffer containing 10 µl of 10× buffer, 2 µl of 10× dNTP, 1 µl of 100 µM Mg lipase primers, 0.5 µl of 100 mM MgSO4 and 2 µl of Vent polymerase from New England Biolabs Inc. After the initiation incubation of 2 min at 94 °C, 40 cycles of PCR consisting of 1 min at 94 °C, 1 min at 65 °C, and 2 min at 72 °C were performed. PCR was completed with a final 10 min at 72 °C. Amplified DNA was separated through a 1% agarose gel and visualized by ethidium bromide staining. The size of the amplified DNA fragment (800 bp) corresponded to an amplification of cDNA rather than genomic DNA. RT-PCR for human L-FABP served as a positive control for Caco-2 RNA and was performed using the following primers: 5′-GGGCA ATCACT GAGAG CAGCA AAGATGTTTAC-3′ and 5′-TTGCT GATCT TCTTG AAGAC AAAGT GC-3′; for an expected size of 1000 bp. The plasmid DNA was purified by Qiagen Miniprep kit. Sequencing of the plasmid DNA was performed using similar concentrations of unbound lipids at the AP and BL surfaces, determined as previously described (3). The net uptake of [3H]-labeled MG, sn-2–18:1, from both TC-mixed and BSA-bound (BL) surfaces of Caco-2 cells was shown in Fig. 1. The net uptake of lipids was greatest from TC-mixed [3H]lipids at the AP surface. A comparison of BSA-bound lipids shows that net uptake was consistently higher at the AP surface than at the BL surface. Uptake of TC-mixed LCFA was greater than TC-mixed MG, with the highest net uptake observed for TC-mixed 18:1. For BSA-bound lipids, in contrast, 16:0 showed higher net uptake than 18:1. BSA-bound sn-2–18:1 has the lowest net uptake at both AP and BL surfaces. As expected, the net uptake of all lipids increased with time of incubation.

Net Uptake of [3H]sn-2-Monoolein, [3H]Oleic acid, and [3H]Palmitic Acid by Caco-2 Cells—Uptake studies were performed using similar concentrations of unbound lipids at the AP and BL surfaces, determined as previously described (3). The net uptake of [3H]-labeled MG, sn-2–18:1, from both TC-mixed and BSA-bound (BL) surfaces of Caco-2 cells was shown in Fig. 1. The net uptake of lipids was greatest from TC-mixed [3H]lipids at the AP surface. A comparison of BSA-bound lipids shows that net uptake was consistently higher than at the BL surface. Uptake of TC-mixed LCFA was greater than TC-mixed MG, with the highest net uptake observed for TC-mixed 18:1. For BSA-bound lipids, in contrast, 16:0 showed higher net uptake than 18:1. BSA-bound sn-2–18:1 has the lowest net uptake at both AP and BL surfaces. As expected, the net uptake of all lipids increased with time of incubation.

Results

Net Uptake of [3H]sn-2-Monoolein, [3H]Oleic acid, and [3H]Palmitic Acid by Caco-2 Cells—Uptake studies were performed using similar concentrations of unbound lipids at the AP and BL surfaces, determined as previously described (3). The net uptake of [3H]-labeled MG, sn-2–18:1, from both TC-mixed and BSA-bound (BL) surfaces of Caco-2 cells was shown in Fig. 1. The net uptake of lipids was greatest from TC-mixed [3H]lipids at the AP surface. A comparison of BSA-bound lipids shows that net uptake was consistently higher than at the AP surface. Uptake of TC-mixed LCFA was greater than TC-mixed MG, with the highest net uptake observed for TC-mixed 18:1. For BSA-bound lipids, in contrast, 16:0 showed higher net uptake than 18:1. BSA-bound sn-2–18:1 has the lowest net uptake at both AP and BL surfaces. As expected, the net uptake of all lipids increased with time of incubation.

Northern Blot Analysis for MG Lipase Expression—Caco-2 cells were collected at various times from 0 to 16 days after confluence. A Sprague-Dawley rat small intestine was collected, and adipose tissue was also obtained to serve as a positive control. Total RNA was extracted by guanidinium thiocyanate extraction. Cells and tissues were homogenized on ice using a high speed Tekmar Tissuemizer (Tekmar, Inc., Cincinnati, OH). 20 µg of total RNA was loaded onto 1% agarose gel,
of BL BSA-bound \([^{3}H]sn-2–18:1\) at all time points \((p < 0.05)\), whereas AP TC-mixed \([^{3}H]sn-2–18:1\) had significantly lower PL incorporation than those of BL BSA-bound \([^{3}H]sn-2–18:1\) at all time points \((p < 0.05)\). To compare AP and BL incubations using the same vehicle for substrate presentation, the AP absorption of \(sn-2–18:1\) from a BSA solution was also examined. Similar results were observed, with the apical TG:PL ratios higher than basolateral incubations at \(2\) and \(6\) h; however, the magnitude of the AP-BL differences were not as dramatic as those observed when the physiologically relevant substrate solutions were used. For apical incubation of BSA-bound \([^{3}H]sn-2–18:1\), TG incorporation was increased over time whereas PL incorporation remained unchanged; thus, the TG:PL ratios at \(2\) and \(6\) h were intermediate between AP TC and BL BSA uptakes (Fig. 2A).

Table II shows the incorporation of \([^{3}H]18:1\) by Caco-2 monolayers. Incorporation of TG tended to increase with time, whereas incorporation into PL tended to decrease with time of incubation. The fractional incorporation into TG was consistently greater for apical TC incubation, and the incorporation into PL was greater for BL BSA incubation. Thus, as was observed for MG, the TG:PL ratio was greater for AP TC-mixed \([^{3}H]18:1\) incubation compared with BSA-bound \([^{3}H]18:1\) incubation at all time points (Fig. 2B). Nevertheless, the extent of metabolic polarity was not as large as that observed for AP versus BL incubations of MG. Moreover, comparison of AP and BL BSA-18:1 solutions showed a higher TG:PL ratio at \(2\) h but, by \(24\) h, the difference was, if any, reversed.

We also examined the metabolic fate of \([^{3}H]16:0\). In agreement with our previous results (3), a higher TG:PL ratio was found for AP BSA relative to BL BSA incubations at \(2\) and \(6\) h of incubation, with no difference at \(24\) h. For AP TC incubations, the relative TG incorporation was also greater compared with the BL BSA incubations (Table III).

\(sn-2–18:1\) has significantly higher TG \% incorporation \((p < 0.05)\) and significantly lower PL \% incorporation \((p < 0.05)\) than \(16:0\) at all time points, whereas \(sn-2–18:1\) had no difference in TG \% incorporation compared with \(18:1\) at all time points, and no difference in PL \% incorporation compared with \(18:1\) at \(6\) and \(24\) h of incubation (Table I–III). Thus, the TG:PL ratio increased with time for both \(sn-2\)-monoolein and oleic acid, whereas it remained unchanged for palmitic acid. The TG:PL ratios are generally higher for \(sn-2\)-monoolein (Fig. 2, A–C). Oleic acid had a very similar TG:PL profile relative to \(sn-2\)-monoolein. In contrast, TG:PL ratios are close to or below \(1.00\) at all time points for both AP TC-mixed and BSA-bound \([^{3}H]16:0\) at both sides, indicating that the palmitic acid substrate is more evenly distributed between TG and PL synthesis (Table III and Fig. 2C).

We further observed that radiolabel from \(sn-2–18:1\) was recovered in the FFA fraction for all treatments and incubation times (Table I), at \(\sim 10–20\%\) of total lipid incorporation. That this was a cell-mediated phenomenon was confirmed by control cell-free incubations, after which no substrate hydrolysis was detected (data not shown). This result suggested that \(sn-2–18:1\) may be hydrolyzed within the Caco-2 cell, or that the \([^{3}H]sn-2–18:1\) was esterified into DG or TG and then subsequently hydrolyzed to yield \([^{3}H]18:1\).

Detection of MG Lipase mRNA by RT-PCR—\(^{3}H\) activity of \(sn-2\)-monoolein was recovered in the FFA fraction, as noted above, indicating that \(sn-2–18:1\) may be hydrolyzed within the Caco-2 cells. We therefore hypothesized that a MG lipase activity may be a candidate to hydrolyze MG into FFA and glycerol within Caco-2. Recently, a MG lipase was cloned from a mouse adipocyte cDNA library (26). We used RT-PCR (non-quantitative) to detect the presence of MG lipase mRNA, as described under “Experimental Procedures.” The results showed that a single 800-bp band, corresponding to MG lipase, was expressed in Caco-2 cells at days 7, 14, and 21 after confluence. No bands were detected in the day 0 and rat jejunum samples; however, it is likely that amplification for these samples was insufficient, as seen for \(\beta\)-actin (Fig. 3), as the Northern blot results below indicate that MGL is expressed throughout Caco-2 differentiation and at low levels in adult rat small intestine (Fig. 4).

cDNA Cloning and Northern Blot Analysis of Human MGL—The human MGL gene was cloned from day 18 Caco-2 cell RNA as described above. The nucleotide sequence obtained for the 1000-bp clone was 83% identical to the murine MG lipase gene (26), and identical to the human lysophospholipase homologue (27). Fig. 4 shows a Northern blot of Caco-2 mRNA from cells at day 0 (confluence) through day 16, and from small intestine. Rat adipose tissue mRNA served as a positive control, and the expected 4-kb mRNA (26) was observed. The results demonstrate that the MGL gene is expressed at low levels at day 0 and increases \(-2.5\)-fold over the course of Caco-2 differentia-
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Caco-2 cells grown on Tranwell filters for 14–18 days after confluence were incubated at 37 °C with either 30 μM \(^{1}H\)sn-2-monolein complexed to 100 μM BSA (at the AP or at the BL surfaces) or with 2 mM \(^{1}H\)sn-2-monolein mixed with 10 mM TC (at the AP surface) for various times. The cellular metabolites of \(^{1}H\)sn-2-monolein were determined by two-step TLC as described under “Experimental Procedures.”

### DISCUSSION

FFA and sn-2-MG are the products of pancreatic TG lipase hydrolysis of dietary TG in the lumen of the small intestine. FFA metabolism in the enterocyte has been intensively studied (12, 17, 30, 31); however, far less is known about sn-2-MG metabolism. In the present study, the metabolism of exogenous sn-2-monolein, oleic acid, and palmitic acid were examined in the human intestinal Caco-2 cell. Culture on permeable filters allowed for a comparison of AP and BL presentation of lipid substrates (32, 33).

Apical net uptake of both TC-mixed lipids and BSA-bound MG and FFA was found to be higher than BL BSA-bound lipid uptake, under conditions of equal unbound lipid concentration (3). This may be related to several factors, including, perhaps, differences in the plasma membrane composition between the AP and BL domains of the enterocytes (34), such as differences in intramembranous protein levels, phospholipid fatty acyl chain unsaturation, cholesterol:PL ratios, and membrane lipid fluidity (34, 35). The presence and distribution of putative transmembrane transporters might also play a role in the greater AP uptake. Previous results showed that the increased AP relative to BL uptake of FFA is not simply because of greater AP plasma membrane surface area in Caco-2, but is, rather, likely to reflect enterocyte-specific FFA transport and metabolism (12).

For similar unbound concentrations, the net uptake of TC-mixed lipids was substantially greater than that of BSA-bound lipids, in agreement with previous reports. For example, Rahnheim et al. (31) have reported that oleic acid and eicosapentaenoic acid (20:5(n-3)) in TC micellar solution were absorbed more efficiently into Caco-2 cells than when bound to BSA. Levin et al. (30) reported similar effects for TC-mixed versus BSA-bound oleic acid in Caco-2 cells. It is generally accepted that micellar solubilization increases uptake of LCFA by increasing their aqueous concentration gradients across the unstirred water layer (1). We were concerned, however, that because bile salts act as detergents when the concentration exceeds the critical micellar concentration, they may have deleterious effects on cell membranes and/or intercellular tight

### TABLE I

| Lipid class | Percentage of total incorporation |
|-------------|---------------------------------|
|             | 2 h                             | 6 h                             | 24 h                            |
|             | TO(AP)  BSA(BL)  BSA(AP)        | TO(AP)  BSA(BL)  BSA(AP)        | TO(AP)  BSA(BL)  BSA(AP)        |
| CE         | 4 ± 1  0.4 ± 0.1  0.1 ± 0.03     | 1.0 ± 0.3                       | 2.0 ± 1.1                       |
| TG         | 37 ± 3  20 ± 6\(^b\)  32 ± 6     | 57 ± 8                          | 34 ± 6\(^b\)                     |
| FA         | 21 ± 3  19 ± 7  21 ± 5          | 23 ± 3                          | 19 ± 3                          |
| DG/C       | 10 ± 7  9 ± 1  10 ± 3           | 4 ± 2                           | 10 ± 5                          |
| MG         | 10 ± 2  12 ± 5  11 ± 2          | 5 ± 1                           | 6 ± 2                           |
| PL         | 18 ± 4  40 ± 4\(^b\)  26 ± 6     | 10 ± 3                          | 29 ± 3\(^b\)                     |
| TG/PL      | 2 ± 0.5  0.5 ± 0.2\(^b\)  1 ± 0.5 | 6 ± 3                           | 1 ± 0.3\(^b\)                   |
| **Mean**   | **2 ± 0.3**  **2 ± 0.3**\(^b\)  **1 ± 0.3** | **3 ± 1**                       |

\(^a\) Mean percentage of total radioactive lipids incorporated into metabolite ± S.D. (n = 3).

\(^b\) p < 0.05 vs. TC(AP).

\(^c\) p < 0.05 vs. BSA(BL).

Western Blot for Human PTL—Tsujita et al. (28) and Mahan et al. (29) have reported that PTL is expressed in the absorptive cells of the rat intestine. Because PTL may, therefore, represent an alternative mechanism whereby exogenous MG could be metabolized independent of the MGAT pathway, the expression of human PTL in Caco-2 was analyzed by Western blot to determine whether these cells express a protein immunoreactive to a polyclonal anti-human PTL antibody. Fig. 5 shows that this antibody does react with purified human PTL with molecular mass of 49 kDa. However, Caco-2 homogenates as well as rat jejunal mucosa homogenates showed no detectable level of human PTL. The expression of L-FABP and I-FABP from the same homogenates served as positive controls (Fig. 5).

**Fig. 2.** TG:PL ratio for \(^{1}H\)sn-2-monolein (A) oleic acid (B), and palmitic acid (C). Caco-2 monolayers were incubated with 2 mM lipids complexed with 10 mM TC at the apical surface (TC(AP)) or at the AP surface (open bar or at the BL surface (BSA, BL) (closed bar). Lipids were extracted from the cell sonicates, and metabolites were analyzed as described under “Experimental Procedures.” Data represent the mean ± S.D. from three experiments. b, p < 0.05 versus TC(AP); c, p < 0.05 versus BSA(BL).
Monoacylglycerol Metabolism in Human Intestinal Caco-2 Cells

Table II
Incorporation of $[^3H]$oleate by Caco-2 monolayers

| Lipid class | 2 h | 6 h | 24 h |
|-------------|-----|-----|-----|
|             | TC(AP) | BSA(BL) | BSA(AP) | TC(AP) | BSA(BL) | BSA(AP) | TC(AP) | BSA(BL) | BSA(AP) |
| CE          | 5 ± 3  | 1.0 ± 0.1 | 2.4 ± 0.5 | 1.0 ± 0.2 | 1.1 ± 0.5 | 1.0 ± 0.2 | 0.3 ± 0.1 | 2.0 ± 1.0 | 1.0 ± 0.3 |
| TG          | 23 ± 9 | 15 ± 5  | 31 ± 5   | 55 ± 2   | 26 ± 6b | 25 ± 1b | 67 ± 6 | 60 ± 9 | 53 ± 13 |
| FA          | 14 ± 7 | 13 ± 2  | 13 ± 2   | 21 ± 2   | 16 ± 1  | 16 ± 3  | 15 ± 3 | 10 ± 2  | 14 ± 6  | 13 ± 7 |
| DG/C        | 11 ± 7 | 13 ± 3  | 9 ± 2    | 4 ± 2    | 13 ± 4  | 7 ± 2   | 10 ± 5 | 2 ± 1   | 3 ± 2   |
| MG          | 13 ± 2 | 14 ± 1  | 13 ± 2   | 5 ± 2    | 9 ± 0.7 | 9 ± 4   | 3 ± 2 | 6 ± 0.5 | 3 ± 1   |
| PL          | 34 ± 5 | 44 ± 2  | 32 ± 1   | 14 ± 2   | 35 ± 8b | 43 ± 1b | 10 ± 1 | 16 ± 3  | 27 ± 8   |
| TG:PL       | 0.7 ± 0.3 | 0.3 ± 0.2 | 1 ± 0.2 | 4 ± 0.2 | 0.7 ± 0.4b | 0.6 ± 0.1b | 7 ± 2 | 4 ± 1   | 2 ± 1b   |

$a$ Mean percentage of total radioactive lipids incorporated into metabolite ± S.D. (n = 3).

$\text{b}p < 0.05$ vs. TC(AP).

$\text{c}p < 0.05$ vs. BSA(BL).

Table III
Incorporation of $[^3H]$palmitate by Caco-2 monolayers

| Lipid class | 2 h | 6 h | 24 h |
|-------------|-----|-----|-----|
|             | TC(AP) | BSA(BL) | BSA(AP) | TC(AP) | BSA(BL) | BSA(AP) | TC(AP) | BSA(BL) | BSA(AP) |
| CE          | 3.7 ± 2.7 | 0.3 ± 0.2 | 2.0 ± 1.2 | 0.3 ± 0.1 | 1.0 ± 0.5 | 1.3 ± 0.3 | 1.0 ± 0.4 | 1.0 ± 0.5 | 0.7 ± 0.5 |
| TG          | 14 ± 10 | 10 ± 6  | 32 ± 1b  | 26 ± 1 | 15 ± 0.1 | 22 ± 2 | 28 ± 9 | 45 ± 10b | 45 ± 2b |
| FA          | 9 ± 0.2 | 15 ± 0.8 | 9 ± 4    | 16 ± 3  | 14 ± 1 | 14 ± 3 | 11 ± 4 | 9 ± 2 | 9 ± 2 |
| DG/C        | 17 ± 2 | 14 ± 4  | 11 ± 1   | 17 ± 7  | 10 ± 1 | 9 ± 1 | 16 ± 3 | 6 ± 4 | 5 ± 3 |
| MG          | 13 ± 0.9 | 20 ± 5  | 14 ± 1   | 9 ± 0.4 | 14 ± 1 | 10 ± 1 | 10 ± 3 | 7 ± 2 | 6 ± 3 |
| PL          | 45 ± 5 | 41 ± 6  | 32 ± 3   | 33 ± 5 | 46 ± 1 | 44 ± 3 | 34 ± 7 | 32 ± 1 | 34 ± 6 |
| TG:PL       | 0.3 ± 0.1 | 0.2 ± 0.6 | 1 ± 0.1b | 0.3 ± 0.01b | 0.3 ± 0.01b | 0.5 ± 0.1 | 0.8 ± 0.3 | 1 ± 0.7 | 1 ± 0.5 |

$a$ Mean percentage of total radioactive lipids incorporated into metabolite ± S.D. (n = 3).

$\text{b}p < 0.05$ vs. TC(AP).

$\text{c}p < 0.05$ vs. BSA(BL).

FIG. 3. RT-PCR to detect gene expression of MG lipase MG lipase mRNA was evaluated by RT-PCR as described under “Experimental Procedures.” Lane 1, Caco-2 cells, day 0 (confluence); lane 2, Caco-2 cells, day 21; lane 3, rat jejunal mucosa; lane 6, rat adipose tissue. The band for MG lipase is 800 bp. The positive controls are human L-FABP (400 bp) and β-actin (300 bp).

FIG. 4. Northern blots for human monoacylglycerol lipase in Caco-2 cells. 20 μg of total RNA was loaded in each lane. The 1000-bp MGL sequence cloned from Caco-2 intestinal cell cDNA, as described under “Experimental Procedures,” was used as the probe. Lanes 1–6, Caco-2 cells, days 0, 2, 4, 8, 12, and 16 after confluence; lane 7, rat intestine; lane 8, rat adipose tissue.

The damage of cell membranes by TC, but rather is presumably representative of physiologically relevant differences secondary to substrate presentation.

In the present study, AP TC-mixed sn-2–18:1 was metabolized differently from BL BSA-bound sn-2–18:1 at all time points examined. TG was the primary metabolite of AP TC-mixed sn-2–18:1, whereas PL was the major metabolite of BL BSA-bound sn-2–18:1. Using human jejunal biopsy samples, Gangl and Renner (11) showed that plasma FFA were preferentially incorporated into PL or oxidized. Although we found little or no oxidation, in agreement with previous results for Caco-2 (3), the present study also showed that radioactivities from BL incubated FFA were primarily incorporated into the PL fraction.

The basic mechanisms underlying the cellular polarity of enterocyte lipid metabolism are not known, but several may be envisioned. As mentioned earlier, the small intestine can syn-
the expression of I-FABP and L-FABP within the enterocyte as a
and MG. Finally, one must also consider the simultaneous
(9), whereas the G3P pathway is localized to the rough ER (39),
smooth ER, and this is the main site of chylomicron assembly
shown that the MG pathway is primarily associated with
(37). Thus, the ACSs could play a role in partitioning of FFA
thesize TG via both the G3P and the CoA-dependent MG path-
ways of acylglycerol synthesis (7, 8). The final reaction in both
MG and G3P pathways is the conversion of DG to TG via DGAT
(7). It is not yet clear whether the DGAT activities in the MG
pathway and G3P pathway are distinct, nor whether intestinal
DGAT might be involved in the metabolic compartmentation of
AP and BL lipids. Interestingly, Owens et al. (36) reported the
presence of two pools of DGAT in liver microsomes, cytosol-
facing and “latent” (ER lumen), and a second DGAT gene has,
in fact, recently been described (10).
Compartmentation of FFA and MG metabolism may also be
related to the presence of multiple acyl-coenzyme A synthetase
(ACS) genes. ACS5 is highly expressed in the small intestine
(37), and ACS1, with widespread tissue distribution, is also
found (38). ACS5 is thought to be involved in PL synthesis and
FFA β-oxidation, and ACS-1 may be linked to TG synthesis
(37). Thus, the ACSs could play a role in partitioning of FFA
between anabolic and catabolic pathways. It has also been
shown that the MG pathway is primarily associated with
smooth ER, and this is the main site of chylomicron assembly
(9), whereas the G3P pathway is localized to the rough ER (39),
affording another possible origin of metabolic polarity of FFA
and MG. Finally, one must also consider the simultaneous
expression of I-FABP and L-FABP within the enterocyte as a
potential source of metabolic compartmentation for their li-
gands, particularly as they are now thought to be involved in
ligand targeting (40).
Palmitic acid was not as efficiently incorporated into the TG
fraction relative to PL as was oleic acid, resulting in a lower
TG:PL ratio for palmitic acid. This is in agreement with previ-
ous studies, which also demonstrated lower incorporation of
16:0 into TG (2, 12). The significantly higher PL synthesis
relative to TG formation for 16:0 may reflect different effects in
the intestinal incorporation of saturated FFA relative to un-
saturated FFA. It has been reported that saturated FFA ester-
fication was significantly less rapid than unsaturated FFA
(linoieic acid) esterification by rat intestinal mucosa (41).
In addition, van Greevenbroek et al. (42) have shown that
saturated palmitic acid was not efficiently incorporated into TG
compared with unsaturated linoleic acid in Caco-2 cells.
The intracellular metabolism of MG showed consistent com-
partmentation into FFA. One possible mechanism is that some
of the MG that is esterified into TG is then hydrolyzed to FFA.
We have shown previously that MGAT activity is present in
Caco-2, albeit at a lower level than in rat jejunum (16). Pre-
sumably, the incorporation of labeled MG into TG is proceeding
via the MGAT pathway. Recently, however, another potential
mechanism for intestinal TG formation was proposed. Tsujita
et al. suggested that PTL may facilitate a CoA-independent
pathway of DG synthesis from MG and FFA in rat intestinal
mucosa, by showing that a CoA-independent MGAT activity
was inhibited up to 65% by addition of the antibodies against
PTL (28). In the present studies, we were unable to detect a
protein immunoreactive to polyclonal anti-human PTL anti-
odies either in Caco-2 cells or in rat jejunal mucosa homoge-
nates. The reason for these discrepant results are not clear;
however, others have also not found PTL in intestine.2
Another mechanism by which 3H activity of sn-2-MG could
appear in the TG as well as the FFA fractions would be via its
hydrolysis and subsequent re-esterification within the Caco-2
cell. Yang and Kuksis (43) have shown that hydrolysis of G3P-
derived TG and resynthesis into TG via the CoA-dependent MG
pathway does appear to occur, possibly by a lysosomal acid
lipase, accounting for ~15% of total CM-TG collected from rats.
In addition, an alkaline lipase activity was found in the rat
intestinal mucosa, with optimal activity using a radioactive
triolein emulsion, requiring both Ca2+ and TC (44). Further,
it has been reported that Caco-2 cells possess an endogenous
lipase activity that is capable of hydrolyzing intracellular TG,
and this lipase activity was shown not to be LPL (45). Thus,
it is possible that these lipase activities, if present in the entero-
cyte, could also, perhaps, hydrolyze MG. A MG hydrolytic
activity (EC 3.1.1.23) was, in fact, partially purified from isolated
rat small intestinal epithelial cells by De Jong et al. in 1978
(46); however, no further reports have appeared. Recently,
a MG lipase was cloned from a mouse adipocyte cDNA library
(26). Using oligonucleotides primers designed from the mouse
MG lipase sequence, we found that Caco-2 cells express MG
lipase. Cloning of the human MG lipase gene from day 18
Caco-2 cells showed that it is 83% identical to the murine gene
(26), and identical to the previously designated but as yet
uncharacterized lysosphospholipase homologue (27).
Northern analysis indicates that the MGL gene is expressed
during early Caco-2 differentiation, and increases to a modest
extent as cells develop the enterocyte-like phenotype. The re-
results show that MGL is expressed at low levels in adult rat
intestine, and preliminary data indicate that higher levels of
expression are present during prenatal and early postnatal age
(data not shown). Caco-2 cells still possess some characteris-
tics similar to fetal cells or crypt cells of the colon (47), and our
previous studies of lipid metabolism during Caco-2 differenti-
tion also suggest that the differentiated phenotype in Caco-2
may be representative of early stages in the enterocyte devel-
oment program (16). It is not known, at present, whether the
MG lipase activity reported by De Jong et al (46) comes
solely from the MGL gene presently detected, or whether more
than one intestinal lipolytic activity for monoacylglycerols may
be present. The presence of MGL expression in Caco-2 cells
offers the opportunity to examine the functional role of MG
hydrolysis in the enterocyte, and the potential for coordinate
and, perhaps, inverse regulation of MG hydrolysis via MGL,
and MG anabolism via monoacylglycerol acyltransferase.
Monoacylglycerol Metabolism in Human Intestinal Caco-2 Cells: EVIDENCE FOR METABOLIC COMPARTMENTATION AND HYDROLYSIS
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