Regulation of MTP expression in developing swine

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Abstract **To define the developmental expression of microsomal triglyceride transfer protein (MTP) large subunit mRNA and protein, samples of small intestine and liver were collected from 40-day gestation fetal, 2-day-old newborn, 3-week-old suckling, and 2-month-old weanling swine. In fetal animals, MTP mRNA expression was high in intestine and liver. Postnatally, jejunal expression paralleled the intake of a high-fat breast milk diet and declined after weaning. Ileal expression was comparable with that of jejunum in 2-day-old animals, but declined to low levels afterward. Hepatic expression declined postnatally and remained low. MTP protein expression generally paralleled mRNA expression, except in fetal intestine in which no 97 kDa protein was detected. In 2-day-old piglets, a high-triacylglycerol diet increased jejunal and ileal MTP mRNA levels, as compared to a low-triacylglycerol diet. To test the roles of glucocorticoids and fatty acids in MTP regulation, a newborn swine enterocyte cell line (IPEC-1) was used. Except at day 2 of differentiation, dexamethasone did not influence MTP expression. Fatty acids either up-regulated or down-regulated MTP expression, depending on the specific fatty acid and duration of exposure. Although programmed genetic cues regulate MTP expression during development, clearly the amount and fatty acid composition of dietary lipid also play regulatory roles.**—Lu, S., M. Huffman, Y. Yao, C. M. Mansbach II, X. Cheng, S. Meng, and D. D. Black. **Regulation of MTP expression in developing swine.** ***J. Lipid Res.*** **2002.** 43: **1303–1311.**

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Microsomal triglyceride transfer protein (MTP), a heterodimeric protein complex possessing lipid transfer activity, has recently been found to function in the small intestine and liver to transport endoplasmic reticulum (ER) membrane-bound lipid, primarily newly synthesized triacylglycerol, to newly translated apolipoproteinB (apoB) in the ER lumen as the first step in triacylglycerol-rich lipoprotein biogenesis (1, 2). In the small intestine, MTP may also facilitate the further lipidation of nascent chylomicrons beyond the first apoB rescue step (3). MTP is a heterodimer consisting of a large subunit (97 kDa), which possesses the lipid transfer activity, and a smaller subunit identical to protein disulfide isomerase (PDI, 55 kDa) (2). Normally, PDI functions in the ER to pair cysteine residues of newly synthesized proteins to form disulfide bonds and has chaperone activity. However, as a component of MTP, PDI appears to maintain the large subunit in soluble form and may play a role in targeting MTP to the ER lumen.

In vivo regulation of MTP expression in liver and small intestine has been studied to a limited extent in adult animal models (hamsters and rats) (2, 4). From these studies, it appears that MTP is regulated by lipid and insulin with some differences in response in the liver and intestine. However, because changes in protein levels do not parallel acute changes in gene expression in these adult animal studies, it is thought that these changes are more related to a chronic adaptation process, rather than a mechanism for acute regulation of hepatic or intestinal lipoprotein production (2). To date, there is a paucity of information on the developmental expression of MTP, with a published study of MTP large subunit expression in the fetal mouse using in situ hybridization (5), another in primary cultures of suckling rat hepatocytes (6), and one study in early-gestation human fetal small intestine (7). Such regulation may have important implications for lipid absorption and metabolism in the neonate (8), because the neonatal mammal is dependent on breast milk with approximately half of total calories derived from lipids. Therefore, the ability to efficiently absorb dietary fat is crucial.

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Abbreviations: ER, endoplasmic reticulum; GM, growth medium; MTP, microsomal triglyceride transfer protein; PDI, protein disulfide isomerase; RACE, rapid amplification of cDNA ends; RQ1, RNA Qualified 1.

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One reason for the paucity of data in this area has been the lack of suitable in vitro and in vivo model systems. Over the past several years, our laboratory has focused on the neonatal swine as a model system for studies of the regulation of intestinal and hepatic apolipoprotein expression and lipid synthesis by dietary lipids during development (8–17). This model offers several advantages for such studies. Intestinal development in the precocial newborn swine is very similar to that of the human infant (18, 19), and there is significant homology with human apolipoprotein and lipoprotein metabolism (9, 10, 20).

In the present study, we have cloned and sequenced the swine MTP large subunit cDNA and generated antibodies to a peptide derived from a portion of this sequence. Using these reagents and our model systems, we have quantitated hepatic and intestinal MTP large subunit mRNA and protein expression in fetal, suckling, and weanling swine. We have also characterized expression in a newborn swine intestinal epithelial cell line cultured with and without dexamethasone and in cells after incubation with long-chain saturated, monounsaturated, and polyunsaturated fatty acids.

MATERIALS AND METHODS

Animals

To obtain intestine and liver from fetal animals, a 40-day gestation (the normal swine gestational period is 114 days) pregnant sow was prepared with general anesthesia. A transverse lower abdominal incision was made, and the bicornuate uterus was exposed. Starting distally and moving toward the uterine bifurcation, each fetus was isolated and exposed. The umbilical cord was tied off and the fetus was removed. Liver and small intestine were harvested and snap frozen in liquid N_2 for later RNA isolation and preparation of tissue homogenates.

Postnatal female swine were obtained from Tyson Farms, Plummerville, AR, and used for intestine and liver harvest on the day of arrival. Two-day-old newborn, 3-week-old suckling, and 2-month-old weanling animals were fasted for 4 to 6 h from their last regular breast milk or chow feeding, followed by euthanasia by intravenous pentobarbital overdose. The abdominal cavity was quickly opened for harvest of intestine and liver samples. Samples of jejunum and ileum were taken 5 cm distal to the ligament of Treitz and 5 cm proximal from the ileocecal valve, respectively, for RNA extraction and protein homogenate preparation. A wedge of the anterior right lobe of the liver was similarly sampled.

To study the short-term (24 h) physiologic regulation of MTP large subunit expression by dietary lipid in newborn swine small intestine and liver, 2-day-old female swine were surgically fitted with duodenal catheters exiting through dorsal swivel tethers as previously described (11). Animals were allowed to recover for 24 h while receiving a glucose-saline (5% glucose in 45 mM NaCl and 20 mM KCl) duodenal infusion at 100 ml/kg/24 h, followed by infusion as previously described of either dilute Vivonex® (Norwich Eaton Pharmaceuticals, Inc., Norwich, NY), a low-fat elemental formula, or Intralipid® (Cutter, Berkeley, CA), a lipid emulsion containing primarily 18-carbon unsaturated fatty acids. Infusions were isocaloric and at a rate of 50 kcal/kg/24 h (11). At the end of the 24 h infusion, the animals were euthanized, and intestinal and liver samples were taken as described above.

Materials

[1,2,3-^{3}H]glycerol (0.2 Ci/mmol) was purchased from DuPont New England Nuclear (Boston, MA). Stearic acid (C18:0, SA), eicosapentaenoic acid (C20:5n-3, EPA), oleic acid (C18:1n-9, OA), essentially fatty acid-free BSA, Triton X-100, phenylmethylsulfonyl fluoride (PMSF), and benzamidine were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell culture

The derivation of the IPEC-1 cell line has been described previously (21). Cells from passages 25 to 80 were used in these studies, and all cell culture was carried out at 37°C in an atmosphere containing 5% CO_2. Undifferentiated IPEC-1 cells were maintained in serial passage in plastic culture flasks (75 cm^2, Corning Glassworks, Corning, NY) in growth medium (GM) [DMEM/F12 medium (Gibco-BRL, Grand Island, NY) supplemented with 5% FBS (Gibco-BRL), insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml) (ITS Premix®, BD Biosciences, Bedford, MA), epidermal growth factor (5 μg/l) (BD Biosciences), penicillin (50 μg/ml), and streptomycin (4 μg/ml) (Gibco-BRL)]. To induce differentiation, undifferentiated cells were harvested by trypsinization, and 2×10^6 cells/well were plated on 75 mm diameter collagen-coated filters (3.0 μm pore size) in Transwell® culture plates (Costar, Corning, Inc., Corning, NY). Cells were maintained in serum-containing GM containing 10^{-7} M dexamethasone (Sigma) for 48 h and then switched to the same medium containing 10^{-7} M dexamethasone, but without FBS. Medium was then changed every 2 days. In experiments testing the effect of dexamethasone on MTP expression, dexamethasone was left out of the medium. We have previously shown that after 10 days, IPEC-1 cells exhibit enterocytic features, including polarization with well-defined microvilli facing the apical medium (21). Cellular membrane integrity was assessed by measurement of apical medium lactate dehydrogenase (Sigma) activity.

Incubation of cells with fatty acids

At 10 days post-plating on Transwell filters in serum-free medium, fresh serum-free medium was added to both the apical and basolateral compartments. The apical medium contained fatty acid complexed with albumin (4:1 molar ratio) at a concentration of 0.8 mM (22). This fatty acid concentration is in the physiologic range, and above this concentration the basolateral secretion of triacylglycerol begins to plateau in IPEC-1 cells (21). Cells were incubated for 6 h to 24 h, followed by harvest of cells. Cell homogenates for Western blotting analysis and total cellular RNA for RT-PCR were prepared as described (21) and stored at −80°C. In lipid radiolabeling experiments in maximally differentiated cells, [^{3}H]glycerol (12 μCi/well) was also added to the apical medium, concomitant with the addition of oleic acid/albumin. After incubation, basolateral culture medium samples were stored at −80°C.

Triacylglycerol and phospholipid radiolabeling with [^{3}H]glycerol

Cells were incubated for 24 h with [^{3}H]glycerol and oleic acid complexed with albumin, and medium was collected and processed as described above. Total lipid in the medium was extracted as previously described (23). Extracts were applied to silica gel G plates and subjected to TLC using petroleum ether-diethyl ether-acetic acid 80:20:1 (v/v/v). Lipid bands were identified by exposure to iodine vapor and scraped off the plate for liquid scintillation counting. Bands corresponding to phospholipid and triacylglycerol were identified by comparison to cochromatographed standards. Secretion of radiolabeled lipid was expressed as specific lipid dpm/well/24 h.

Swine MTP large subunit cDNA

Total RNA was isolated from 2-day-old piglet jejunal enterocytes. 5′ and 3′ rapid amplification of cDNA ends (RACE) PCR was conducted using the SMART RACE® cDNA amplification kit (Clontech, Palo Alto, CA). Oligonucleotides 5′-AATGATGGCGGCAACCTGCTTCC-3′ and 5′-TGACCTACCAGGCTCATCAAGAC-3′ were used for 5′ and 3′ RACE PCR, respectively. The complete MTP large subunit mRNA sequence was generated by end-to-end PCR.

Analysis of MTP large subunit mRNA by semi-quantitative RT-PCR

Total RNA was extracted from piglet intestinal and liver samples and IPEC-1 cells (24). Aliquots (2 μg to10 μg) were treated with 0.5 units of RNA qualified 1 (RQ1) DNase (Promega, Madison, WI) at 37°C for 60 min in 50 μl 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 10 mM NaCl, 10 mM dithiothreitol (DTT), 20 units RNase inhibitor (RNAsin, Promega). The RNA was then sequentially extracted with phenol-chloroform and chloroform, precipitated with ethanol, washed once (with 70% ethanol), and resuspended in 20 to 40 μl H_2O. For reverse transcription, 5 μg total RNA was used. Reverse transcription was performed at 42°C for 15 min in a final volume of 20 μl in buffer containing 10 mM Tris-HCl (pH 8.3), 90 mM KCl, 1 mM $MnCl_2$, 200 μM of each deoxynucleotide triphosphate (dNTP), 0.5 μg oligod$T_{(15)}$ as primer, and 15 units Avian Myeloblastosis virus (AMV) reverse transcriptase (Promega). Following reverse transcription, the single-strand cDNA was amplified using the Qiagen Taq PCR core kit (Qiagen, Santa Clarita, CA) with 1 μl cDNA and 100 pmol of each specific primer (MTP527 and MTP862 for MTP large subunit, Actin F and Actin R for β-actin, and B2MGF and B2MGR for β_2-microglobulin) in a total volume of 50 μl. After incubation for 3 min at 95°C, PCR was performed for 21 cycles for β_2-microglobulin, 23 cycles for β-actin, and 25 cycles for MTP large subunit in a thermal cycler (Perkin-Elmer, Boston, MA) as follows: 60 s at 94°C, 10 s at 94°C, 15 s at 58°C, and 45 s at 72°C. For each RNA sample, a negative control was run to check for DNA contamination using AmpliTaq (Perkin-Elmer), leaving the sample on ice during reverse transcription. Additionally, each reaction contained a tube with all the above buffers and enzymes but without RNA to exclude PCR product contamination. The optimal number of PCR cycles for each set of primers was established by constructing curves of number of cycles versus PCR product band density in agarose gels. Cycle numbers were selected in the linear portion of the curves. After RT-PCR, 40% of the reaction products were subjected to 1.5% agarose electrophoresis. Expected product sizes were confirmed as follows: MTP large subunit, 336 bp; β-actin, 475 bp; β_2-microglobulin, 172 bp. Agarose gels containing PCR products were imaged on the Gel-Doc 2000 (Bio-Rad, Hercules, CA), followed by digital densitometric quantitation using National Institutes of Health (NIH, Bethesda, MD) Image software. Results were expressed as a ratio of MTP large subunit to β_2-microglobulin (animals and cells) or β-actin (neonatal animals receiving intraduodenal infusions) arbitrary densitometric units.

Oligonucleotides

MTP527: 5′-TGACCTACCAGGCTCATCAA-3′ (sense, nt 527–547), MTP862: 5′-GGATGGCCGTGTACTTAGAA-3′ (antisense, nt 862–842), ActinF: 5′-GACCTGACCGACTACCTCAT-3′ (sense, nt 184–203), ActinR: 5′-CGATCCACACCGGAGTACTTG-3′ (antisense, nt 658–639), PMG2BR: 5′-TGC CGG TTA GTG GTC TCG AT-3′ (sense, nt 344–363), PMG2BF: 5′-GAA GAT GAA CGC GGA GCA GT-3′ (antisense, nt 211–192)

Quantitation of MTP large subunit protein by Western blot analysis

Rabbit anti-swine MTP large subunit antibody was generated using a synthetic polypeptide [N-YSASVKGHTTGLSL-C] (**Fig. 1B**) by Alpha Diagnostic, San Antonio, TX. This sequence was selected after analysis of the full-length swine cDNA sequence to select a region predicted to code a unique, accessible epitope (Alpha Diagnostic). The generated antiserum was used directly as a 1:1000 (v/v) dilution for Western blot analysis of MTP large subunit protein.

Swine tissue (30 mg to 50 mg) or IPEC-1 cells from Transwell filters were lysed in 0.5 ml of radioimmunoprecipitation buffer with protease inhibitors for whole-cell extraction. Twenty micrograms of lysate was electrophoresed on an 8% SDS-PAGE gel, followed by transfer to nitrocellulose filters. Western blotting was conducted using the enhanced chemiluminescence Western blot kit according to their protocol (Amersham Pharmacia Biotech, Piscataway, NJ). Bands corresponding to MTP large subunit protein were subjected to scanning densitometry (Bio-Rad), followed by densitometric quantitation using NIH Image software. Competition experiments were performed using fetal liver and intestine samples incubated with antibody and increasing amounts of MTP peptide.

Alkaline phosphatase measurement

IPEC-1 cells were lysed with 200 μl of mammalian protein extraction reagent (Pierce, Rockford, IL). Cell lysates were centrifuged, and supernatants were stored at −20°C until ready for assay. Alkaline phosphatase activity was measured using the Great EscAPe® SEAP kit (Clontech). Activity was expressed as fluorescent units × 10^3/μg cell protein.

Protein measurement

Cell homogenate protein was determined by the Bradford method (25).

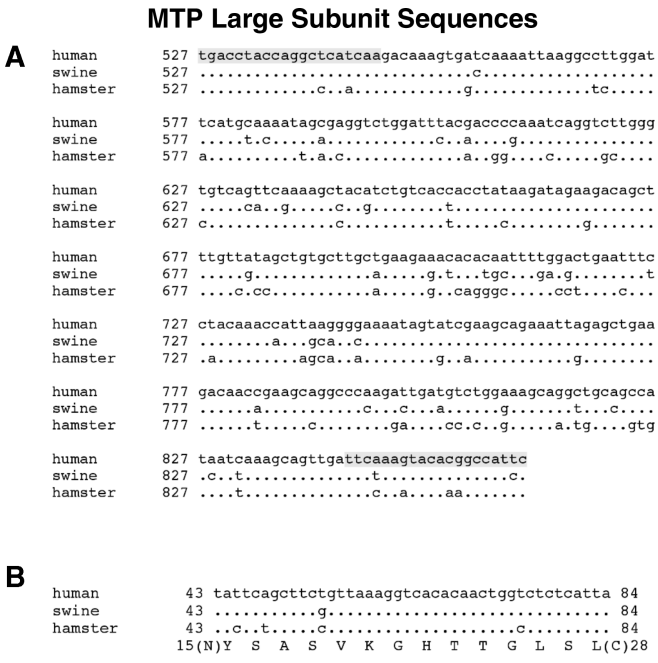

Fig. 1. Microsomal triglyceride transfer protein (MTP) large subunit sequences. A: Partial cDNA sequence of swine MTP large subunit used to generate PCR products for semi-quantitative RT-PCR. Nucleotides are numbered from the first nucleotide of the complete coding sequence. The corresponding human and hamster sequences are also shown for comparison. Sequences used to generate PCR primers are highlighted. B: The cDNA sequence used to generate the peptide used to produce rabbit anti-swine MTP large subunit polyclonal antibodies. Nucleotides are numbered from the first nucleotide of the complete coding sequence. The amino acid residues are numbered from the first N-terminal amino acid.

Statistical analysis

Data in experimental groups were analyzed by one-way ANOVA, followed by the Fisher least significant difference test to compare specific groups. Statistical significance was set at a two-tailed P value of <0.05.

RESULTS

Swine MTP large subunit cDNA sequence

Figure 1A shows the partial cDNA sequence of swine MTP large subunit used to generate PCR products for semi-quantitative RT-PCR. Nucleotides are numbered from the first nucleotide of the complete coding sequence. The corresponding human and hamster sequences are also shown for comparison. Sequences used to generate PCR primers are highlighted. Figure 1B shows the sequence used to generate the peptide used to produce rabbit anti-swine MTP large subunit polyclonal antibodies. The protein sequence coded by the region demonstrated 100% amino acid homology with the corresponding human and hamster sequences (4, 26).

MTP large subunit mRNA and protein expression in developing swine liver and small intestine

Figure 2 shows MTP large subunit mRNA levels in jejunum, ileum, and liver of fetal, newborn, suckling, and weanling swine, as measured by RT-PCR. In fetal animals, MTP mRNA expression was high in intestine and liver. Postnatally, jejunal expression paralleled the intake of a high-fat breast milk diet and declined after weaning. Ileal expression was comparable with that of jejunum in 2-day-old animals, but declined to low levels afterward. Hepatic expression declined postnatally and remained low.

MTP protein expression, as analyzed by Western blotting, in piglet intestine and liver generally paralleled mRNA expression in postnatal animals (**Fig. 3**). Although MTP large subunit protein expression in fetal liver paralleled mRNA expression, in fetal intestine, no band was detectable of the appropriate size for MTP large subunit (approximately 97 kDa) (**Fig. 4A**). Instead, a prominent band of lower apparent molecular weight (approximately 85 kDa) was detected. To determine whether this band might represent a lower-molecular-weight form of MTP large subunit, Western blot analysis of the fetal hepatic and intestinal samples was repeated with the addition of increasing amounts of the peptide used to generate the antibody. In the Western blots shown in Fig. 4B, the 97 kDa band in fetal liver was easily competed out with added peptide. However, in fetal intestine, the 85 kDa band was not competed out with increasing amounts of added peptide. We suspect that this band represents a different protein with partial sequence homology to the region of MTP large

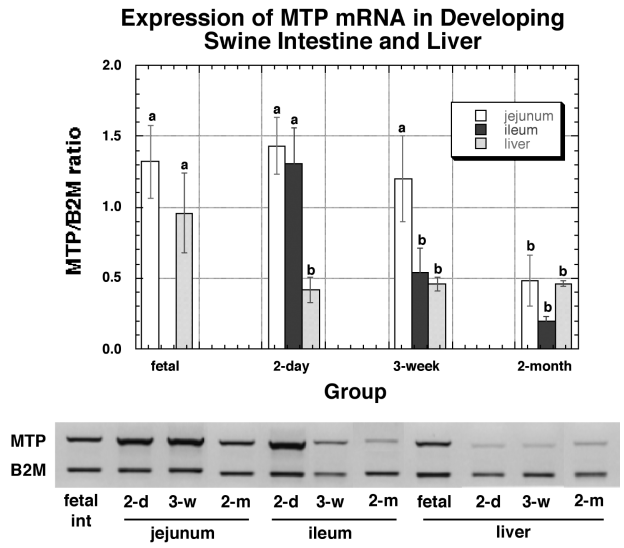

Fig. 2. MTP large subunit mRNA in developing swine intestine and liver. The graph shows jejunal, ileal and hepatic MTP large subunit mRNA levels expressed as MTP large subunit to β_2-microglobulin (B2M) ratios (y axis). Piglet developmental groups are indicated on the x axis. Bars represent the means of data from 3 to 4 animals ±SEM. Note that in fetal animals, the RNA from the entire small intestine (jejunum + ileum) was used for analysis. By ANOVA, values for developmental groups for each organ were significantly different ($P < 0.05$). By post hoc analysis, bars sharing a letter are not different, and those not sharing a letter are significantly different ($P < 0.05$). A 1.5% agarose gel containing RT-PCR products for MTP large subunit and B2M from jejunum, ileum, and liver from a representative animal in each developmental group is shown at the bottom of the figure.

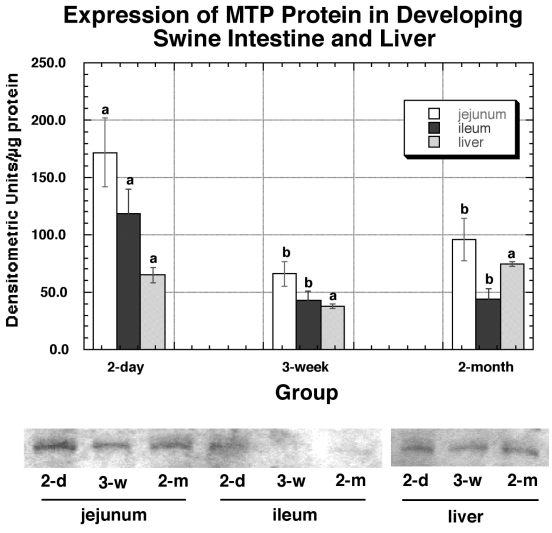

Fig. 3. MTP large subunit protein in developing swine intestine and liver. Homogenates from 2-day-old newborn (2-d), 3-week-old suckling (3-w), and 2-month-old weanling (2-m) piglet jejunum, ileum and liver were subjected to Western blot analysis as described in Materials and Methods. Twenty micrograms of homogenate protein was loaded per well. MTP large subunit protein levels are expressed as densitometric units per μg protein (y axis). Piglet developmental groups are indicated on the x axis. Bars represent the means of data from three animals ±SEM. By ANOVA, values for developmental groups for each organ were significantly different ($P < 0.05$). By post hoc analysis, bars sharing a letter are not different, and those not sharing a letter are significantly different ($P < 0.05$). Western blots from jejunum, ileum, and liver from a representative animal in each developmental group are shown at the bottom of the figure.

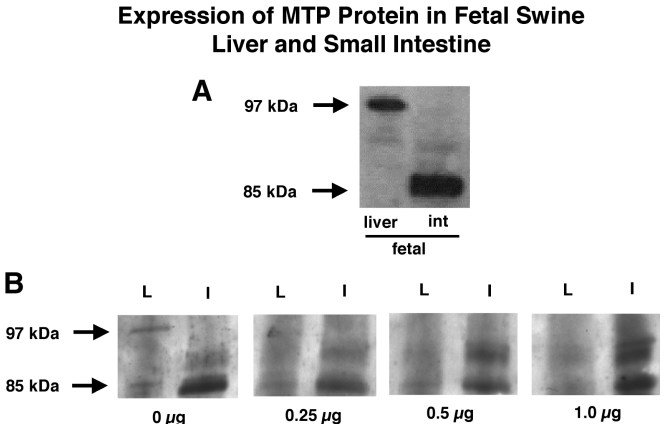

Fig. 4. Expression of MTP large subunit protein in fetal swine liver and small intestine. Western blot analysis using a rabbit anti-swine MTP large subunit peptide antibody was performed as described in Materials and Methods. Twenty micrograms of homogenate protein was loaded per well. A: A band with an apparent molecular weight of 97 kDa was identified as the MTP large subunit in fetal liver. An 85 kDa band was found in fetal small intestine. B: Western blot analysis performed using fetal liver (L) and intestine (I) homogenates with the addition of increasing amounts of the MTP peptide used to generate the antibody.

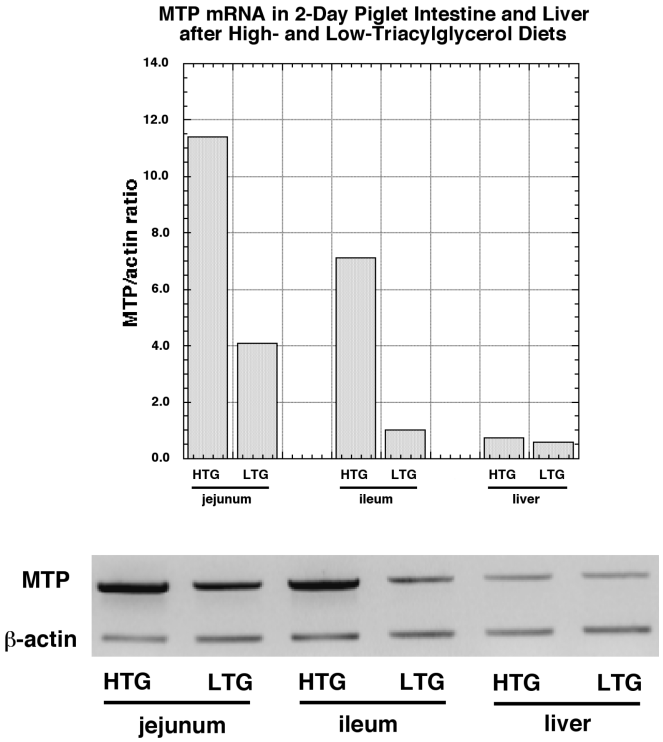

Fig. 5. MTP large subunit mRNA in 2-day-old piglet intestine and liver after high- (HTG) and low- (LTG) triacylglycerol diets, as described in Materials and Methods. The graph shows MTP large subunit mRNA levels expressed as MTP large subunit to β-actin ratios (*y* axis). Experimental conditions are indicated on the *x* axis. The accompanying 1.5% agarose gel containing RT-PCR products for MTP large subunit and β-actin is shown at the bottom of the figure.

subunit protein used to generate the antibody. On examination of additional fetal samples, we did find some liver samples with a small amount of the 85 kDa protein, as is evident in the samples used for the competition experiment shown in Fig. 4B.

Acute regulation of MTP large subunit mRNA by dietary lipid in neonatal swine

Figure 5 shows the effect of the intraduodenal administration of high- and low-triacylglycerol diets for 24 h on MTP large subunit mRNA levels in 2-day-old swine intestine and liver. Jejunal large subunit mRNA level was increased 3-fold and the ileal level increased 7-fold after 24 h with the high-triacylglycerol infusion, as compared with the low-triacylglycerol infusion. However, no change in hepatic mRNA abundance was observed over this time frame.

MTP large subunit mRNA and protein expression in IPEC-1 cells

To better define factors that might mediate the regulation of MTP large subunit gene expression in newborn swine intestinal epithelial cells, we used an in vitro system consisting of IPEC-1 cells. This cell line was derived from a newborn unsuckled piglet, and we have previously characterized the apical uptake, cellular processing, and basolateral secretion of several fatty acids, as well as associated changes in apolipoprotein secretion (21, 23, 27). Because of the similarities of undifferentiated and differentiated IPEC-1 cells to fetal and newborn intestinal cells during development, we defined MTP large subunit expression in these cells at various time points from the undifferentiated state through maximal differentiation 10 days after plating on collagen-coated filters. Plasma cortisol levels in the piglet are high at birth and decline to adult levels by 5 days of age (28). Therefore, to test whether corticosteroids might play a role in the regulation of MTP expression, particularly in the perinatal period, we analyzed MTP large subunit expression in IPEC-1 cells with and without added dexamethasone. **Figure 6A** shows MTP large subunit mRNA levels in IPEC-1 cells at various time points from the undifferentiated state at the time of plating on Transwell filters (time 0) through the change to serum-free medium at day 2 through the maximally differentiated state at day 10. This experiment was performed with and without the addition of 10^{-7} M dexamethasone to the culture medium, beginning at the time of plating. Except for lower expression at day 2 in the cells treated with dexamethasone, there appeared to be no significant difference at any of the other time points. Figure 6B shows corresponding MTP large subunit protein levels in cells treated with dexamethasone, which generally parallel mRNA levels.

To address the issue of whether the lack of difference in MTP expression between the dexamethasone-treated and untreated cells might be due to lack of differentiation and impaired lipid transport in the untreated cells, additional experiments were carried out to measure cellular alkaline

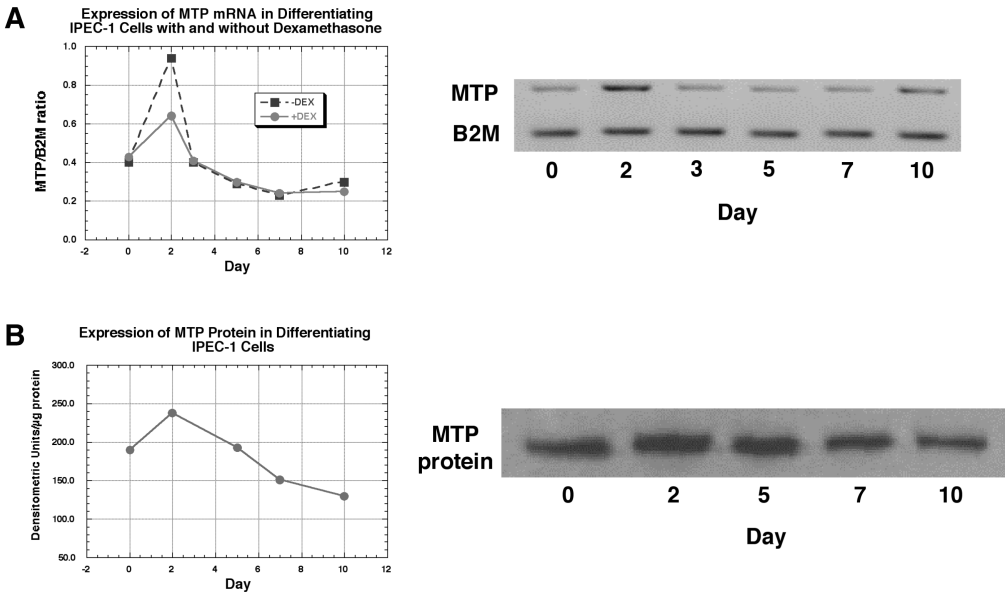

Fig. 6. MTP large subunit mRNA and protein in differentiating IPEC-1 cells. A: The graph shows the expression of MTP large subunit mRNA determined by RT-PCR in differentiating IPEC-1 cells with and without 10^{-7} M dexamethasone added to the culture medium from the time of plating. Undifferentiated cells were plated on collagen-coated filters in Transwell® culture plates in medium containing 5% FBS. At 48 h, cells were switched to serum-free medium. MTP large subunit mRNA levels are expressed as MTP large subunit to B2M ratios (*y* axis). Days in cell culture are indicated on the *x* axis. The accompanying 1.5% agarose gel containing RT-PCR products for MTP large subunit and B2M from dexamethasone-treated cells is shown next to the graph. B: The graph shows the expression of MTP large subunit protein in differentiating dexamethasone-treated IPEC-1 cells, as determined by Western blot analysis as described in Materials and Methods. Twenty micrograms of cell homogenate protein was loaded per well. MTP large subunit protein levels are expressed as arbitrary densitometric units per μg total protein (*y* axis). Days in cell culture are indicated on the *x* axis. The accompanying Western blot showing bands for MTP large subunit from dexamethasone-treated cells is shown next to the graph.

phosphatase activity as an index of terminal differentiation measured at 5, 7, and 10 days of culture with and without dexamethasone on collagen-coated Transwell filters. There was no difference in activity between treated and untreated cells at all time points (minus dexamethasone: 278, 303, and 381; plus dexamethasone: 272, 307, and 361 units $\times 10^3$/μg protein at 5, 7, and 10 days, respectively). Lipid transport was assessed in dexamethasone-treated and untreated cells by incubating treated and untreated cells at 10 days post-plating with 0.8 mM oleic acid and [^{3}H]glycerol added to the apical medium compartment for 24 h, followed by measurement of secretion of labeled triacylglycerol and phospholipid into the basolateral medium. Dexamethasone treatment did not result in significant differences in labeled triacylglycerol (228 vs. 180 dpm/well/24 h, minus vs. plus dexamethasone, respectively) or phospholipid (1,811 vs. 2,092 dpm/well/24 h, minus vs. plus dexamethasone, respectively) basolateral secretion. These data, coupled with the MTP expression data, strongly suggest that glucocorticoids do not significantly modulate differentiation, lipid transport, or MTP expression in immature enterocytes.

We next examined the influence of various fatty acids on MTP large subunit expression in IPEC-1 cells. To mimic the swine perinatal milieu, we used maximally differentiated, dexamethasone-treated cells in this experiment. We chose to test three specific fatty acids: oleic (18:1), stearic (18:0), and eicosapentaenoic (20:5). We have previously shown that oleic acid is the fatty acid most efficiently taken up and esterified into triacylglycerol and secreted into the basolateral culture medium as triacylglycerol-rich lipoproteins by IPEC-1 cells (21, 23). Stearic acid is efficiently taken up and esterified by IPEC-1 cells, but is inefficiently secreted as a component of lipoprotein particles (23). Eicosapentaenoic acid is both inefficiently esterified and secreted by IPEC-1 cells (27). The three fatty acid classes represented by these three fatty acids are all important in neonatal nutrition. **Figure 7** shows MTP large subunit mRNA and protein levels in differentiated IPEC-1 cells after 24 h incubation with 0.8 mM oleic (18:1), stearic (18:0), and eicosapentaenoic (20:5) acids complexed with albumin. Control cells were incubated with albumin only. Compared with oleic acid, which induced MPT large subunit mRNA expression relative to control cells, stearic and eicosapentaenoic acids reduced expression, with the lowest level of expression after eicosapentaenoic acid incubation. Protein levels (Fig. 7, bottom) generally paralleled mRNA levels. Because incubation time has previously been shown to be important for the effect of eicosapentaenoic acid on lipid synthesis and secretion in Caco-2 cells (29), we studied MTP large subunit mRNA expression at 6 and 24 h of incubation with ei-

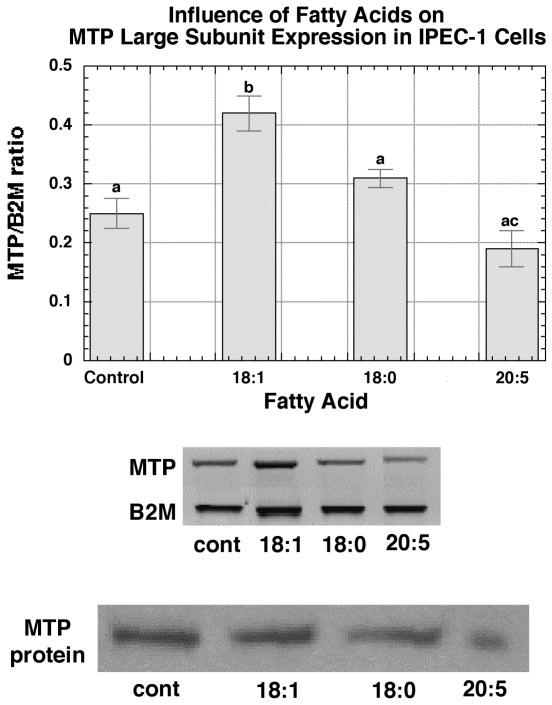

Fig. 7. Influence of fatty acids on MTP large subunit mRNA and protein in IPEC-1 cells. Differentiated cells were incubated for 24 h with either albumin only (control) or oleic acid (18:1), stearic acid (18:0), or eicosapentaenoic acid (20:5) complexed with albumin (4:1 molar ratio) at a fatty acid concentration of 0.8 mM added to the apical medium. The graph shows IPEC-1 cell MTP large subunit mRNA levels measured by RT-PCR expressed as MTP large subunit to B2M ratios (*y* axis). Experimental groups are indicated on the *x* axis. Bars represent the means of data from 3 wells ±SEM. By ANOVA, values for each experimental condition were significantly different ($P = 0.0002$). By post hoc analysis, bars sharing a letter are not different, and those not sharing a letter are significantly different ($P < 0.003$). A 1.5% agarose gel containing RT-PCR products for MTP large subunit and B2M from a representative sample in each experimental group is shown in the middle of the figure. Shown at the bottom of the figure is a representative cell homogenate sample from each group, which was subjected to Western blot analysis using a rabbit anti-swine MTP large subunit peptide antibody as described in Materials and Methods. Twenty micrograms of cell homogenate protein was loaded per well.

ther oleic or eicosapentaenoic acid (**Fig. 8**). Whereas mRNA levels gradually increased during incubation with oleic acid, eicosapentaenoic acid induced a striking increase at 6 h several-fold higher than that induced by oleic acid. By 24 h, expression with eicosapentaenoic acid incubation was down to half that associated with oleic acid incubation. Because this early increase in expression induced by eicosapentaenoic acid is not sustained, it is of doubtful importance in the long-term regulation of MTP protein and activity levels.

DISCUSSION

A switch from a maternal nutrient source via the placenta to an enteral high-fat breast milk source of nutrition characterizes the period of transition from the intrauterine fetal to extrauterine neonatal environments. The newborn mammal must be equipped to efficiently absorb dietary fat, predominantly in the form of triacylglycerol, and to produce chylomicrons to deliver this lipid to peripheral tissues. MTP plays an essential role in the assembly of nascent chylomicrons in the ER of the enterocyte. Therefore, MTP expression in adequate amounts in neonatal small intestine is essential during the critical suckling period. In the present study, we have defined the expression of MTP large subunit mRNA and protein in intestine and liver of fetal, newborn, suckling, and weanling swine.

The finding of a relatively high level of intestinal MTP large subunit mRNA expression in the fetus in the face of undetectable protein of the appropriate apparent molecular weight was unexpected. A prominent band of lower apparent molecular weight (approximately 85 kDa) was detected in fetal intestine by Western blot analysis. However, competition experiments with the peptide used to generate the antibody suggested that this smaller protein might be a different protein with partial sequence homology to MTP large subunit. The dissociation of mRNA and protein levels suggests that MTP protein levels may be regulated at the translational level in fetal intestine.

The high level of MTP large subunit expressed in fetal liver may reflect the central role of the liver in lipoprotein

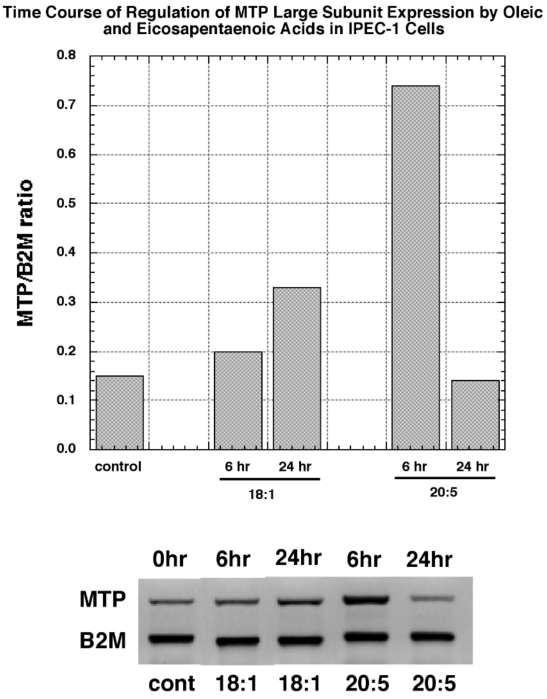

Fig. 8. Time course of regulation of MTP large subunit expression by oleic and eicosapentaenoic acids in IPEC-1 cells. Differentiated cells were then incubated for 6 h and 24 h with albumin only (control), oleic acid (18:1), or eicosapentaenoic acid (20:5) complexed with albumin (4:1 molar ratio) at a fatty acid concentration of 0.8 mM added to the apical medium. The graph shows IPEC-1 cell MTP large subunit mRNA levels measured by RT-PCR expressed as MTP large subunit to B2M ratios (*y* axis). Experimental conditions are indicated on the *x* axis. The accompanying 1.5% agarose gel containing RT-PCR products for MTP large subunit and B2M is shown at the bottom of the figure.

synthesis and secretion in the fetus. Shelton et al. (5) studied MTP large subunit mRNA expression in the developing fetal mouse by in situ hybridization. This group found that during early embryonic development, expression appeared in the yolk sac first, followed by expression in fetal liver at gestational day 9.5. Intestinal expression appeared later at gestational day 12.5 and attained robust levels exceeding that of liver, which persisted into the postnatal period. Early expression in yolk sac probably reflects the importance of that organ as a source of lipoproteins in early fetal life. Our results are consistent with those of this previous study. The importance of MTP in the transport of lipid in the fetus is underscored by the fact that mice homozygous for targeted deletion of the MTP large subunit gene do not survive early fetal life (30).

Regulation of MTP large subunit expression in postnatal developing swine intestine appeared to parallel the degree of intake of dietary fat in the form of breast milk. Interestingly, ileal MTP large subunit mRNA levels in 2-day-old animals were comparable to those in jejunum and declined sharply thereafter. This may have been due to some degree of ileal lipid absorption in newborn animals receiving a high-fat breast milk diet, which may have exceeded the absorptive capacity of the proximal small intestine. Thus, early increased ileal MTP large subunit expression may represent an important compensatory mechanism for scavenging proximally unabsorbed lipid. This hypothesis is supported by the finding that ileal MTP mRNA expression was markedly inducible by an intraduodenal high-fat infusion, as compared with a low-fat infusion, in 2-day-old newborn animals. In contrast to intestinal MTP large subunit expression, hepatic expression declined to low levels immediately postpartum and remained low in all postpartum groups.

Two-day-old piglets receiving an intraduodenal infusion of a high-fat formula containing primarily long-chain unsaturated fatty acids over a 24 h period demonstrated a striking up-regulation of jejunal MTP large subunit mRNA expression with no change in hepatic expression over this time frame. This finding demonstrates that, although programmed genetic cues may be responsible for some degree of developmental regulation of MTP large subunit expression, dietary lipid clearly plays an important regulatory role in the neonatal period. A high-fat diet has been shown to acutely (24 h) up-regulate intestinal MTP large subunit expression in adult hamsters without an acute hepatic response over this time frame (4). Perhaps the fact that the fatty acids are delivered peripherally by chylomicrons, rather than by the portal vein to the liver, might be a factor in the apparent lack of change in hepatic expression.

To better define factors that might mediate the regulation of MTP large subunit gene expression in newborn swine intestinal epithelial cells, we used an in vitro system consisting of IPEC-1 cells. We defined MTP large subunit expression in these cells at various time points from the undifferentiated state through maximal differentiation 10 days after plating on collagen-coated filters. Plasma cortisol levels in the piglet are high at birth and decline to adult levels by 5 days of age (28). Therefore, to test whether corticosteroids might play a role in the regulation of MTP expression, particularly in the perinatal period, we analyzed MTP large subunit expression in these cells with and without added dexamethasone. There were no differences in MTP large subunit expression in treated and untreated cells, except for an isolated increase at day two in both the dexamethasone-treated and untreated cells, with a greater increase in the untreated cells. This initial increase might be explained, other than by the presence or absence of dexamethasone, by the fact that the cells were kept in serum-containing medium for the first 48 h after plating on the filters and then were kept in serum-free medium for the remainder of the culture period. A factor in the FBS, such as fatty acids, other hormones, or growth factors, might have been responsible for this increase followed by the decline in expression after switching to serum-free medium. There were no differences between the treated and untreated cells with regard to alkaline phosphatase activity as a marker for differentiation or lipid transport assessed by incubation with labeled glycerol and oleic acid. Despite the inherent limitations in translating cell culture studies to the in vivo situation, these findings suggest that dexamethasone does not induce MTP large subunit expression in immature enterocytes. These results are consistent with the fact that the MTP large subunit gene promoter does not contain a glucocorticoid response element (31).

We next examined the influence of various fatty acids on MTP large subunit expression in IPEC-1 cells. To mimic the swine perinatal milieu, we used maximally differentiated, dexamethasone-treated cells in this experiment. We chose to test three specific fatty acids with relevance to infant nutrition: oleic (18:1), stearic (18:0), and eicosapentaenoic (20:5). In general, levels of MTP large subunit expression paralleled overall efficiency of triacylglycerol secretion induced by the different fatty acid types in IPEC-1 cells in previous studies showing oleic acid as the most efficient, stearic acid as intermediate, and eicosapentaenoic acid as least efficient in stimulating basolateral triacylglycerol secretion (23, 27, 32). Thus, the degree of MTP expression does appear to be acutely regulated by the type of absorbed fatty acid and proportional to triacylglyerol secretion efficiency. These results are also consistent with the marked induction of MTP large subunit mRNA expression in piglet jejunum by a high-fat intraduodenal infusion of lipid containing mainly 18-carbon unsaturated fatty acids as described in the present report. We conclude that not only the amount of dietary lipid, but also the fatty acid class, in the neonatal diet plays an important role in the regulation of intestinal MTP large subunit expression.

In summary, the expression of the MTP large subunit gene in developing swine small intestine and liver follows a pattern suited to accommodate the needs of the animal. All of the results of the present study should be interpreted in light of the fact that changes in MTP large subunit mRNA levels have not been found to correlate well with actual lipid transfer activity, because MTP is not a

secreted protein and has an approximate half-life of approximately 4.4 days, as determined in HepG2 cells (33). However, regulatory factors, such as dietary composition, which may exert a sustained influence over days or weeks, would likely result in corresponding changes in MTP activity. Although programmed genetic cues undoubtedly play a major role in the developmental regulation of MTP gene expression, clearly the amount and fatty acid composition of dietary lipid also play important regulatory roles.

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