Compensatory Increase in Hepatic Lipogenesis in Mice with Conditional Intestine-specific Mttp Deficiency*

Received for publication, September 28, 2005, and in revised form, November 15, 2005 Published, JBC Papers in Press, December 13, 2005, DOI 10.1074/jbc.M510622200

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Microsomal TG transfer protein (MTTP) is required for the assembly and secretion of TG (TG)-rich lipoproteins from both enterocytes and hepatocytes. Liver-specific deletion of Mttp produced a dramatic reduction in plasma very low density lipoprotein-TG and virtually eliminated apolipoprotein B100 (apoB100) secretion yet caused only modest reductions in plasma apoB48 and apoB48 secretion from primary hepatocytes. These observations prompted us to examine the phenotype following intestine-specific Mttp deletion because murine, like human enterocytes, secrete virtually exclusively apoB48. We generated mice with conditional Mttp deletion in villus enterocytes (Mttp-IKO), using a tamoxifen-inducible, intestine-specific Cre transgene. Villus enterocytes from chow-fed Mttp-IKO mice contained large cytoplasmic TG droplets and no chylomicron-sized particles within the secretory pathway. Chow-fed, Mttp-IKO mice manifested steatorrhea, growth arrest, and decreased cholesterol absorption, features that collectively recapitulate the phenotype associated with abetalipoproteinemia. Chylomicron secretion was reduced dramatically in vivo, in conjunction with an ~80% decrease in apoB48 secretion from primary enterocytes. Additionally, although plasma and hepatic cholesterol and TG content were decreased, Mttp-IKO mice demonstrated a paradoxical increase in both hepatic lipogenesis and very low density lipoprotein secretion. These findings establish distinctive features for MTTP involvement in intestinal chylomicrosen assembly and secretion and suggest that hepatic lipogenesis undergoes compensatory induction in the face of defective intestinal TG secretion.

The mobilization and secretion of triglyceride (TG)3-rich lipoproteins from mammalian enterocytes and hepatocytes are critically dependent on the integrated function of at least two dominant genes. These include the microsomal triglyceride transfer protein (MTTP), a resident endoplasmic reticulum protein that facilitates the transfer of neutral lipid to a large hydrophobic acceptor protein, apolipoprotein B (apoB) (1). ApoB in turn functions as a requisite structural component of the surface of TG-rich lipoproteins and plays a vital role in plasma lipoprotein metabolism (2). Their physiological importance is exemplified through the phenotypes associated with abetalipoproteinemia and homozygous familial hypobetalipoproteinemia, where structural defects in either the MTTP or apoB gene, respectively, lead to a syndrome of hypocholesterolemia (including low levels of high density lipoprotein (HDL)), mild fat malabsorption with fasting lipid accumulation in the small intestine along with hepatic steatosis (3, 4).

Exploration of the mechanisms underlying these phenotypes has been advanced through studies in murine genetic models in which either the Apob or Mttp gene was deleted. Germ line deletion of either the Apob or Mttp gene, however, resulted in embryonic lethality (5–7), the result of defective lipid delivery to the developing embryo, necessitating alternative approaches to the study of their genetic disruption in the adult animal. In regard to the effects of Mttp deletion, this approach was particularly informative. Heterozygous Mttp knock-out mice grew normally, demonstrate no apparent intestinal phenotype, no effects on plasma TG levels, and only marginally elevated hepatic TG content (5, 8), suggesting that half-normal levels of MTTP are well tolerated. Liver-specific deletion of Mttp gene expression, by contrast, produced a much more dramatic phenotype, with almost complete elimination of hepatic TG secretion, reduction in plasma TG and cholesterol levels, and the accumulation of large hepatocyte lipid droplets, with a ~3-fold increase in hepatic TG content (7, 9, 10). An additional feature of the liver-specific Mttp deleter mice reported by Young and colleagues was the divergent responses observed in hepatocyte secretion of the apoB isoforms. Specifically, these workers (7) demonstrated that serum apoB100 levels were decreased by ~90%, and the secretion of apoB100 from primary murine hepatocytes was virtually eliminated. By contrast, the secretion of apoB48 was essentially unchanged, and there were no significant changes in plasma apoB48 levels (7). These findings raised the question of whether the effects of MTTP ablation would be intrinsically less dramatic in murine small intestine, where apoB48 is overwhelmingly the dominant isoform expressed (11). A corollary question posed in the current study was the extent to which intestinal apoB48 secretion requires normal MTTP abundance/activity because evidence from either genetic deletion (7) or pharmacologic inhibition of MTTP in murine hepatocytes (12, 13) indicated only marginal effects of MTTP deletion on apoB48 secretion.

The role of intestinal apoB gene expression in chylomicrosen assembly and secretion has been inferred from the study of apoB knock-out mice (which otherwise die during embryonic development) rescued by crossing into a hemizygous human apoB transgenic line (HuBtg+/-, ApoB-/-). The offspring of these crosses express apoB in the liver, but not in the small intestine (14). Neonatal HuBtg+/-, ApoB-/- mice developed lipid malabsorp-
tion, fat-filled enterocytes with large cytoplasmic lipid droplets, and one-half to two-thirds of the animals died before 3 weeks of age (14). Nevertheless, the surviving mice appeared to grow normally while consuming a chow diet and eventually weighed the same as their littermates. These latter findings suggested that intestinal TG absorption may be compromised at levels of low dietary fat intake, despite the absence of intestinal apoB. Although cholesterol absorption in these surviving HuBTg apoB-/- mice was completely eliminated and serum cholesterol levels slightly lower, serum TG levels were unchanged compared with HuBTg apoB+/+ mice (14). Thus, given the modest effects of intestinal apoB deletion on TG absorption in the surviving adults (on a chow diet), it seemed reasonable to raise the question of whether TG absorption would be affected to a similar extent in young adult animals with intestine-specific Mttp deletion. A corollary question posed in the current study was the extent to which alterations in intestinal Mttp expression might influence systemic cholesterol and TG metabolism.

To address these questions, we have generated mice with conditional deletion of Mttp in the small intestinal epithelium. The findings reveal subtle distinctions between the effects of intestinal Mttp and Apob deletion and demonstrate an unexpected compensatory adaptation in hepatic lipogenesis in the setting of defective chylomicron formation and secretion.

### MATERIALS AND METHODS

**Animals—**Mttpfloxflo×Cre mice (7) (50% C57BL/6, ~50% 129/SvJ) were crossed with transgenic villin-Cre-ErT2 deleter mouse (15) (C57BL/6) mice to generate a compound line, Mttpfloxflo×villin-Cre-ER T2 (Mttpflo×villCre), in a background of (~75% C57BL/6 and ~25% 129/SvJ). Cre recombinase expression in villus enterocytes was induced by intraperitoneal injection of 1 mg/100 μl tamoxifen (Sigma) 1 mg/day for 5 days, as described previously (15). Experiments were performed on mice between 8 and 15 weeks of age, unless otherwise noted, and were undertaken 3 weeks after the tamoxifen injection. Growth analysis was undertaken in groups of mice starting at 4 weeks of age, as noted in the relevant figure legend. Genotyping was performed by PCR using the following primers: for Mttpfloxflo, sense, 5'-GCTCTAAGAGAAGGAGTTTAAG-3'; antisense, 5'-GCTCTTTCAAGAGAGAAGG-3'; for villin-Cre-ErT2, sense, 5'-CAAGCTGCGTACGCCGGGC-3'; antisense, 5'-CGGCAACATCTTCAAGGTTTC-3'. In some experiments, where noted, wild type and Apobec-1-/- mice (C57BL/6) were used as a source of primary intestinal enterocytes.

**Histologic and Ultrastructural Analysis of Tissues—**Frozen sections of intestine were stained by oil Red O and examined by light microscopy. For electron microscopic examination, intestinal samples from the proximal jejunum were prepared after perfusion fixation with freshly prepared 1.5% glutaraldehyde and 4% polyvinylpyrrolidone in 0.05% calcium chloride, 0.1 M sodium cacodylate, pH 7.4, for 10 min. Tissues were stained in 2% aqueous uranyl acetate and sections viewed by electron microscopy exactly as detailed previously (16). For immunohistochemical detection of Cre staining, the proximal intestine was fixed in 10% formalin, sectioned, and stained with a 1:1,500 dilution of rabbit anti-Cre IgG (Novagen, Madison, WI).

**Enterocyte Isolation, Lipid Determination, and Metabolic Labeling—**Enterocytes were isolated from the entire small intestine as described previously (17) and pooled. Fresh cells were used for pulse-chase experiments; alternatively, cell pellets were frozen at ~80 °C for further experiments. For determination of enterocyte lipid content, ~10^7 enterocytes were lysed in 1 ml of phosphate-buffered saline by sonication, whereupon 600 μl of lysate was extracted into 5 ml of hexane/isopropyl alcohol, 3:2. The hexane phase was collected, dried under nitrogen, and resuspended in 1% Triton X-100 for enzymatic assay. Determinations of TG, cholesterol, free fatty acid, and phospholipid content were undertaken enzymatically using an L-type triglyceride H kit, Cholesterol E kit, NEFA C kit, or phospholipid B kit, respectively (Wako Chemicals, Neuss, Germany). Aliquots of enterocyte lysate were used for protein content determination by the Bio-Rad protein assay kit. Pulse-chase experiments were conducted as described previously (17). In brief, freshly isolated enterocytes (10^7/ incubation) were pulse labeled for 30 min with 250 μCi of [35S]-

### Intestine-specific Mttp Deleter Mice

| Gene     | Forward primer                          | Reverse primer                          |
|----------|----------------------------------------|----------------------------------------|
| ABCA1    | 5'-AGGTTTTCCTTTGTCCTAGATGACATGG-3'     | 5'-TGGCCAAAGGTTGCGGCAACAAGAG-3'       |
| ABCG5    | 5'-TGGCTGACAAACAGACAGGTGCT-3'         | 5'-GCTTCCGAGCCAGATGAGT-3'            |
| ABCG8    | 5'-AGCTACCTTGGCCAGGTGATGAGT-3'        | 5'-ACCATCCGAGAAAGTGTCCT-3'           |
| ACAT2    | 5'-GGCTTGGGCTTGAGTTGTTGAGT-3'         | 5'-GGATGCGCAACAGACAGTTT-3'           |
| ApoA1    | 5'-AGGAATAGGAATTGGAAGATTGGAAGT-3'     | 5'-AGGAATAGGAATTGGAAGATTGGAAGT-3'    |
| ApoA-IV  | 5'-GGCTTGGGCTTGAGTTGTTGAGT-3'         | 5'-GGATGCGCAACAGACAGTTT-3'           |
| CD36     | 5'-TGCTGACAAACAGACAGGTGCT-3'         | 5'-GCTGCAACAGAGCAGCCTTAC-3'          |
| DGAT1    | 5'-CTGGCAAGAGGACAGCATCTTCA-3'         | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| DGAT2    | 5'-AGCCATCGTGGCCAGGACAGCTC-3'         | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| FANS5    | 5'-ACTTACATCGACGACAGCATCTTAC-3'       | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| FATP4    | 5'-GCTGCAAGAGGACAGCATCTTCA-3'         | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| HMG5     | 5'-GCTGCAAGAGGACAGCATCTTCA-3'         | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| HMG5     | 5'-TGGGCAACTTCGGAAGGGCTTA-3'          | 5'-GCTGCAAGAGGACAGCATCTTCA-3'        |
| I-FABP   | 5'-ACTTACATCGACGACAGCATCTTAC-3'       | 5'-GCTGCAAGAGGACAGCATCTTCA-3'        |
| LDLR     | 5'-CTGACCGACAGGAGTATGCTTCA-3'         | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| L-FABP   | 5'-CCGAGGAGACTTGGCAGGACATCT-3'        | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| LMRA     | 5'-GCTGCAAGAGGACAGCATCTTCA-3'         | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| MTP      | 5'-TGATGACACAGGCTTGGCAGGATCT-3'       | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| NPC1L1   | 5'-CCGAGGAGACTTGGCAGGACATCT-3'        | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| PPARα    | 5'-TGGGCAACTTCGGAAGGGCTTA-3'          | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| S1a      | 5'-GGCCGTTTACGGAGGACAGCATCT-3'        | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| S1b      | 5'-GGCCGTTTACGGAGGACAGCATCT-3'        | 5'-TGGTTGAGTGCGGTCTCTCT-3'           |
| SREBP2   | 5'-AGACCCCGTTCCTTGTGCTTAC-3'          | 5'-GGGCTGGAATGCAAGAGAAAGT-3'         |

**TABLE 1**

Oligodeoxyribonucleotide primer sequences for real-time Q-PCR

**TABLE 2**

Intestine-specific Mttp Deleter Mice
protein labeling mix (PerkinElmer Life Sciences) and chased for 120 min in Dulbecco’s modified Eagle’s medium supplemented with 10 mM methionine and 5 mM cysteine. A mixed micellar lipid solution (0.4 mM sodium taurocholate, 0.54 mM sodium taurodeoxycholate, 0.3 mM phosphatidylcholine, 0.45 mM oleic acid, 0.26 mM monoolein) was added to both pulse and chase medium for all of the experiments. At the end of the chase, medium and cells were collected and aliquots of lysate and medium immunoprecipitated with rabbit anti-mouse apoB IgG. Quantitation of 35S incorporation into apoB48 was conducted using 4–15% PAGE separation of the immunoprecipitation reactions, which were then quantified using PhosphorImager analysis (Molecular Dynamics, Sunnydale, CA). In some experiments, where noted, isolated enterocytes were labeled as above with 250 μCi of 35S-protein labeling mix/ml for 2 h with or without 10 μM MTTP inhibitor BMS197636 (a generous gift from Dr. John Wetterau at Bristol-Myers Squibb Company). 35S-apoB synthesis and secretion were analyzed in cell lysates and media as described above.

**Real Time Quantitative Reverse Transcription-PCR (Real Time Q-PCR)**—RNA was extracted from isolated enterocytes or liver tissue using TRIzol (Invitrogen) and treated with DNase. Reverse transcription was performed using the SuperScript II First-strand Synthesis System (Invitrogen), with 3 μg of total RNA and random hexamers, to generate cDNA. For Cre detection, cDNA products were amplified with Cre-specific primers: 5’-ATGGAAAATAGCGATCGCTGCCAG-3’ (forward), 5’-ACCAGGCCAGGTATCTGTGACCAG-3’ (reverse). Real time Q-PCR assays were performed in triplicate on an ABI Prim 7000 Sequence Detection System using SYBR Green PCR Master Mix. The mRNA level of individual genes was quantified and normalized to Hsp40. Relative mRNA abundance of individual genes was calculated as -fold change compared with its mRNA level in pooled preparations of isolated enterocytes or livers from 5 Mttpf/f mice. The primers used for real time Q-PCR are listed in Table 1.

**Detection of Enterocyte MTTP and ApoB Protein by Western Blotting**—Enterocyte lysates were prepared as described previously (17). Aliquots of lysates representing 100 μg of total protein were separated by 4–15% PAGE. Western blots were performed with rabbit antiserum against mouse apoB (1:4,000) or Goat anti-serum against MTTP (1:8,000), and ECL detection reagents. Quantitation was performed by Kodak 440CF imager system. Reactivity against Hsp40 was used as an internal control (StressGen).

**Cholesterol Absorption and Fecal Lipid Content**—Cholesterol absorption was measured by a fecal dual-isotope ratio method, essentially as described previously (18). Mice were gavaged with 150 μl of corn oil mixed with 1 μCi of [14C] cholesterol (PerkinElmer Life Sciences) and 2 μCi of β-[3H]sitostanol (American Radiolabeled Chemicals, Inc.). Mice were then housed individually in fresh metabolic cages, and feces were collected over the next 2 days. The ratio of 14C to 3H in each fecal sample was determined, corrected for the ratio in the dosing mixture, and the percent of cholesterol absorption calculated as described previously (18). The lipid content of feces was determined gravimetrically after chloroform/methanol extraction.

**Determination of Intestinal Apolipoprotein and Triglyceride Secretion in Vivo**—Mice were fasted for 16 h, weighed, and injected intravenously with 500 mg/kg body weight Tyloxapol (Sigma). Immediately after tyloxapol administration, the mice received an intragastric bolus of 0.5 ml of lipid emulsion containing 400 μl of 20% Intralipid, 90 μl of corn oil, and 10 μCi of [3H]triolein (American Radiolabel chemicals). Blood samples were collected at 0, 1, 2, 3, and 4 h after injection and plasma TG measured enzymatically. Aliquots of plasma were separated by 4–15%
SDS-PAGE and apoB and apoE were detected by Western blot. To ensure a linear response, 1/1000 l of serum at time zero was run alongside 0.5/1000 l from the 3 h time point and the images quantitated using a Kodak 440CF Imager System. The increase of plasma apoB48 at 3 h was expressed as -fold increase of apoB48 compared with time zero. In other experiments, where indicated, 200/1000 l of plasma obtained 4 h after tyloxapol injection and intragastric lipid bolus was size fractionated by fast performance liquid chromatography (FPLC). 20 fractions were collected and used for Western blot analysis of apoB48 distribution. Lipids were extracted from each fraction, separated by TLC, and radiolabeled TG quantitated by scintillation counting.

**Determination of Hepatic Lipid Content and Secretion and ApoB Secretion in Vivo**—Approximately 50 mg of liver was homogenized and used to prepare lipid extracts for enzymatic assay as described previously (19). Hepatic TG and apoB secretion were determined as described previously (20). Mice were fasted for 4 h and then injected via tail vein with 500 mg/kg tyloxapol and 500 lCi of 35S-protein labeling mix. Blood samples were collected before injection and every 30 min for 2 h. Plasma TGs and cholesterol were measured enzymatically, as above. Newly synthesized apoB and albumin were estimated by PhosphorImager analysis after 4–15% SDS-PAGE of 2/1000 l of plasma, as described previously (20). To determine VLDL TG secretion, 50 l of plasma obtained 2 h after injection was overlaid with 850 l of 0.15 M NaCl and d < 1.006 (VLDL) and d > 1.006 fractions subsequently separated by ultracentrifugation at 40,000 rpm, 16 h at 14 °C in a Beckman MLA-130 rotor model. TG content was determined enzymatically in the isolated fractions.

**RESULTS**

**Conditional, Intestine-specific Mttp Deletion**

Floxed Mttp mice were crossed into the ER-T2 villin-Cre deletor line and recombination induced by tamoxifen administration. Reverse transcription-PCR analysis of intestinal and hepatic RNA (Fig. 1A) confirmed intestine-restricted Cre expression, and nuclear translocation was confirmed by immunochemical localization in heterozygous mice (Fig. 1B, compare left two panels). Biallelic inactivation of Mttp was associated with gross lipid accumulation (Fig. 1B, right panel), described in detail below.
Enterocyte MTTP mRNA and protein abundance were decreased after tamoxifen administration, with a range of expression ranging from ~50% of wild type levels down to undetectable (Fig. 1C). As detailed below, a phenotype associated with Mttp-IKO mice (MTTP+/−, Fig. 1C) was demonstrated with enterocyte MTTP mRNA and protein expression of less than 20% of wild type levels.

**Functional Characterization of Mttp-IKO Mice: Effects on Weight Gain and Intestinal Lipid Absorption**

Starting at approximately 1 week after tamoxifen administration, Mttp-IKO mice demonstrated a plateau in weight gain (Fig. 2A) associated with the onset of steatorrhea (Fig. 2, B and D). Regression analysis

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**FIGURE 3. Intestinal phenotype in Mttp-IKO mice.** A, gross appearance of small intestine showing engorgement and distention with residual lipid. B, oil Red O staining of proximal intestine demonstrating lipid staining of villi. C, electron micrographs (×12,000) of absorptive enterocytes from mice of the indicated genotype, revealing the presence of lipid droplets (LD) in the apical portions adjacent to the microvillus border (MV). D, electron micrographs (×24,000) revealing numerous electron-dense particles within the endoplasmic reticulum of MTTP+/− mice (arrowheads, upper panel), contrasted with their virtual absence in MTTP−/− (Mttp-IKO) mice (arrows, lower panel). Arrows indicate the membranes of the apical endoplasmic reticulum. E, electron micrographs (×36,000) of perinuclear (N) Golgi from MTTP+/− enterocytes (left panel) demonstrating abundant lipoprotein particles of chylomicron size within secretory vesicles (SV). Because of fixation artifact, these relatively protein-poor nascent chylomicrons often coalesce when compressed together within tightly packed secretory vesicles, making them appear larger than when they are separated individually within the endoplasmic reticulum. In sharp contrast, the Golgi from MTTP−/− enterocytes (right panel) completely lack secretory vesicles containing nascent chylomicrons, although some Golgi cisternae contain occasional irregularly shaped lipid-rich material, staining similarly to the lipid droplets (LD). F, lipid content of pooled enterocytes from seven to nine mice of the indicated genotype. TG, cholesterol (Chol) and free fatty acid (FFA) were measured enzymatically; **, p < 0.01.
of 53 animals (encompassing control, floxed, and Mttp-IKO mice) revealed a significant negative correlation between MTTP mRNA abundance and fecal fat, with an evident shift when relative MTTP mRNA abundance fell below 20% control (Fig. 2B). The presence of steatorrhea (>7%) on a chow diet excluded animals with residual MTTP expression greater than 20% of control levels, providing a convenient functional screen to identify Mttp-IKO (or MTTPfl/fl) mice.

Cholesterol absorption, measured by dual isotope ratio methodology, revealed a positive correlation with enterocyte MTTP mRNA abundance (Fig. 2C). The findings demonstrate a 60–70% reduction in cholesterol absorption in Mttp-IKO mice (Fig. 2E), suggesting that cholesterol absorption, although decreased, was not eliminated. Even in a subgroup of 11 animals with less than 3% residual MTTP expression, there was still 10.5% cholesterol absorption detectable (comparable with the Mttp-IKO group as a whole (Fig. 2E)), which represents values approximately one-third of controls. These findings contrast with results from mice producing apoB in the liver but not the small intestine (14), where cholesterol absorption was undetectable.

**Morphologic Characterization of Mttp-IKO Mice**

After a 4-h fast, the entire small intestine of Mttp-IKO mice was visibly engorged with lipid (Fig. 3A), and oil Red O staining of frozen sections revealed confluent lipid droplets from the lower villus to the tip (Fig. 3B). These findings contrast with the findings in Mx1-Cre transgenic mice crossed into the Mttpflox/flox mice, where fat-filled enterocytes were confined to the lower half of the villus (7).

We next analyzed the proximal small intestine of Mttp-IKO mice using electron microscopy. The apical portions of Mttp-IKO mice contained numerous large lipid droplets, although virtually no VLDL or chylomicron-sized lipoprotein particles were seen within the endoplasmic reticulum or Golgi (Fig. 3, C–E). Occasional, small, electron-dense particles were observed within the Golgi apparatus of Mttp-IKO mice, the functional significance of which is unknown. These findings are reminiscent of the findings of Raabe and co-workers (7) and suggest an important element of conservation in the effects of Mttp disruption in murine hepatocytes and enterocytes. Accompanying these morphologic changes, isolated enterocytes from Mttp-IKO mice demonstrated a 12-fold increase in TG content and a 2-fold increase in free fatty acid content (Fig. 3F). Cholesterol content, by contrast, was unchanged (Fig. 3F).

**Functional Characterization of Mttp-IKO Mice**

*Effects on Plasma Lipids and Intestinal Chylomicron Secretion—Previous studies in mice with conditional deletion of hepatic Mttp gene expression demonstrated a striking reduction in plasma TG levels (7). By contrast, HuBTg/fl, Apob−/− mice, expressing no small intestinal apoB, exhibit normal plasma TG concentrations (14). These findings might imply that the maintenance of plasma TG levels is influenced...*
predominantly by changes in hepatic but not intestinal lipoprotein secretion. Contrary to this prediction, however, plasma lipid levels in Mttp-IKO mice revealed decreased TG, cholesterol, and free fatty acid levels (Fig. 4A), accompanied by a striking decrease in HDL cholesterol (Fig. 4B).

To examine the dynamic process of intestinal lipoprotein secretion in vivo, animals were injected with tyloxapol and a ~100-mg lipid bolus administered by intragastric gavage containing radiolabeled TG. The serum TG content rose only marginally in Mttp-IKO mice (Fig. 4C), and FPLC separation revealed a striking decrease in radiolabeled TG in chylomicron/VLDL fractions (Fig. 4D). Complementing the findings of decreased newly synthesized TG, both cholesterol (Fig. 4E) and TG mass (Fig. 4F) were decreased in lipoprotein fractions obtained from in Mttp-IKO mice.

To pursue further the alterations in intestinal apoB-containing lipoprotein secretion after a lipid bolus, we examined serum apoB48 abundance using Western blotting. The findings reveal comparable fasting levels of apoB48 but a decrease in apoB48 abundance in plasma at 3 h postbolus administration in Mttp-IKO mice with control animals (Fig. 5, A and B). ApoB48 distribution, estimated after FPLC separation of pooled postprandial samples, revealed a decrease in chylomicron-associated fractions and a shift toward particles in the LDL/HDL density range. We will return to the question of the hepatic contribution to this profile of newly secreted lipoproteins in a later section.

Effects on Enterocyte Apolipoprotein Synthesis and Secretion—A central conclusion of studies in mice with liver-specific inactivation of Mttp was that apoB100 secretion was virtually eliminated, whereas apoB48 secretion was barely affected (7). These findings raised the obvious question of whether apoB48 secretion would be altered by targeted deletion of Mttp within villus enterocytes. To address this question, we isolated primary murine enterocytes and undertook short term pulse-chase studies. Studies in an unselected group of Mttp-IKO mice (<20% Mttp mRNA) demonstrate that apoB48 accumulates intracellularly with apoB48 secretion reduced ~50% compared with control animals (data not shown). Further studies in a cohort of Mttp-IKO mice with <5% residual MTTP mRNA revealed an ~80% reduction in apoB48 secretion (Fig. 6A), again with comparable recovery (the sum of immunoprecipitable apoB counts in lysate plus medium) of newly synthesized apoB48 (Fig. 6A, lower panel). These findings demonstrate that intestinal apoB48 secretion is greatly reduced but not eliminated in Mttp-IKO mice. Newly synthesized apoB48 was recovered in a d < 1.21 fraction (but not the d < 1.006 fraction) from the media in Mttp-IKO mice (data not shown), indicating detectable apoB48 secretion into lipid-poor particles. Enterocyte synthesis and secretion of apoA-I and apoA-IV, two major intestinal apoproteins associated with circulating HDL, was unchanged in Mttp-IKO mice (data not shown). Fasting apoB48 content and de novo apoB48 synthesis were comparable in Mttp-IKO mice and control animals (Fig. 6B and data not shown), suggesting that the retained, newly synthesized apoB48 in Mttp-IKO mice is eventually degraded.

The finding that enterocyte apoB48 secretion was reduced in Mttp-IKO mice compared with control animals raised the question of whether the disproportionate effects in liver-specific Mttp deltor mice, with respect to apoB100 versus apoB48 secretion, reflect an intrinsic property of apoB100 or whether cell-specific factors account for the differences in enterocyte versus hepatocyte apoB48 secretion. To begin to explore this important question, we prepared isolated enterocytes from wild type (>95% apoB48) and Apobec-1<sup>−/−</sup> animals (apoB100 only) and undertook short term incubations in the presence of a pharmacologic MTTP inhibitor at doses that decreased TG secretion ~80% (higher doses were toxic to the cells). The results demonstrate that apoB48 secretion was reduced ~50% in wild type enterocytes incubated with the MTTP inhibitor (Fig. 6C). By contrast, apoB100 secretion was reduced by ~90% in association with decreased recovery, consistent with the prediction that retained apoB100 undergoes degradation within the 2-h period employed (Fig. 6C).

From a physiological perspective, these results establish that enterocyte apoB48 secretion is modulated by genetic or pharmacologic decreases in MTTP abundance or activity.

Insights into the Mechanisms and Pathways Involved in Altered Intestinal Lipid and Lipoprotein Metabolism—To begin to understand the mechanisms underlying some of the adaptations involved in intestinal lipid metabolism and lipoprotein formation after Mttp disruption, a panel of candidate mRNAs was examined by real time Q-PCR, including genes involved in cholesterol absorption, fatty acid transport, and complex lipid synthesis and secretion. The findings demonstrate decreased mRNA abundance in Mttp-IKO mice of several genes implicated in cholesterol absorption, including ABCA1, ABCG5/8, NPC1L1, and the multifunctional transporter CD36 (Fig. 7). By contrast, mRNA expression of several candidate fatty acid transporter/binding proteins was increased in Mttp-IKO mice, particularly L-FABP (Fig. 7). Genes involved in cholesterol synthesis (HMG-CoA reductase) and esterification (ACAT2) as well as complex lipid esterification pathways (MAGAT2, DGAT2) also demonstrated increased mRNA abundance in Mttp-IKO mice (Fig. 7). Sar1A and Sar1B mRNAs, genes involved in the latter stages of prechylomicron fusion with transport vesicles in the
secretory pathway (22), were also increased in Mttp-IKO mice (Fig. 7).

By contrast, there was no change in mRNA abundance for apoB, apoA-I, or apoA-IV in Mttp-IKO mice. These findings are noteworthy because microarray studies in liver-specific Mttp-deleter mice demonstrated that most genes involved in hepatic lipid metabolism were unchanged (9).

Adaptations to Hepatic Lipid Metabolism in Mttp-IKO Mice—Recent reports of compensatory alterations in one lipogenic organ after tissue-specific manipulation of gene expression in a distinct organ (23, 24) prompted us to examine the effects on hepatic lipid metabolism after intestinal Mttp disruption. Hepatic TG, cholesterol, and free fatty acid content were significantly reduced in Mttp-IKO mice (Fig. 8A), consistent with decreased intestinal lipid delivery. Unexpectedly however, we found a paradoxical increase in hepatic TG secretion in these animals (Fig. 8D). This increase in hepatic TG secretion appeared to be in VLDL, as inferred from the distribution of TG in the less dense fraction after ultracentrifugation in Mttp-IKO mice (Fig. 8C). The increase in hepatic TG secretion in Mttp-IKO mice was accompanied by an increase in hepatic apoB100 secretion (Fig. 8D). The decrease in newly synthesized apoB48 in the plasma of Mttp-IKO mice likely reflects decreased intestinal secretion, as illustrated above (Figs. 5 and 6).

These findings strongly suggest the existence of compensatory alterations in hepatic lipid metabolism accompanying intestine-specific Mttp deletion. To examine this possibility more directly, we isolated primary hepatocytes from Mttp-IKO mice and controls and undertook metabolic labeling using [1-14C]acetate. Lipid classes were extracted from both cell lysates and media and newly synthesized lipid quantitated. The findings demonstrate a striking increase in radiolabel incorporation into cellular TG, cholesterol, and phospholipids and increased TG secretion into the media (Fig. 8E). These changes were noted in the setting of decreased hepatocyte TG mass in isolated hepatocytes (Fig. 8E), confirming the observations in whole liver (Fig. 8A).

To begin to explore the mechanisms and genetic pathways by which these changes are mediated, we again turned to a real-time Q-PCR assay of a panel of candidate genes involved in hepatic lipid metabolism. The findings reveal increased mRNA abundance of fatty acid synthase, SREBP1c, and SREBP2 and a striking increase in HMG-CoA reductase, along with increased expression of ABCG5/8, SR-B1, LDLR, and CD36 mRNA abundance (Fig. 9). By contrast, expression of MTTP and apoB was unchanged in Mttp-IKO mice. These findings will of course require more extensive analysis to resolve the functional significance of each pathway.

DISCUSSION

In this study, we have exploited an approach to conditional, intestine-specific Mttp deletion to explore the role of MTTP in intestinal lipoprotein formation and secretion in the adult animal. This approach
bypassed the difficulties associated with embryonic lethality of the germ line deletion of Mttp, which have heretofore precluded examination of the intestinal phenotypes associated with abetalipoproteinemia (3, 4). Our findings in chow-fed Mttp-IKO mice recapitulate key elements of the phenotype associated with homozygous abetalipoproteinemia, including arrested growth, steatorrhea, enterocyte lipid droplet accumulation, and the virtual elimination of TG-rich lipoproteins from within organelles of the secretory pathway (25). In addition, Mttp-IKO mice demonstrate reduced serum TG and cholesterol levels, particularly HDL cholesterol, and decreased cholesterol absorption.

The current findings add new information concerning intestinal lipoprotein secretion, extending findings from earlier studies in mice that synthesize apoB in the liver but not in the intestine (14). However, although some elements of the intestinal phenotype of the Mttp-IKO mice were somewhat predictable, our findings reveal subtle yet important differences from the phenotypes associated with intestinal deletion of Apob. The striking phenotype associated with intestinal Mttp deletion in adult, chow-fed mice, including steatorrhea and growth arrest, particularly evident in female Mttp-IKO mice, contrasts with the milder phenotype encountered in the surviving adult HuBTg<sup>+</sup>/0, Apob<sup>−/−</sup> mice (14). These latter mice appeared to grow normally, reaching weights indistinguishable from their littermate controls, despite evidence of fat-filled villus enterocytes at necropsy. These results were interpreted to suggest that the HuBTg<sup>+</sup>/0, Apob<sup>−/−</sup> mice are able to absorb small amounts of dietary TG, or at least sufficient quantities to maintain weight gain and to prevent the development of essential fatty acid deficiency, despite the absence of intestinal apoB (14). The current findings, on the other hand, indicate a more substantial defect in TG absorption in the absence of intestinal MTTP. However, despite the relatively complete block in TG absorption, cholesterol absorption in the Mttp-IKO mice was decreased by ~70%, but not eliminated as was the case in adult HuBTg<sup>+</sup>/0, Apob<sup>−/−</sup> mice (14). These latter findings suggest that intestinal cholesterol absorption occurs almost exclusively via apoB-dependent lipoprotein secretion, but that ~one-third of cholesterol absorption occurs independently of MTTP expression. These suggestions are broadly consistent with the conclusions of Hussain and colleagues (26, 27), who proposed, based on the findings of pharmacologic inhibition of MTTP activity in Caco-2 cells, that as much as 30% of cholesterol secretion occurs via an apoB-independent pathway, possibly involving ABCA1-dependent pathways.

Along these lines, the current studies reveal a significant decrease in Mttp-IKO mice of mRNA expression for many candidate genes implicated in cholesterol absorption, including ABCA1, ABCG5/G8, NPC1L1, and CD36. In addition, we demonstrated significant up-regulation of mRNAs for several genes involved in intestinal lipid metabolism, including ABCA1, ABCG5/G8, NPC1L1, and CD36. These findings are of interest in view of the previous demonstration that whole body cholesterogenesis was up-regulated in HuBTg<sup>+</sup>/0, Apob<sup>−/−</sup> mice, although to our knowledge altered patterns of intestinal gene expression were not explored in that setting. However, hepatic microarray analysis in the liver-specific Mttp-deleter mice revealed no compensatory changes in most genes involved in hepatic lipid metabolism (and specifically not in HMG-CoA reductase), suggesting an important divergence in the response of intestinal and hepatic cholesterol metabolism to alterations in MTTP expression. The signals mediating alteration of these mRNAs in Mttp-IKO mice remain unknown, but our findings exclude changes in enterocyte cholesterol content as the trivial explanation.

In regard to the functional distinctions in lipid malabsorption between the Mttp-IKO and HuBTg<sup>+</sup>/0, Apob<sup>−/−</sup> mice, homologous recombination at the Mttp locus after tamoxifen administration was sporadic, with up to 20% residual MTTP mRNA or protein detectable, even in those functionally characterized (on the basis of steatorrhea) as Mttp-IKO. However, it is worth noting that studies (examples below) using pharmacologic MTTP inhibitors generally report an ~80% inhibition, and even the liver-specific Mttp deleter mice retained up to 5% MTTP mRNA/activity (7). As a practical matter, ~50% of the tamox-
ifen-injected mice were functionally Mttp-IKO. The likely cause of this variable deletion is the mosaic pattern of Cre expression, (data not shown and Ref. 15), a pattern noted with other intestinal (e.g. L-FABP) promoters (28). Although a horizontal gradient (duodenum-ileum) of MTTP mRNA and protein expression was found in wild type mice, the decrease in MTTP mRNA and protein in Mttp-IKO mice affected all regions without evidence of selective sparing (data not shown). These findings suggest that Mttp deletion is complete in 100% of the villi in which homologous recombination has occurred, whereas residual MTTP expression is accounted for by a few patches of cells in which Cre expression has not been activated. This caveat noted, we observed similar values for cholesterol absorption (i.e. ~30% of control values) in both the entire cohort of Mttp-IKO mice as well as in a subgroup of 11 animals with >97% Mttp deletion, supporting the conclusion that a fraction of cholesterol absorption may be independent of MTTP. In addition, the findings with respect to decreased enterocyte apoB48 secretion were qualitatively similar in the entire cohort of Mttp-IKO mice (~50% decrease) compared with a selected group of Mttp-IKO mice with less than 5% residual MTTP (~80% decrease).

The findings with regard to intestinal apoB48 secretion in Mttp-IKO mice are particularly intriguing in regard to the apoB isoform-specific requirements for MTTP. Previous results in liver-specific Mttp deletor mice demonstrated that the secretion of apoB100 was virtually eliminated, whereas the secretion of apoB48 was only slightly decreased (7, 13). We note, however, that other workers found the secretion of both apoB100 and apoB48 to be eliminated in liver-specific Mttp deletor mice (29). Nevertheless, the findings in liver-specific Mttp deletor mice are consistent with earlier findings of Wang and colleagues in McA rat.
hepatoma cells (30) and more recent findings of Kulinski and colleagues (12), who used pharmacologic inhibition of MTTP activity in wild type murine primary hepatocytes to examine the synthesis and secretion of apoB isoforms. These latter workers demonstrated an ~90% decrease of apoB100 secretion with MTTP inhibition, with minimal effects on apoB48 secretion (12). The current findings reveal a similar pattern with respect to apoB100 secretion from murine enterocytes, which was decreased by ~90% after the addition of the MTTP inhibitor. However, unlike the findings in hepatoma cells and primary murine hepatocytes from liver-specific Mttp deleter mice, apoB48 secretion from murine enterocytes was decreased significantly (by 50–80%) with either pharmacologic or genetic deletion of MTTP. These new findings collectively reinforce the paradigm that apoB100 secretion, whether from enterocytes or hepatocytes, is profoundly sensitive to limiting abundance or activity of MTTP. A role for the LDL receptor has been established in salvaging apoB100 secretion from hepatocytes of liver-specific Mttp deleter mice but is unlikely to play a significant role in regulating either hepatic or, by inference, intestinal apoB48 secretion (13). However, the current findings demonstrate that, unlike the situation in murine hepatocytes or hepatoma cells, intestinal apoB48 secretion is modulated by MTTP activity or abundance.

The finding that a significant fraction of intestinal apoB48 is presumably capable of undergoing adequate folding, transport, and secretion in the absence of MTTP raises the corollary questions of the nature of the particle(s) secreted and their associated lipid. Previous studies in murine primary hepatocytes demonstrated that lipidation of apoB48 is decreased by MTTP inhibitors, shifting the density of microsomal apoB48 toward lipid-poor particles (12). The current findings demonstrate that the residual secretion of apoB48 in Mttp-IKO mice occurs most likely in association with dense, lipid-poor particles. The nature of these particles is unknown, but it is reasonable to speculate that they would correspond to the so-called “first step” primordial intracellular particle described by Hamilton and colleagues (16). Our ultrastructural studies revealed no large, VLDL- or chylomicron-size particles within the endoplasmic reticulum or Golgi cisternae of Mttp-IKO mice, but small, HDL-sized particles would be undetectable even at the highest magnification.

An unanticipated consequence of intestine-specific Mttp deletion was the decrease in plasma and hepatic lipid levels and the accompanying increases in hepatic lipogenesis. These changes were particularly unexpected because previous studies in adult HuBTg+/0, Apob−/− mice had demonstrated no significant changes in plasma cholesterol or TG levels and no alteration in hepatic TG content. Further examination of metabolic adaptations in HuBTg+/0, Apob−/− mice revealed that maintenance of plasma lipid levels was accomplished through more rapid turnover and utilization of plasma nonesterified fatty acid as a substrate for hepatic TG production rather than an alteration in de novo lipogenesis (31). This adaptation was accomplished without changes in the circulating levels of plasma nonesterified fatty acids or hepatic TG content. By contrast, the current findings in Mttp-IKO mice demonstrate a decrease in plasma free fatty acid levels, decreased hepatic TG content, and a striking increase in hepatic lipogenesis together with evidence for up-regulation of several lipogenic genes, including SREBP1c, fatty acid synthase, and HMG-CoA reductase. These findings suggest that maintenance of the hepatic TG pool in HuBTg+/0, Apob−/− mice is accomplished through a combination of apoB-independent fatty acid absorption and a shift in the utilization of adipose fatty acid stores. By contrast, intestinal fatty acid delivery appears substantially blocked in the Mttp-IKO mice, resulting in compensatory up-regulation of hepatic lipogenesis associated with decreased hepatic TG pool size. Taken together, the findings point to an important and unexpected dialogue between intestinal TG secretion and hepatic lipid metabolism, the basis for which will be the focus of future investigation. The accompanying increases in hepatic mRNA abundance of HMG-CoA reductase and ABCG5/G8 also suggest that compensatory alterations take place with respect to hepatic cholesterol production and mobilization. These current findings imply that selective, tissue-specific inhibition of MTTP, in this instance intestinal deletion, may not be without consequences for other tissues, specifically the liver. Further study will thus be required before intestinal MTTP inhibition can be proposed as a therapeutic option (32), for example in regulating plasma lipid levels or in patients with extreme obesity.

Acknowledgments—We acknowledge the assistance of the Morphology and Mouse Cores of the Washington University Digestive Diseases Research Core Center.
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