Conditional Intestinal Lipotoxicity in Apobec-1−/− Mttp-IKO Mice

A SURVIVAL ADVANTAGE FOR MAMMALIAN INTESTINAL APOLIPOPROTEIN B mRNA EDITING

Received for publication, June 29, 2007, and in revised form, September 4, 2007 Published, JBC Papers in Press, September 12, 2007, DOI 10.1074/jbc.M705386200

Yan Xie‡, Jianyang Luo‡, Susan Kennedy‡, and Nicholas O. Davidson*†

From the Departments of ‡Medicine and ‡Pharmacology and Molecular Biology, Washington University School of Medicine, St. Louis, Missouri 63110

Mammalian small intestinal lipid absorption requires the coordinated interactions of apolipoprotein B (apoB) and the microsomal triglyceride transfer protein (Mttp). The observation that apoB100 displays greater dependence on Mttp availability than does apoB48 prompted us to examine the phenotype of Mttp deletion in an Apobec-1−/− background (i.e. apoB100 Mttp-IKO). 20% apoB100 Mttp-IKO mice died on a chow diet, and >90% died following high fat feeding (versus 0 and 11% apoB48 Mttp-IKO mice, respectively). Intestinal adaptation occurred in apoB48 Mttp-IKO mice in response to high fat feeding, evidenced by increased bromodeoxyuridine incorporation and villus lengthening, changes that did not occur in apoB100 Mttp-IKO mice. There was an exaggerated unfolded protein response (UPR), which became more pronounced in apoB100 Mttp-IKO mice upon initiation of high fat feeding. Tauroursodeoxycholate administration abrogated the UPR but produced an unexpected acceleration in the onset of lethality in apoB100 Mttp-IKO mice. The findings demonstrate that there is activation of the UPR with lethal lipotoxicity in conditional intestinal apoB100 Mttp-IKO mice. Together the data provide the first plausible biological evidence for a survival advantage for mammalian intestinal apoB mRNA editing.

Lipid absorption and the secretion of triglyceride (TG)²-rich lipoproteins (chylomicrons) from enterocytes of the mammalian small intestine are critically dependent on the coordinated interactions of two dominant genes. These include the structural protein apolipoprotein B (apoB) and the resident endoplasmic reticulum (ER) protein microsomal triglyceride transfer protein (Mttp) (1). Proper lipidation of apoB is achieved through the lipid transfer functions of Mttp, and this step is essential to maintaining the conformational integrity of the nascent apoB protein as it enters the luminal ER and initiates nascent lipoprotein biogenesis (1). Limiting lipid availability or defective expression and/or function of Mttp leads predictably to a failure of lipidation of the nascent apoB polypeptide, which in turn causes its misfolding and presecretory degradation (reviewed in Ref. 2). The physiological relevance of this pathway is illustrated by the phenotype associated with abetalipoproteinemia where mutations in the Mttp gene are associated with failure of lipid export from both enterocytes and hepatocytes and serum levels of apoB are extremely low or undetectable (3).

The requirements for Mttp-mediated lipidation of the apoB protein exhibit an important pattern of isoform dependence. Studies in hepatoma cells and in primary murine hepatocytes using pharmacological inhibitors of Mttp have demonstrated that apoB100 is dramatically more susceptible to presecretory degradation with limiting Mttp than is the shorter isoform, apoB48 (4, 5). These findings were reproduced in mice with conditional genetic deletion of Mttp in which apoB100 secretion from isolated hepatocytes was virtually eliminated with minimal effects on the secretion of apoB48 (6, 7), although it is worth noting that other workers demonstrated a comparable decrease in the secretion of both isoforms from hepatocytes in their Mttp deletor mice (8). These findings collectively imply a functional, isoform-specific divergence in the environmental modifiers and genetic interactions of apoB with Mttp that has important ramifications for intestinal lipid transport.

The species- and tissue-specific production of two apoB isoforms in mammals is the result of post-transcriptional C to U RNA editing of the nuclear apoB mRNA (9, 10). ApoB mRNA editing is a developmentally regulated event in the mammalian small intestine (11–14), mediated by a program of increased abundance of intestinal apobec-1 (the catalytic deaminase) along with a developmentally orchestrated expression profile of additional auxiliary factors (15, 16) that likely modulate the stoichiometry of the holo-editing enzyme complex (11, 12, 16). This developmental program results in progressively increasing proportions of the edited apoB mRNA during late gestation and early neonatal development along with corresponding increases in the production of apoB48 (11, 13, 17). However,

* This work was supported by National Institutes of Health Grants HL-38180 and DK-56260 (to N. O. D.) and by Digestive Disease Research Core Grant DK-52574, particularly the morphology core. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: GI Division, Washington University School of Medicine, 660 South Euclid, St. Louis, MO 63110. Tel.: 314-362-2027; Fax: 314-362-2033; E-mail: nod@wustl.edu.

‡ The abbreviations used are: TG, triglyceride; apoB, apolipoprotein B; Mttp, microsomal triglyceride transfer protein; IKO, intestine-specific knockout; ER, endoplasmic reticulum; UPR, unfolded protein response; Q-PCR, quantitative reverse transcription-PCT; TUDCA, tauroursodeoxycholic acid; BrdUrd, bromodeoxyuridine.
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despite considerable work on the developmental and nutritional regulation of apoB mRNA editing, the biological significance of this adaptation remains unknown. Targeted deletion of Apobec-1 yielded apoB100-only mice that appear phenotypically unremarkable (18) with only a subtle defect in intestinal lipid transport kinetics but no overt malabsorption or lipid accumulation within either intestinal enterocytes or hepatocytes (19, 20). These findings, along with complementary results from other gene targeting approaches that generated apoB100-only mice (21), suggest that apoB100 and apoB48 are for the most part interchangeable with respect to lipid mobilization from small intestinal enterocytes.

We recently generated mice with conditional deletion of intestinal Mttp in a wild-type Apobec-1 background (i.e. apoB48 Mttp-IKO) and showed that enterocytes were grossly lipid filled along with decreased (but not eliminated) secretion of apoB48 (22). On a chow diet, these animals gain less weight than littermate controls but survive for several months following induction of the conditional deletor allele. In view of the isoform-specific dependence on Mttp availability in modulating the presecretory degradation of apoB100 versus apoB48, we undertook to examine the phenotype of conditional Mttp deletion in the Apobec-1−/− background (i.e. apoB100 Mttp-IKO). We reasoned that targeted deletion of Mttp in the apoB100-only background might exacerbate intestinal lipid accumulation even beyond what was observed previously in the apoB48 Mttp-IKO line (22). We further examined the effects of exposure to high fat intake either in the adult setting or during suckling in both the apoB48 Mttp-IKO and apoB100 Mttp-IKO lines. The results of these studies suggest that Mttp insufficiency produces conditional intestinal lipotoxicity, which was markedly exaggerated in the apoB100-only background. The findings collectively provide the first biological evidence for a survival advantage to apoB mRNA editing in the mammalian intestine.

MATERIALS AND METHODS

Animals—Mttpflox/flox villin-Cre-ERT2 deletor mice were generated as previously described (22) and maintained in a mixed genetic background (~75% C57BL/6, ~25% 129/SvJ) and referred to hereafter as apoB48 Mttp-IKO mice. This line was crossed into the Apobec-1−/− background (18), which is maintained in a congenic C57BL/6 background, to generate apoB100 Mttp-IKO mice, again in a mixed genetic background (~75% C57BL/6, 25% 129/SvJ). Genotyping was performed as previously detailed (22). Cre recombinase expression in villus enterocytes was induced in adult mice by injection of tamoxifen (Sigma, 50 μg/g body weight daily for 5 days) as previously described (22). This injection protocol was modified for the neonatal mice, because 100% wild-type pups died following either five or three daily injections of tamoxifen (data not shown). As illustrated in the legend to Fig. 1, we settled on a single dose of 20 μg/g tamoxifen, because this was tolerated by Mttp wild-type mice. Animals consumed a regular low-fat mouse chow (PicoLab rodent chow) or, where indicated, a high fat Western diet containing 20% milk fat and 0.15% cholesterol (Harlan Teklad, TD 88137), a high polyunsaturated fat diet containing 20% safflower oil (ICN Research Diets, 960244), or a chow diet supplemented with 2% cholesterol (ICN Research Diets, 904691). In selected animals, where indicated in the figure legend, tauroursodeoxycholic acid (TUDCA, Calbiochem, 580549) was administered by daily intraperitoneal injection (250 mg/kg (23) twice daily upon initiating high fat intake and continued for the duration of the experiment. Animals were studied at 10 days, and the indicated tissues were harvested for study.

BrdUrd Incorporation and Histological Analysis—Animals were injected intraperitoneally with BrdUrd (120 mg/kg) and 12 mg/kg fluorodeoxyuridine 2 h prior to sacrifice. Intestinal samples were taken from corresponding regions of the proximal and distal small intestine in mice of all genotypes to make appropriate comparisons. Samples were fixed in 10% buffered formalin and embedded in 2% agar for optimal orientation prior to sectioning and staining with hematoxylin. BrdUrd incorporation was assessed by immunostaining with anti-BrdUrd IgG (Accurate Chemical, H8365) and secondary staining with anti-rat IgG (Jackson Laboratories). Quantitation was conducted by counting stained nuclei in fully visualized crypts, by individuals blinded to the genotype. Villus length was quantitated using National Institutes of Health ImageJ software and reflected assessment of at least 50 individual villi per mouse from representative (generally three to four mice per genotype) animals by an individual blinded to genotype.

Lipid Determinations and Western Blotting—Mucosal scrapings were prepared from the small intestine and frozen at −80 °C until analyzed. Aliquots of frozen tissue were extracted, and individual lipid classes were assayed as previously detailed (22). Mucosal samples were also subjected to protein analysis following denaturing preparation of tissue lysates and SDS-PAGE. Samples were probed for Mttp and apoB abundance as previously described (22). Antibodies to eIF2α (total and phosphorylated) were purchased from Cell Signaling Technology Inc. (catalog numbers 9722 and 3597).

Real-time Q-PCR—RNA was extracted from mucosal scrapings using TRIzol (Invitrogen) as previously detailed, and cDNA was generated using the SuperScript II first-strand synthesis system (Invitrogen). Real-time Q-PCR was performed in triplicate on an ABI Prism 7000 Sequence Detection System using SYBR green and the commercial Master Mix. The relative mRNA abundance of individual genes was normalized to 18 S RNA using primers detailed in Table 1.

Statistical Analyses—Survival analyses were conducted using Kaplan-Meier statistics and performed using Prism 4.0 (GraphPad, San Diego, CA). Other comparisons were made using paired or unpaired t-statistics using as supplied by the manufacturer (Microsoft Excel). All data are presented as means ± S.E. for the indicated number of independent observations.

RESULTS

High Fat Feeding Exacerbates Conditional Lethality in ApoB100-Mttp IKO Mice—We observed that 20% of apoB100 Mttp-IKO mice (2 of 10) died within ~2 weeks following Cre induction while consuming a low fat chow diet (Fig. 1A). However, there was a striking phenotype associated with intake of a high fat (Western) diet where >90% apoB100 Mttp-IKO mice (17 of 18) died, compared with ~11% mortality in the apoB48
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TABLE 1
Oligodeoxyribonucleotide primer sequences for real-time Q-PCR

| Gene       | Forward primer       | Reverse primer       |
|------------|----------------------|----------------------|
| Atf4       | 5'-caggttaaagacatttcctggagct-3' | 5'-ttcgcgtgtaaagagccaatt-3' |
| Bax        | 5'-gtgctgtcacaagaaggtctc-3' | 5'-gcctccaggtcattgcttc-3' |
| Casp3      | 5'-ctggacgatgctgatgcttgac-3' | 5'-tgctaaaaagcgagaacactca-3' |
| Chop       | 5'-ccaccaacacgagacyaggctt-3' | 5'-gaagatcctctacgccatgcttc-3' |
| Grp78      | 5'-acccggagagcagcagctctc-3' | 5'-ccaccaacacgagacyaggctt-3' |
| Ireα       | 5'-cgcacgcttgactggtccttc-3' | 5'-gagtccgcagcagctctacgccatgcttc-3' |
| Ireβ       | 5'-cgagttaagcacattcctgg-3' | 5'-gagtccgcagcagctctacgccatgcttc-3' |
| Xltp-1(s)  | 5'-gactgctgaccaggaggctc-3' | 5'-gagtccgcagcagctctacgccatgcttc-3' |

FIGURE 1. Conditional lipotoxicity following Mttp deletion in apoB100-only mice. Mttp-IKO mice of the indicated apoB genotype (either wild-type, i.e. apoB48 or Apobec-1/-/-, i.e. apoB100) and their respective littermate Mttp/-/- controls were studied for the indicated times following induction of intestinal Cre expression (22). Adult animals (8–12 weeks) were allowed ad libitum access to the following. A, low fat mouse chow; 10 apoB48 Mttp-IKO mice and 10 apoB100 Mttp-IKO mice were studied along with 5 apoB48 and 5 apoB100 littermate Mttp/-/- controls. B, high saturated fat Western diet; 18 apoB48 Mttp-IKO mice and 18 apoB100 Mttp-IKO mice were studied along with 8 apoB48 and 8 apoB100 littermate Mttp/-/- controls. 2 of 18 apoB48 Mttp-IKO mice died while 17 of 18 apoB100 Mttp-IKO mice died. All of the littermate controls survived. C, high polysaturated fat diet; 5 apoB100 Mttp-IKO mice were studied along with 5 apoB100 littermate Mttp/-/- controls. All the Mttp-IKO mice died while all the controls survived. D, 2% cholesterol supplemented low fat mouse chow diet; 5 apoB100 Mttp-IKO mice were studied along with 5 apoB100 littermate Mttp/-/- controls. All the Mttp-IKO mice and all the controls survived. E, conditional Mttp deletion in neonatal mice. Neonatal mice of the indicated genotype were administered tamoxifen (20 μg/g) by a single intraperitoneal injection at 4 days of age (arrow). 11 apoB100 Mttp-IKO pups were studied (all of which died) and 14 apoB48 Mttp-IKO pups (11 of which eventually died, although with delayed onset of lipotoxicity). 7 apoB48 littermate Mttp/-/- controls and 4 apoB100 littermate Mttp/-/- controls were also studied, all of which survived.

Mttp-IKO mice (where only 2 of 18 died) (Fig. 1B). The effect of Western diet feeding on the mortality observed in apoB100 Mttp-IKO mice was reproduced by feeding a high polysaturated fat diet (Fig. 1C), suggesting that either saturated or unsaturated fat feeding yield similar phenotypes in this background. By contrast, intake of a 2% cholesterol diet appeared to be well tolerated by apoB100 Mttp-IKO mice (Fig. 1D), suggesting that augmented cholesterol intake is not responsible for the toxic effects of the Western diet. We further examined the effects of conditional Mttp deletion in neonatal mice. This proved to be technically challenging owing to the toxic effects of tamoxifen in all genotypes of neonatal mice (data not shown). However, using a dosing schedule that was tolerated in wild-type animals, we found that there was increased sensitivity to Mttp deletion in the apoB100 genotype, resulting in increased lethality (Fig. 1E).

Conditional Lipotoxicity in ApoB100-Mttp IKO Mice Does Not Reflect Differences in Intestinal Lipid Content—In seeking to understand the mechanisms underlying the lethality associated with Mttp deletion in the apoB100 background, we reasoned that the previously described isoform-dependent differences in apoB degradation with limiting Mttp might be reflected by augmented lipid accumulation in small intestinal mucosa from apoB100-Mttp-IKO mice. However, this was not the case. Mucosal samples from animals of the four genotypes analyzed at 7, 14, and 28 days following high fat intake revealed a consistent pattern of increased TG in the setting of Mttp deletion, but with no differences between the apoB genotypes (Fig. 2, A–C). The values for mucosal TG content in these high fat fed animals (~600–1000 μg/mg) are comparable to those seen previously in chow fed Mttp-IKO mice (22), suggesting that there is an apparent threshold for enterocyte TG accumulation. Mucosal cholesterol and free fatty acid content increased at later times following Mttp deletion, but again there was no striking difference by apoB genotype (Fig. 2, A–C). These findings suggest strongly that quantitative differences in intestinal lipid accumulation alone are unlikely to account for the toxicity observed in apoB100-Mttp-IKO mice.

Intestinal Adaptation Is Defective in ApoB100-Mttp IKO Mice—We were intrigued by the possibility that differences in intestinal adaptation might account for the phenotypes observed in the setting of fat malabsorption following Mttp deletion. To pursue this possibility, we turned to an examination of BrdUrd incorporation along with morphometric evaluations of villus length in high fat fed animals of the various genotypes. The findings demonstrate a significant increase in crypt proliferation in apoB48 Mttp-IKO mice versus their littermate apoB48 controls (Fig. 3). By contrast, crypt proliferation as revealed by BrdUrd incorporation was not increased in apoB100 Mttp-IKO mice (Fig. 3), suggesting that intestinal adaptation fails to occur in this genotype. These findings were confirmed independently by quantitating villus length in com-
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A. Mucosal lipid content in Western diet-fed mice reveals comparable increases in Mttp-IKO mice of either apoB48 or apoB100 genotype. Animals of the indicated genotype were fed a high fat Western diet for either 7 days (A), 14 days (B), or for 4 weeks (C). At the conclusion of the feeding period, mucosal extracts were prepared for lipid extraction and quantitation of individual classes ("Materials and Methods"). A. 7-day Western diet feeding was examined in 3 apoB100 Mttp-IKO mice, 7 apoB48 Mttp-IKO mice, 4 apoB100 littermate Mttp+/+ controls, and 5 apoB48 littermate Mttp+/+ controls. B. 14-day Western diet feeding was examined in 5 apoB100 Mttp-IKO mice, 5 apoB48 Mttp-IKO mice, 5 apoB100 littermate Mttp+/+ controls, and 5 apoB48 littermate Mttp+/+ controls. C. 4-week Western diet feeding was examined in 3 apoB100 Mttp-IKO mice, 5 apoB48 Mttp-IKO mice, 3 apoB100 littermate Mttp+/+ controls, and 4 apoB48 littermate Mttp+/+ controls. Note that the 3 surviving apoB100 Mttp-IKO mice were derived from a different cohort than presented in Fig. 1, where only 1 animal survived. *, p < 0.05; **, p < 0.01; †, nmol/mg protein. ‡, p < 0.05 between apoB100 Mttp-IKO mice and apoB48 Mttp-IKO mice.

B. Intestinal proliferation is increased in apoB48 Mttp-IKO mice but not apoB100 Mttp-IKO mice following 7-day Western diet feeding. Animals of the indicated genotype were injected with BrdUrd prior to sacrifice, as detailed under "Materials and Methods." Intestines were fixed and sectioned and stained with anti-BrdUrd for analysis of crypt proliferation. A, apoB48 Mttp+/+ control; B, apoB48 Mttp-IKO; C, apoB100 Mttp+/+ control; and D, apoB100 Mttp-IKO. Representative crypts from three to four animals per genotype were analyzed. E. bar graphs representing BrdUrd incorporation by genotype, shown as mean ± S.E. * indicates significance p < 0.05; †, p < 0.05 between apoB100 Mttp-IKO mice and apoB48 Mttp-IKO mice.

C. A UPR Is Exaggerated in ApoB100 Mttp-IKO Mice—Previous studies have demonstrated that pharmacological Mttp inhibition or Mttp deletion greatly enhances apoB100 degradation in both enterocytes and hepatocytes (4, 5, 20, 22). We reasoned that the presence of incompletely lipidated apoB would result in the accumulation of degradation products that would in turn trigger an UPR and contribute to the lipotoxicity observed in apoB100 Mttp-IKO mice. Accordingly, we examined the expression of several of the known effector genes involved in this response (26–28). The findings demonstrate induction of Atf4, Chop, and Grp78 mRNA in Mttp-IKO mice of both apoB genotypes at both 7 and 14 days (Fig. 6, A and B). Additionally, there was a striking induction of these three genes in the surviving apoB100 Mttp-IKO mice at 4 weeks (Fig. 6C), suggesting that there is a progressive increase in the UPR in these few surviving animals. In addition, there was increased intestinal mRNAs were not informative at earlier time points in Mttp-IKO mice of either apoB genotype (Fig. 5). These findings suggest that alterations in villus apoptosis do not account for the differences in adaptation observed at 7 days in Mttp-IKO mice.
Alleviating ER-associated Stress and the UPR Exacerbates Lipotoxicity in ApoB100 Mttp-IKO Mice—Recent work in ob/ob mice has indicated that alleviation of ER stress produces abrogation of the UPR, which is accompanied by improvements in the metabolic profile of these animals, including decreased hepatic steatosis (23). In view of the findings above demonstrating induction of the UPR in apoB100 Mttp-IKO mice, we administered TUDCA to these (apoB100 Mttp-IKO) mice upon initiation of high fat feeding with the anticipation that alleviating ER stress would abrogate the lethality observed following intake of the Western diet. Surprisingly, the findings demonstrated quite the opposite. ApoB100 Mttp-IKO mice treated with TUDCA died earlier than untreated mice (Fig. 7A). TUDCA was effective in abrogating the UPR as inferred by the reduction in mRNA abundance for Xbp-1s in the treated apoB100 Mttp-IKO mice (Fig. 7B). However, pharmacological alleviation of ER stress accompanying TUDCA treatment was accompanied by decreased BrdUrd incorporation into intestinal crypts of apoB100 Mttp-IKO mice (Fig. 7C) and gross morphological disorganization of villi in both the proximal (Fig. 7, D and E) and distal small intestine (Fig. 7, F and G). It is worth emphasizing that TUDCA was administered intraperitoneally in these animals and that the phenotype most likely reflects the effects of systemic distribution rather than endoluminal uptake of this bile salt.

DISCUSSION

The findings herein demonstrate a dramatic and lethal phenotype in the Apobec-1−/− background following conditional intestinal deletion of Mttp. The results complement and extend the phenotypic characterization of adult mice following conditional Mttp deletion (22) in several important areas. First, the findings demonstrate that there is an apparent maximum threshold for murine intestinal TG accumulation, because mucosal lipid content in Mttp-IKO mice consuming a high fat...
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There is extensive evidence that indicates apoB100 is exquisitely sensitive to limiting availability or function of Mttp. This evidence is derived from a variety of in vivo studies as well as in isolated murine hepatocytes and hepatoma cell lines (5–7, 29, 30). Previous findings in isolated murine enterocytes prepared from wild-type or Apobec-1−/− mice demonstrated that pharmacological inhibition of Mttp decreased apoB48 secretion but virtually eliminated secretion of apoB100 (22). These findings collectively suggest that there is a hierarchy of sensitivity to limiting Mttp, which is recapitulated in murine enterocytes expressing different apoB genotypes. In attempting to reconcile these prior observations with the findings presented in the current report, we recalled that the functional expression of these distinct mammalian intestinal apoB genotypes is itself a regulated event. Intestinal apoB gene expression is detectable in the developing murine and human embryo, in both instances with the dominant isoform initially apoB100 (11, 17). During fetal maturation, a developmentally regulated transition occurs to the adult apoB genotype (i.e. predominantly apoB48), mediated by increased expression of apobec-1, which is virtually complete prior to birth (13, 14). This developmental pattern roughly coincides with the expression profile of Mttp, which increases in mammalian intestine prior to the onset of suckling (31, 32), although to our knowledge simultaneous profiling of these two processes has not been formally detailed. These developmentally orchestrated events would presumably facilitate an optimal response to high fat intake in the neonatal suckling period, by simultaneously maximizing lipid mobilization with apoB48 during a transition period of potentially limiting Mttp. This suggestion, along with recent findings from Hussain and col-

diet was not appreciably different from that in Mttp-IKO mice fed a chow diet (22). Furthermore, this threshold effect was recapitulated in both apoB genotypes. These findings, taken in conjunction with results (not shown) demonstrating >95% decrease in Mttp gene expression in mucosal extracts, suggest that intestinal Mttp deletion is virtually complete in both the apoB48 and apoB100 lines. Secondly, and by corollary to the findings summarized above, there is a critical intestinal adaptation that takes place in high fat fed Mttp-IKO mice in the apoB48 background, which includes increased crypt proliferation and villus lengthening. This adaptation fails to occur in apoB100 Mttp-IKO mice. Thirdly, an ER stress response is evident upon high fat feeding in Mttp-IKO mice with a sustained and progressive UPR in the surviving apoB100 Mttp-IKO mice. Pharmacological intervention to abrogate ER stress produced an accelerated lethality in the apoB100 Mttp-IKO mice, associated with reduced crypt proliferation and gross villus distortion. Each of these observations merits additional discussion.

The current findings demonstrate the expected increase in mucosal TG accumulation following conditional Mttp deletion but revealed no systematic differences by apoB genotype. These observations are consistent with prior findings in Apobec-1−/− mice that demonstrated a subtle defect in intestinal chylomicon secretion rate following bolus lipid administration both in vivo and in isolated enterocytes (19, 20). One important conclusion from these earlier studies was that for the most part apoB100 and apoB48 function interchangeably in regard to intestinal lipid absorption, because Apobec-1−/− mice appear to grow normally and without evidence of fat malabsorption when fed a high fat diet (19). Similar conclusions were reached by Farese and colleagues (21), who used Apob gene targeting to create an apoB100-only line that also demonstrated indistinguishable intestinal lipid absorption kinetics compared with either littermate controls or to their apoB48-only mice, without overt steatorrhea even while consuming a high fat diet. Taken together, these findings support the concept that intestinal apoB100 functions efficiently in directing triglyceride-rich lipoprotein assembly and bulk lipid export while in an Mttp-sufficient background.

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leagues (33) demonstrating rapid, cyclic variations in Mttp gene expression in murine tissues, permit us to speculate that apoB48 would provide a functional advantage for intestinal lipid export under situations where Mttp was limiting, perhaps developmentally or during diurnal cycling. The findings reported herein demonstrate that neonatal mice are exquisitely sensitive to Mttp deletion and indicate that the lethality is exaggerated in the apoB100-only background. By extension, the results point to an unanticipated biological advantage for mammalian apoB mRNA editing. We would predict with this scenario that the diurnal or dietary-induced cycling of Mttp gene expression in the mammalian intestine could result in lipotoxic injury in an apoB100 background, exacerbated for example by hypoxic or other environmental stressors. This would be particularly relevant during the neonatal and suckling period. It is worth noting that even though adult mammalian small intestinal apoB mRNA editing is generally ~90% complete, the horizontal (i.e. duodenal-ileal) and vertical (i.e. crypt-villus) distribution of the remaining 10% unedited apoB transcripts (encoding apoB100) is entirely unknown.

An important but unresolved question arising from the current findings is the nature of the stimulus mediating the adaptive response to high fat feeding noted in the apoB48 Mttp-IKO mice. As detailed above, we consider it unlikely that quantitative differences in mucosal lipid accumulation alone account for this effect, because there were no differences between chow fed and high fat fed Mttp-IKO mice or between Mttp-IKO mice of the different apoB genotypes. It is possible that exacerbation of the steatorrhea accompanying introduction of high fat feeding in Mttp-IKO mice results in adaptive signaling and there is certainly evidence of a proliferative response in intestinal enterocytes following intestinal bypass surgery or resection that may reflect increased luminal fatty acid content (24). TUDCA administration to apoB100 Mttp-IKO mice abrogated the ER stress response but also reversed the adaptive changes in intestinal proliferation and shortened the time to lethality. This suggests the intriguing possibility that components of the UPR may actually be essential to the adaptive response to intestinal Mttp deletion in apoB100-only mice, a suggestion consistent with the observation that the few long term apoB100 Mttp-IKO survivors all demonstrated dramatic up-regulation of genes involved in this response (Fig. 6C). This suggestion is also consistent with the independent observation that transient expression of the phosphorylated form of eIF2α induced a preconditioned, stress-resistant state that rendered cells less susceptible to other stress-induced signals (34). It is also worth noting that TUDCA has been shown to stabilize mitochondrial membranes and thereby to reduce apoptosis in isolated rat hepatocytes (35).
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Whether such a membrane-stabilizing effect occurs within enterocyte mitochondria following parenteral TUDCA administration is unknown. Nevertheless, our findings suggest that, even were this to occur, the outcome would be dramatically worse following TUDCA treatment.

A related question concerns the nature of the one or more signals that trigger the UPR following high fat feeding in Mttp-IKO mice. Multiple lines of evidence suggest that saturated fatty acids, and particularly palmitate, are potent inducers of ER stress in cultured cell lines, and considerable attention has focused on the likely pathways by which this response is mediated (36–39). These pathways include activation of Xbp-1s as well as other downstream pathways (23, 38), some of which have been demonstrated to intersect with pro-apoptotic pathways such as Bax (40), that are demonstrated in the current studies to be up-regulated in apoB100 Mttp-IKO mice. Along these lines, it is tempting to speculate that the increased expression of Bax in mucosal RNA from surviving apoB100 Mttp-IKO mice at 4 weeks may reflect the progressive UPR observed in these animals and that this integrated response may be an effective adaptation, rather than a manifestation of progressive toxicity. Other work has demonstrated that cholesterol accumulation can also initiate an UPR and macrophage apoptosis (41). We found that the lethal effects of high fat feeding were if anything more severe in polyunsaturated fat-fed (versus Western diet fed) apoB100 Mttp-IKO mice, but that cholesterol supplementation appeared to be tolerated. This latter conclusion should be interpreted with caution, however, because cholesterol absorption is severely limited in Mttp-IKO mice (22). Another possibility is that the elimination of apobec-1 itself, rather than the apoB100-only status of these animals, imposes a further critical level of post-transcriptional regulation of target genes involved in intestinal adaptation. Evidence in this regard would be analogous to the finding that apobec-1 is an AU-rich RNA-binding protein that mediates alterations in cox-2 mRNA stability, which in turn modify the production of pro-proliferative prostanoids such as prostaglandin E2 (42).

In summary, the findings illustrate a dramatic intestinal phenotype associated with Apobec-1 deletion in Mttp-IKO mice. The results represent the first demonstration of a functional survival advantage to apoB48 versus apoB100 and establish a biologically plausible role for mammalian intestinal apoB mRNA editing. The mechanisms and signaling pathways by which these adaptive responses are mediated in the different apoB genotypes will require further work and will be the focus of future reports.

Acknowledgments—We are most grateful to our colleagues Valerie Blanc, Deborah Rubin, Libby Newberry, Zhouji Chen, and Soo-Jin Cho for stimulating discussions.

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