Why does the gut choose apolipoprotein B48 but not B100 for chylomicron formation?

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Lo CM, Nordskog BK, Nauli AM, Zheng S, vonLehmden SB, Yang Q, Lee D, Swift LL, Davidson NO, Tso P. Why does the gut choose apolipoprotein B48 but not B100 for chylomicron formation? Am J Physiol Gastrointest Liver Physiol 294: G344–G352, 2008. First published November 15, 2007; doi:10.1152/ajpgi.00123.2007.—Chylomicrons produced by the human gut contain apolipoprotein (apo) B48, whereas very-low-density lipoproteins made by the liver contain apo B100. To study how these molecules function during lipid absorption, we examined the process as it occurs in apobec-1 knockout mice (able to produce only apo B100; KO) and in wild-type mice (of which the normally functioning intestine makes apo B48, WT). Using the lymph fistula model, we studied the process of lipid absorption when animals were intraduodenally infused with a lipid emulsion (4 or 6 mol/l of triolein). KO mice transported triacylglycerol (TG) as efficiently as WT mice when infused with the lower lipid dose; when infused with 6 mol/l of triolein, however, KO mice transported significantly less TG to lymph than WT mice, leading to the accumulation of mucosal TG. Interestingly, the size of lipoprotein particles from both KO and WT mice were enlarged to chylomicron-size particles during absorption of the higher dose. These increased-size particles produced by KO mice were not associated with increased apo AIV secretion. However, we found that the gut of the KO mice secreted fewer apo B molecules to lymph (compared with WT), during both fasting and lipid infusion, leading us to conclude that the KO gut produced fewer numbers of TG-rich lipoproteins (including chylomicron) than the wild-type animals. The reduced apo B secretion in KO mice was not related to reduced microsomal triglyceride transfer protein lipid transfer activity. We propose that apo B48 is the preferred protein for the gut to coat chylomicrons to ensure efficient chylomicron formation and lipid absorption.

very low-density lipoprotein; lymph

TRIACYLGlycerol (TG) is the major component of human dietary fat. After consumption, TG molecules are hydrolyzed by pancreatic lipase yielding the products 2-monoglycerol (2-MG) and free fatty acids (FA), which are then able to be absorbed by the intestinal enterocytes either by passive diffusion or through carrier-mediated processes (5, 20, 27, 35). Following uptake by the enterocytes, the 2-MG and FA are resynthesized back to form TG through the MG pathway (17, 26). TG is then packaged with phosphatidylcholine, cholesteryl ester, free cholesterol, and apolipoproteins (apo) B48, A1, and AIV forming either chylomicrons or very-low-density lipoprotein-sized (VLDL) lipoproteins (36). The secretion of these respective intestinal lipoprotein particles is regulated by the physiological state of the small intestine; specifically, during the fasting state or during active lipid absorption. In periods of fasting, VLDL-sized lipoproteins are the predominant particles secreted by the small intestine (23, 31). After ingestion of a meal rich in TG, the small intestine continues to form VLDL; however, the predominant TG-rich lipoprotein particles secreted by the gut are the larger chylomicron particles (13, 32). An interesting question dealing with the mechanism of chylomicron formation is whether the intestine produces a larger number of chylomicrons, or an equivalent number of chylomicron particles that are larger, during active lipid absorption. The answer is that the number of chylomicron particles produced by the small intestine remains unchanged during active lipid absorption; to accommodate the large amount of lipid to be transported by the gut, the small intestine produced larger chylomicrons during this period (6, 13).

TG synthesized by the liver is packaged with apo B100 to form VLDL particles, which are subsequently secreted to plasma circulation. Using isolated hepatocytes in vitro, it has been demonstrated that incubation with oleic acid results in an increased production and secretion of TG into the culture media (9, 24). Additionally, a positive correlation was observed among hepatic TG secretion and apo B100 secretion (3, 10). Thus oleate supplementation increased the number of apo B particles secreted by the liver, but not the size, since the products secreted by the liver were consistently the smaller VLDL particles (8, 15, 38). Clearly, there are differences in the ways the gut and liver respond physiologically to increased lipid flux. The question at hand is whether apo B48 and apo B100 themselves play a role in mediating these differing physiological responses. The purpose of this study is to answer the question of whether or not there is a possible functional advantage of exclusive apo B48 expression in the small intestine for the purpose of chylomicron formation leading to the most efficient handling of dietary lipid loads.

Tissue-specific production of apo B48 is mediated by posttranscriptional cytidine deamination of the nuclear apo B transcript by an RNA-specific cytidine deaminase, apobec-1 (29). Targeted deletion of the apobec-1 gene yields mice in which only apo B100 is produced in both the liver and the small intestine; thus circulating lipoprotein particles contain exclusively apo B100 (14, 21–22). Earlier studies have shown no significant difference in total plasma TG concentrations between ad libitum fed or 4-h fasted knockout (KO) mice and

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wild-type (WT) mice (fed a standard chow diet), and that the KO mice appeared to be totally capable of absorbing both lipid and fat-soluble vitamins normally (14, 22). Thus it was originally concluded that apo B48 is not required for the assembly of intestinal lipoproteins and that apo B100 can serve as a substitute for apo B48 in the intestine, still resulting in normal lipid transport (12, 38). In contrast to these conclusions, however, was the observation that KO mice had higher TG concentrations in plasma chylomicron fractions and that KO mice accumulated lipid droplets within enterocytes after being fed a high-fat chow diet (16). These data seem to hint that lipids ingested by a KO animal are not absorbed and transported as efficiently as they are in WT mice and that the apo B100 chylomicrons are not as efficiently catabolized in circulation as the chylomicrons assembled with apo B48. More recent studies of apobec-1 KO mice bred into pure C57 genetic background substantiated these subtle differences in intestinal chylomicron assembly and secretion from isolated enterocytes. Thus it is necessary to reevaluate whether or not apo B48 and B100 are indispensable for transport lipid into lymph more efficiently than the apo B100-producing intestine (WT) absorbs and transports lipid into lymph more efficiently than the apo B100-producing intestine and whether the differences previously observed are reflective of the quantity of lipid presented for absorption.

MATERIAL AND METHODS

Animal Surgery and Postoperative Care

Male apobec-1 KO mice, backcrossed as described (14, 37) to yield animals that are almost of pure C57BL/6 genetic background. Six animals of each genotype were used in the modest-dose triolein study and seven mice of each genotype were used for the high-dose triolein study. The main mesenteric lymph duct of each animal was cannulated with a polyvinylchloride tube, which was then secured with a drop of Krazy Glue (4). A second polyvinylchloride tube was introduced into the stomach through the fundus and threaded into the duodenum. The fundal incision was closed by purse-string suture. After surgery, the animals were infused via duodenal catheter with a saline solution containing 5% glucose at a rate of 0.3 ml/h to compensate for fluid and electrolyte loss due to lymphatic drainage. The animals were allowed to recover for at least 24 h in restraining cages situated in a warm chamber (~30°C) prior to the start of the experiments. All procedures were approved by the University of Cincinnati Internal Animal Care and Use Committee and complied with the NIH Guide for the Care and Use of Laboratory Animals.

TG Infusion

Lipid was infused into KO mice (experimental group) and WT mice (control group) at two different doses. The lower dose lipid emulsion contained 4 μmol/h triolein (labeled with [9,10-3H (N)]oleic acid, 1 μCi/0.3 ml, Perkin Elmer, Boston, MA), 0.78 μmol/0.3 ml of cholesterol and phospholipid (PL; 0.78 μmol/0.3 ml) in a 19 mM sodium taurocholate solution. This lipid emulsion was infused intraduodenally to the KO and WT mice at a rate of 0.3 ml/h for 6 h.

Lymphatic, Mucosal, and Luminal Lipid Content Determined by Radioactive Assay

Fasting lymph was collected for 1 h prior to lipid infusion, and hourly lymph samples were collected for 6 h after the start of lipid infusion. At the end of the infusion period, the stomach, small intestine, and colon were taken from each animal and luminal lipids were collected by washing the lumen of the organs three times with a 0.5% sodium taurodeoxycholate solution. The small intestine (from duodenum to ileum) was divided into four equal-length segments and each of the four mucosal samples was used in the determination of radioactive lipid content. Radioactive luminal lipid content was determined by liquid scintillation counting.

Particle Size of Chylomicron and VLDL

To study the size of the lipoprotein particles, fresh lymph samples from three animals each of both WT and KO groups were stained with 2% phosphotungstic acid (pH 6.0) for 5 min and examined with a transmission electron microscope (TEM, JEOL, Peabody, MA) and images were documented with an AMT Advantage Plus CD camera (31). Eight hundred lipoprotein particles per group were measured by manual counting using a magnifying glass for further magnification of printed images of the respective fields of view. All images were evaluated blindly, without the examiner knowing the animals’ genotype, to avoid any bias in the sizing of the lipoprotein particles.

Lipoproteins in the diameter range between 200 and 800 Å were classified as chylomicrons (31).

Lymph Lipoprotein and Apolipoproteins Determination

Samples of mouse lymph were collected for 1 h and aliquoted (i.e., 1/60 of the volume collected over 60 min) to prepare the equivalent of a 1-min lymph collection. The rationale for using the 1-min sample is to overcome the variations in lymph flow between different time points, both within the same animal and also between animals of the same group. An equal volume of 2× SDS sample buffer was then added to each sample. Samples were boiled for 5 min and then loaded into a 4–20% Tris-HCl gradient gel (Bio-Rad Laboratories, Hercules, CA). Gels were run at a constant voltage (60 V) until the protein standards were well separated. Proteins were then transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories) for 2 h at a constant current of 350 mA. After blocking nonspecific binding sites on the membranes for 1 h with a 5% solution of nonfat milk in Tris-buffer saline with 0.1% Tween (TBS-T), membranes were then incubated with polycytoplasmic rabbit anti-rat apo B antibody (1:7,500 dilution in 5% nonfat milk in TBS-T) or with polyclonal goat anti-rat apo AIV antibody (1:7,500 dilution in 5% nonfat milk in TBS-T) or with polyclonal goat anti-rat apo AIV antibody (1:7,500 dilution in 5% nonfat milk in TBS-T). After incubation with the primary antibody, the blots were washed with 1X nonfat milk in TBS-T and then incubated either with horseradish peroxidase-conjugated goat anti-rabbit antibodies or with horseradish peroxidase-conjugated rabbit anti-goat antibodies (Dako, Cytomation) diluted 1:5,000 with 0.1% Tween (TBS-T), membranes were then incubated with polyclonal rabbit anti-rat apo B antibody (1:7,500 dilution in 5% nonfat milk in TBS-T) or with polyclonal goat anti-rat apo AIV antibody (1:7,500 dilution in 5% nonfat milk in TBS-T).

Detection of apo B was achieved by using the enhanced chemiluminescence system (ECL, Western Blotting Detection Reagents, Amersham Biosciences, Buckinghamshire, UK), and X-OMAT AR films (Kodak) were used for development and visualization of the membranes. Net apo B secretion during each subsequent hour of infusion was quantified by subtracting the 0 h (fasting) lymph apo B content from relative apo B levels at each hourly time point.

MTP Lipid Transfer Activity

After 4 h of triolein infusion (6 μmol triolein·0.3 ml⁻¹·h⁻¹, 0.78 μmol/0.3 ml of cholesterol, PL 0.78 μmol/0.3 ml in a 19 mM sodium
Lymphatic transport of the [3H]TG in KO mice was not of lipid infusion (44% of hourly infused lipid) (Fig. 2). The output became steady at the third hour after the beginning of fat absorption in either the WT or KO mice. From the second to sixth hour of lipid infusion, transport of the [3H]TG than WT mice in the first hour; however, this difference is not considered significant (Fig. 1). Unlike what we and others have observed in rats, lymph flow rate did not change during fat absorption in either the WT or KO mice (33).

Statistical Analysis

All values are means ± SE. Student’s t-test and two-way repeated ANOVA were performed for comparison of all groups throughout the 6-h lipid infusion. Statistical analyses were performed via GraphPad Prism (version 3.0, San Diego, CA), and differences were considered significant if P values were <0.05.

RESULTS

Modest Dose (4 μmol/h) Triolein Study

Lymph flow rate. The fasting lymph flow rate was 0.22 ml/h in the WT animals and 0.26 ml/h in the apobec-1 KO animals, a difference that is not statistically significant (Fig. 1). Unlike what we and others have observed in rats, lymph flow rate did not change during fat absorption in either the WT or KO mice (33).

Lymphatic [3H]TG output. KO animals had lower lymphatic transport of the [3H]TG than WT mice in the first hour; however, this difference is not considered significant (P = 0.064) (Fig. 2). From the second to sixth hour of lipid infusion, a similar increase in output was observed in both groups and the output became steady at the third hour after the beginning of lipid infusion (~58% of hourly infused lipid) (Fig. 2). Lymphatic transport of the [3H]TG in KO mice was not different from that of the WT animals during the subsequent 6-h infusion period (P = 0.93; not significant). Total recovery of lymphatic radioactive TG in the KO mice (45.56 ± 4.42%) was slightly lower than the WT animals (52.28 ± 3.12%), but again, this difference is not statistically significant (Fig. 3).

Luminal and mucosal recovery of [3H]TG. There was no statistical difference in the luminal recovery of [3H]TG between the WT and the KO animals at the end of the 6-h infusion of the modest dose of lipid infusate (Fig. 3). However, the accumulation of TG in the mucosa of KO mice (19.28 ± 2.50%) was significantly higher than that of WT mice (11.90 ± 1.02%, P < 0.02). These findings suggest that whereas WT and KO mice absorb the digestion products of ingested TG with comparable efficiency, the KO mice are less efficient in delivering the absorbed lipids to lymph as chylomicrons, resulting in greater accumulation of lipids in the mucosa of KO animals. When the recovery of radioactive mucosal lipids from the four equal-length intestinal segments was analyzed, most of the radiolabeled TG was detected in the proximal jejunal segment of the small intestine and some remained in the second segment; only trace amounts of the [3H]TG remained in the third and fourth intestinal segments in both KO and WT animals (Fig. 4). The KO mice (14.06 ± 1.43%) accumulated significa-

Fig. 1. Hourly lymphatic flow rate during continuous intraduodenal infusion of 4 μmol/h triolein. Six mice of both [knockout (KO) and wild-type (WT)] genotypes were used in each study (n = 6). Lymphatic flow was monitored during the 6-h lipid infusion period. Values are means ± SE. *P < 0.05.

Fig. 2. Lymphatic [3H]triglyceride (TG) transport into lymph during continuous intraduodenal infusion of 4 μmol/h of triolein. Mice were implanted with intraduodenal and lymph cannulas and were intraduodenally infused with a lipid emulsion containing labeled [3H]TG continuously for 6 h (n = 6). Lymph was collected hourly following the start of lipid infusion and analyzed. Values are means ± SE. *P < 0.05.

Fig. 3. [3H]TG content in the WT and KO mice at the end of 6-h continuous infusion of 4 μmol/h triolein. Luminal contents, small intestinal mucosa, and lymph were harvested at the end of the experiment and radioactive lipids were determined by scintillation counting (n = 6). Values are means ± SE. *P < 0.05.
cantly more radioactive TG than the WT mice (9.20 ± 0.85%) in the first segment of the small intestine. Thus the duodenum and the proximal jejunum are the major sites for lipid absorption in both KO and WT mice, but the KO mice are less efficient in exporting absorbed lipid to lymph.

Negative staining and sizing of the lymph lipoproteins. With slightly less lymphatic TG transport observed in KO mice compared with WT animals, we wondered whether this difference could be attributed to a difference in the size of the chylomicron particles produced by the enterocytes of the KO vs. the WT animals. In this study, fresh lipoproteins from lymph of both WT and KO mice were negatively stained and sized (31). The KO and WT animals secreted predominantly VLDL particles during fasting (99.0% and 99.3%, respectively) (Fig. 5A). In response to lipid feeding, the pattern of lipoprotein secretion in WT animals shifted from mostly VLDL-sized particles to particles of chylomicron size (lymph samples from the third and fourth hour were analyzed; data are shown in Fig. 5B). Of the total numbers of lymph lipoproteins counted, ~39% were in the chylomicron size range in the WT animals (the rest in the VLDL range), whereas the KO mice only had 12% of their total lipoprotein particles as chylomicrons during the third to fourth hour of lipid infusion. Thus there was more of a shift in size in the lymphatic lipoproteins secreted by WT mice during active fat absorption, whereas, in contrast, lymph lipoproteins secreted by KO mice mostly remained as VLDL-sized particles. This observed decrease in the production of chylomicron-sized particles is possibly reflective of the observation that KO mice seem to be slightly less efficient than WT mice in transporting absorbed TG as chylomicrons during active fat absorption.

High-Dose (6 μmol/h) Triolein Study

Lymph flow rate. As shown in Fig. 6, the fasting lymph flow was 0.23 ml/h in the WT and 0.28 ml/h in the KO animals, but this difference is not statistically significant. Following lipid infusion, the rate of lymph flow in both groups of mice fell; with the effect more marked in the KO animals. At the third hour following the start of lipid infusion, a significant difference was observed in the rate of lymph flow between the WT and the KO animals (P < 0.016).

Lymphatic [3H]TG output. As shown in Fig. 7, the KO mice transported lipids less efficiently than WT mice at the second hour (P = 0.017) and third hour (P = 0.003) of lipid infusion. The largest difference was observed at the third hour, when both groups reached their maximum TG output; the WT group...
measured 48.16 ± 3.91% of initial dose, whereas the KO mice only reached 26.75 ± 4.33% (P < 0.003) (Fig. 7). The total output of lymphatic TG of the KO mice (25.04 ± 3.86%) was significantly lower (P < 0.012) than that of the WT mice (38.65 ± 2.51%) (Fig. 8). These data showed that KO mice did not transport TG into lymph as efficiently as WT animals when the dose of the lipid emulsion infused increased by 50%.

**Lymph, luminal, and mucosal recovery of [3H]TG.** By the end of the 6 h lipid infusion period, there was a greater level of radioactive TG accumulation in the mucosa of KO mice than in WT mice (39.57 ± 3.86 vs. 21.66 ± 5.94% respectively, P < 0.026). This increase in overall mucosal accumulation of [3H]TG can be attributed to the vast difference in radioactivity observed in segment 1 (duodenum + proximal jejunum) of the small intestine in the KO mice (28.58 ± 3.73%) relative to the WT animals (16.05 ± 4.19%) (P < 0.045) (Fig. 9). These observations demonstrate that KO mice are clearly less efficient than WT mice in transporting absorbed lipid into lymph as chylomicron particles, resulting in a marked accumulation of the radioactive TG in the intestine, especially in the first mucosal segment.

**Negative staining and sizing of the lymph lipoproteins.** As shown in Fig. 10, A and B, respectively, both the KO and the WT mice secreted large chylomicron-sized lipoprotein particles to the lymphatic circulation during active lipid absorption after the beginning of infusion of the 6 μmol/h TG emulsion. The size of lipoproteins secreted by the KO mice was not significantly different from that secreted by WT mice (P < 0.68). Overall, during the third to fourth hour, 37% of the total number of secreted particles was of chylomicron size in the KO animals, compared with 32% for WT animals during the same period (Fig. 10C). We can draw two conclusions from these data: 1) apo B100-producing enterocytes of apobec-1 KO animals are indeed capable of secreting chylomicron-size particles; and 2) despite the ability to secrete chylomicrons, the apobec-1 KO animals are still significantly less capable of delivering the absorbed TG into lymph than the WT animals.

**Lymphatic apo B and apo AIV secretion.** We next examined whether decreased apo B secretion might account for the differences in chylomicron-mediated triglyceride transport observed between WT and KO mice. To compare the output from various animals, we examined the apo B content of timed lymph collections (a sample equivalent to a 1-min lymph sample was taken from the total lymph collected over 60 min). The hourly lymphatic apo B mass output of the WT group was higher than that of KO mice during fasting as well as throughout the 6-h lipid-infusion period (Fig. 11A). The mass of apo B secreted by the KO mice was 20% less than that secreted by WT animals for the 6 h of lipid infusion (Fig. 11B). Collectively, one can infer from the data that the reason KO mice secrete less TG than WT mice during the infusion period is probably due to the lower number of apo B100 chylomicron particles secreted by the KO group; this is certainly a plausible explanation for the decrease in efficacy of KO animals in their transportation of TG to lymph. Furthermore, we examined whether KO rats produced larger particles with more TG due to increased apo AIV mass. The hourly output of apo AIV mass by the KO
mice did not differ significantly from that secreted by WT mice during fasting and during lipid infusion (Fig. 11A). These observations suggest that KO mice secrete fewer but larger chylomicrons without changing the apo AIV mass output following lipid infusion.

**MTP lipid transfer activity.** Next, we determined whether the reduced apo B secretion in the KO animals relative to the WT animals was related to less activity of MTP, an enzyme intimately involved in lipidation of prechylomicrons in endoplasmic reticulum (ER). Figure 12 showed that KO...
mice had 29.4 ± 4.0% MTP lipid transfer activity in the duodenum plus jejunum whereas WT groups displayed 28.6 ± 1.6% after 4 h of 6 μmol/h infusion of TG (the difference is not statistically significant from the KO animals). These data imply that MTP lipid transfer activity is not responsible for the less TG lipidation in ER and thus results in reduced apo B secretion in KO mice.

**DISCUSSION**

Apo B48 found in chylomicrons produced by the wild-type intestine clearly plays a critical role in the transportation of lipids from intestinal enterocytes to the lymphatic circulation. To examine the different physiological roles of apo B48 and apo B100, we used the lymph fistula model to compare lipid transport in the apobec-1 KO and the WT animals. The study using a modest dose of infused TG (4 μmol/h) demonstrated that the lymphatic flow rate, uptake, and lymphatic output of TG in the KO mice were not statistically different from those observed in WT mice, except for the slightly lower TG output of the KO group, which was observed during the first hour. After the second hour of lipid infusion, KO animals seemed to transport lipids as well as the WT mice, and these observations are consistent with earlier conclusions that KO mice absorbed fat just as well as WT mice (14, 22), which was further supported by the fact that plasma lipid concentrations between the two groups was not significantly different (14, 22). Interestingly, the present study revealed that KO mice accumulated significantly more mucosal TG than the WT animals; these new data imply that the KO mice are actually less efficient in TG transport. Secondly, we also observed that the majority of the mucosal radioactive TG is found in the first quarter of the small intestine in both KO and WT mice. Therefore, the duodenum and the jejunum are the predominant sites of lipid uptake (33) for both groups of animals, leading us to conclude that there is no shift in the major location of lipid absorption to the distal small intestine in KO animals relative to the WT controls.

To further investigate a possible deficit in the capacity to transport lipids as chylomicrons in KO mice, a larger dose of radioactive TG (6 μmol/h) was infused in KO and WT groups of mice. The KO mice transported lipids to the lymphatic circulation at a significantly lower rate than the WT mice (26.75 ± 4.33 vs. 48.16 ± 3.90%, respectively) at 3 h into the infusion period. Associated with the reduced lymphatic transport of the absorbed TG, there was a significant increase in the amount of radioactive TG retained in the mucosa of the KO animals relative to the WT animals (P < 0.026). This finding coincides with the observation that the KO mice had slightly higher mucosal TG content compared with WT mice following exposure to a 2-wk ad libitum feeding of a high-fat chow diet (16). Therefore, the gut producing exclusively apo B100 in the KO mice appears to be generally less capable of transporting absorbed TG into lymph than the apo B48-producing gut of WT animals. There are three possibilities for this reduced capacity to transport absorbed lipids to the lymph: 1) a decreased ability to synthesize and secrete chylomicrons, which may be associated with the apo B100-producing gut; 2) an inability to increase the number of secreted chylomicrons to adequately handle a lipid load when they are assembled with apo B100; or 3) a combination of both possibilities.

In the present study, the WT mice produced larger chylomicrons to facilitate lipid transport after infusion of a modest or a high dose of TG; these findings are in agreement with our previous observations in rats (32). In contrast, the KO animals produced and transported mostly VLDL-sized particles to lymph during infusion of a modest dose of TG. However, when we increased the dose of infused TG fed by 50%, we found that both KO and WT animals secreted larger chylomicrons. Furthermore, when we examined the size distribution of the lipoprotein particles found in the lymph of both groups by electron microscopy, no difference in the size distribution of TG-rich lipoproteins in lymph was observed. This finding is in agreement with the earlier observations of Morrison et al. (21). The findings are also consistent with our own data obtained from isolated enterocytes in vitro, harvested from WT and KO mice, in which large chylomicron particles were observed in the media of cells of both genotypes (37). From the morphology data, we can conclude that when the KO and WT mice were infused with a modest dose of lipid, there was an apparent lack in the ability of KO animals to secret larger TG-rich chylomicron particles. However, this apparent deficiency was diminished when a larger dose of the TG emulsion was infused. Therefore, it can be concluded that the reduced lymphatic transport of absorbed lipids seen in KO animals is not due to a lack in the ability to make chylomicrons, but rather due to less of an affinity for chylomicron formation when handling lipid loads, clearly highlighting a physiological difference between apo B100 and apo B48.

Further evaluation was undertaken to answer the question of whether the impaired lipid transport seen in KO mice is a result of a reduced number of secreted TG-rich lipoproteins following administration of the high-dose lipid infusate. Previous studies have demonstrated that one VLDL-sized particle contains one apo B100 molecule and that one molecule of apo B48 is associated with one chylomicron particle (11, 25). Accordingly, the mass of lymphatic apo B secretion was used as a surrogate measure to evaluate the relative production of chylomicron and VLDL particles by the gut (13). This approach revealed that the gut that produces exclusively apo B100 secretes less total apo B mass (therefore a significant less number of chylomicron particles) than the gut that produces apo B48 in response to administration of the higher TG dose.
Previous reports showed that increased apo AIV expression in IPEC-1 cells produced larger lipoprotein particles (19). We speculated that increased apo AIV mass may be associated with larger chylomicrons secreted by KO mice following high-dose TG infusion. However, our present study showed that apobec-1 KO mice produced a comparable amount of apo AIV mass but reduced apo B mass in the lymph after high-dose TG treatment. This observation is consistent with normal apo AIV synthesis and secretion in enterocytes of KO mice (29). The data suggest that apo AIV secretion is not influenced by apo B isoform composition of intestinal lipoproteins.

Dietary fatty acids are absorbed and resynthesized in the small intestine (30). Apo B and MTP are essential for the assembly and secretion of apo B-containing lipoproteins (2, 7). MTP transfers lipid to the apo B containing prechylomicrons in the lumen of the ER (2, 7). MTP expression is highest in duodenum and jejunum and its expression decreases in the ileum (28). MTP lipid transfer activity in duodenum and jejunum of KO mice did not differ from the WT mice. The observation implies that reduced MTP lipid transfer activity is not responsible for the reduced number of chylomicron particles produced by the gut of the KO mice after the high-dose TG infusion. The mechanism resulting in reduced apo B secretion of KO mice is not yet clear and will require further evaluation. We propose, however, that reduced apo B mass secretion (which in turn reduces the number of TG-rich lipoproteins produced by KO enterocytes) is responsible for the diminished efficacy of lipid transport in the KO mice.

In conclusion, apobec-1 KO mice can absorb and transport a modest dose of dietary TG as well as WT mice, but the ability of KO mice to transport lipid becomes limited upon administration of higher doses of TG. The reduction in lymphatic transport of absorbed TG is associated with an observed enterocyte TG accumulation. The deficit in lymphatic transport of absorbed lipid by the KO animals relative to the WT controls is likely a result of the reduction of lymphatic apo B secretion by the gut, in turn leading to a reduction in the number of TG rich lipoprotein particles produced. The reduced production of apo B is not associated with a change in the MTP lipid transfer activity in ER. We are able to conclude from this study that an important functional adaptation associated with the exclusive production of apo B48 by the gut is the facilitation of the formation and secretion of chylomicrons. The unique ability of apo B48 to direct chylomicron formation and secretion ensures that the naturally occurring gut is optimally suited for the transport of large amounts of absorbed dietary TG to the lymphatic system.

GRANTS

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