Is apolipoprotein A-IV rate-limiting in the intestinal transport and absorption of triglyceride?

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Apolipoprotein A-IV (apoA-IV) is synthesized by the intestine and secreted when dietary fat is absorbed and transported into lymph associated with chylomicrons. We have recently demonstrated that loss of apoA-IV increases chylomicron size and delays their clearance from the blood. There is still uncertainty, however, about the precise role apoA-IV on the transport of dietary fat from the intestine into the lymph. ApoA-IV KO mice do not have a gross defect in dietary lipid absorption, as measured by oral fat tolerance and fecal fat measurements. Here, using the *in vivo* lymph fistula mouse model, we show that the cumulative secretion of triglyceride (TG) into lymph in apoA-IV KO mice is very similar to that of WT mice. However, the apoA-IV KO mice do have subtle changes in TG accumulation in the intestinal mucosa during a 6 hour continuous, but not bolus, infusion of lipid. There are no changes in the ratio of esterified to free fatty acids in the intestinal mucosa of the apoA-IV KO, however. When we extended these findings, by giving a higher dose of lipid (6 µmole per h) and for a longer infusion period (8 hours), we found no effect of apoA-IV KO on intestinal TG absorption. This higher lipid infusion most certainly stresses the intestine, as we see a drastically lower absorption of TG (in both WT and KO mice), but the loss of A-IV does not exacerbate this effect. This supports our hypothesis that apoA-IV is not required for TG absorption in the intestine. Our data suggest that the mechanisms by which the apoA-IV KO intestine responds to intestinal lipid may not be different from their WT counterparts. We conclude that apoA-IV is not required for normal lymphatic transport of TG.
KEYWORDS – apolipoprotein A-IV (apoA-IV), chylomicron, intestinal lipid transport, lymph fistula mouse model

ABBREVIATIONS – apolipoprotein A-IV (apoA-IV), knockout (KO), triglyceride (TG), wild-type (WT)
INTRODUCTION

Apolipoprotein A-IV (apoA-IV) is a lipid-binding protein that is synthesized and secreted by enterocytes in response to lipid absorption (1, 5, 9). ApoA-IV is secreted associated with nascent chylomicrons into intestinal lymph (1, 12, 14); once those chylomicrons enter the plasma and undergo lipolysis, a significant fraction of the apoA-IV dissociates (as a result of the shrinkage of the surface area of the chylomicrons) and is found within the HDL and lipoprotein-free fraction (10, 19, 24). ApoA-IV expression is highly responsive to intestinal lipid absorption and its expression requires chylomcron secretion (11, 30, 35). Therefore, the presence of apoA-IV in the periphery is uniquely linked to the intestinal formation and secretion of lipid.

Many physiological functions have been ascribed to apoA-IV, including anti-oxidant (20), anti-inflammatory factor (26), anti-atherosclerotic factor (2, 3), as well as a mediator of reverse-cholesterol transport (4, 22), and acute satiety factor (7, 25). Although these are potentially important functions of apoA-IV, some of these roles are also ascribed to other apolipoproteins and may be redundant functions rather than its primary role. Additionally, we have recently demonstrated an important role for apoA-IV in mediating glucose homeostasis by stimulating insulin secretion (27), and in modulating chylomicron metabolism (13).

Based on observations in apoA-IV KO mice in our own lab, we have not found a difference in intestinal lipid absorption in the absence of apoA-IV expression (13).

Recently, however, Simon and Weinberg (21) have observed in everted gut sacs that apoA-IV subtly modulates triglyceride (TG) transport throughout the small intestine, and...
that this in turn regulates the gene expression of lipid trafficking and possibly gut hormone secretion.

This intriguing and apparent discrepancy in the regulation of intestinal lipid absorption by apoA-IV led us to ask whether we could more precisely determine the effect of apoA-IV on TG absorption in the intestine by using the well-established lymph fistula mouse model. This model system has tremendous advantages over in vitro approaches including: 1) it is an in vivo model, complete with intact circulatory system as well as neural and hormonal influences; 2) the ability to sample lymph continuously after lipid-infusion; 3) this model is free from the complications of anesthesia on both chylomicron formation and lymph flow, since the animals in our model are conscious; and (4) lastly, the lymph we collect and analyze from the intestine represents the physiological response of the intestine to luminal lipid, as it is secreted into the mesenteric lymphatic vessel prior to entering the circulation through the left subclavian vein.

In this in vivo model, our findings reveal that loss of apoA-IV does not impact overall TG absorption and secretion by the intestine into lymph. We used several experimental conditions designed to capture subtle differences in TG transport; these were a high and low lipid dose, infusion of either a bolus or continuous lipid dose, and extending the time of lymph collection. In none of these conditions does apoA-IV have a significant physiological effect on lipid trafficking. In apoA-IV KO mice receiving a bolus dose of lipid, however, there is an accumulation of TG in the M1 (the first quarter of the small intestine) segment of the intestinal mucosa, but this is not accompanied by
changes in the esterification status of lipid in those segments. This evidence suggests
that apoA-IV does not modulate overall lipid absorption, but that the physiological
effect of apoA-IV KO may rather be to modulate chylomicron composition or the
subsequent metabolism of those chylomicrons. Our data support an intriguing role for
apoA-IV in mediating plasma lipid metabolism that is independent of a change in
intestinal lipid transport.
MATERIALS AND METHODS

Animals. Generation of the apoA-IV KO mouse was described previously and these animals were kindly provided to us by Dr. Jan Breslow of Rockefeller University (32). The apoA-IV KO mice used in this study were extensively backcrossed onto the C57BL/6 genetic background, and all mice were genotyped by PCR. Male apoA-IV KO and WT mice, approximately 3 months of age, were housed (3-4 per cage) in a temperature-controlled (21 ±1°C) vivarium on a 12-h light-dark cycle. All animals received free access to water and chow diet (LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories). All animal procedures were performed in accordance with the University of Cincinnati Internal Animal Care and Use Committee and in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Cannulation of intestinal lymph. Prior to surgery, mice were fasted overnight with free access to water. Under isoflurane anesthesia, the superior mesenteric lymphatic duct was cannulated with polyvinyl chloride tubing, with slight modifications to the procedure described previously (13, 34). The cannula was secured with a drop of cyanoacrylate glue (Krazy Glue, Columbus, OH). A duodenal cannula was also placed and secured by purse-string suture. After surgery, a saline solution containing 5% glucose was infused via duodenal catheter 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 24 h in restraining cages in warmed chambers (28 - 30°C) prior to the start of the experiments. Animals usually nestle together to keep warm, and so it is imperative that
the animals are warmed post-operatively, since they were housed singly. Although the animals were restrained, they had considerable freedom of movement.

**Lipid Infusion and Lymph Collection.** Three groups of apoA-IV KO and wild-type lymph fistula mice were studied, and these were: 1) mice receiving a bolus infusion of lipid, a group which received a single 300 μl duodenal bolus containing 220 μl Liposyn II (Hospira Inc. Lake Forest, Illinois, USA), 80 μl 0.9% saline, with 30 μCi [³H]-triolein; 2) mice receiving a 4 μmol/h continuous dose of lipid received 0.3 ml of an emulsion containing 1 μCi [³H]-triolein, 0.78 μmol cholesterol, 0.87 μmol phospholipid, and 4 μmol triolein in 19 μmol sodium taurocholate per hour for 6 h; and finally 3) mice receiving a 6 μmol/h continuous dose of lipid received 0.3 ml of an emulsion containing 1 μCi [³H]-triolein, 0.78 μmol cholesterol, 0.87 μmol phospholipid, and 6 μmol triolein in 19 μmol sodium taurocholate per hour for 8 h. Lymph was collected hourly in microcentrifuge tubes on ice for 6 h. Each lymph sample contained 10% by volume of an antiproteolytic cocktail (0.25 M EDTA, 0.80 mg/ml aprotinin) and 80 U/ml heparin. Lymph was collected from n=8 apoA-IV KO mice and n=6 WT mice for the bolus and 4 μmol/h continuous infusion groups, respectively, and lymph from n=4 apoA-IV KO and WT mice was collected for the 6 μmol/h continuous infusion group.

**Triglyceride Measurement.** Lymphatic TG was determined using 2 approaches: total lymph TG was measured using a standard assay kit (Randox, Crumlin, UK) and the total
$^{3}$H-TG counts in lymph and the intestinal mucosa were assayed by scintillation counting, as described previously (13).

**Collection of Tissue**

At the end of the 6 h infusion and lymph collection period, the mice were anesthetized with the ketamine–xylazine mixture, and the stomach, small intestine, and colon were rapidly isolated. The luminal contents of the intestine were isolated by rinsing the intestine 3 times with a 10 mM sodium taurocholate in saline. The small intestine was then cut into 4 equal segments, designated as M1–M4 (M1 being the most proximal and M4 being the most distal). Tissue samples were homogenized using a Polytron homogenizer, and radioactivity of each sample was measured by scintillation counter. The radioactive lipid in lymph was also quantitated by liquid scintillation counting.

**Folch Extraction of Tissue Lipids and Thin-Layer Chromatography**

100 mg of tissue was added to 5 ml of chloroform/methanol (2:1, v/v) for Folch extraction, as described previously (6). Lipids extracted from M1–M4 were separated on silica gel 60 plates using a solvent system of petroleum ether/diethyl ether/glacial acetic acid with 25:5:1 volume ratio. After visualizing the samples and the co-migrating reference standards by staining with iodine vapor, samples were scraped into scintillation vials, and 1 mL of absolute alcohol was added to scintillation liquid to elute the radioactive lipid from the gel (OptiFluor for aqueous samples) for counting of radioactivity (18).
Statistics. Overall statistical significance was determined by two tailed, unpaired t-tests, unless otherwise noted; differences were considered significant at $P < 0.05$. All data are presented as the mean ± the SEM. Statistics were performed using GraphPad Prism (version 6.0).
RESULTS

**Bolus Dose (300 µl bolus of 20% Liposyn II)**

To determine whether apoA-IV plays a role in TG transport by the small intestine, we analyzed the lymphatic output of TG in apoA-IV KO and WT mice after a duodenal bolus of lipid. A bolus dose approximates a third of the daily fat that is normally consumed by a mouse. As shown in Figure 1, the fasting lymph flow rates were not different between WT and apoA-IV KO mice. Thirty minutes after lipid infusion, lymph flow increased in both WT and KO mice, but then rapidly returned to baseline levels. Over the 6 h study period, the loss of apoA-IV did not alter the lymph flow.

In addition, apoA-IV KO and WT mice had no significant difference in lymphatic output of \[^{3}H\]-TG post-infusion (Figure 2A). In both apoA-IV KO and WT mice we observed a steady increase in the secretion of \[^{3}H\]-TG into lymph starting 1 h post-infusion, with peak TG transport occurring at approximately 3 h post-infusion. TG mass transport, which includes both labeled \[^{3}H\]-TG and unlabeled endogenous TG secreted from the intestine into the lymph, also did not differ from WT mice throughout the 6 h study (Figure 2B). TG output increased immediately following infusion (30 min) and peaked at approximately 2-3 h for both WT and apoA-IV KO mice. When we calculated the amount of exogenous TG transported in lymph over the 6 h period between the WT and KO animals, about 14 mg of lipid was transported over the 6 h in both groups of animals. When we calculated the amount of TG transported in lymph based on the chemical assay (mass transport), between 15 to 16 mg of TG was transported into lymph.
in both the WT and the KO animals. Thus, both the radioactive exogenous lipid out and
the mass TG output (exogenous plus endogenous) agreed with each other well.

We asked whether there might be a difference in the fate of TG after the bolus
infusion of lipid. Tissue samples from the duodenum, jejunum, and ileum (M1, M2, M3,
M4, respectively) were isolated from each mouse at the end of the lymph collection
period. As shown in Figure 3A, the greatest amount of TG recovered was from the M2
segment (jejunum) in both WT and apoA-IV KO mice, and there were no differences in
accumulation.

TG not already secreted into lymph and remaining in the stomach, lumen,
mucosa, or the colon was also measured (Figure 3B). There were no significant
differences in the amount of TG recovered between WT and apoA-IV KO mice, with the
highest recovery in the lumen of the intestine. Only a small amount of $^{3}$H-TG was
recovered from the colon of both groups of animals, which corroborates our previously
published data showing that there is no overall change in dietary lipid absorption
between apoA-IV KO and WT mice (and thus no difference in fecal lipid recovery). This
also suggests that there is not a delay in the movement of lipid along the intestine of
apoA-IV KO mice after a bolus infusion.

As shown in Figure 4, approximately 86% of the TG infusion was recovered from
either lymph or within the tissues of the GI tract, with no difference in recovery
between apoA-IV KO and WT mice. This strongly suggests that the loss of apoA-IV does
not inhibit or delay lipid absorption by the intestine after a bolus infusion of lipid.
Continuous Dose (4 µmol/h)

- It has been suggested that a significant function of apoA-IV may be in the synthesis of chylomicrons in the intestine (8, 10, 16). In a carefully designed study, Lu et al. (15) showed elegantly that overexpression of apoA-IV in IPEC cells enhanced their chylomicron secretion by increasing the size of the chylomicrons secreted.

- Consequently, when we failed to detect a difference in the amount of lipid transported after a single bolus dose of lipid, it seemed reasonable that a difference might be observable under a different infusion paradigm. Whereas a bolus infusion will deliver lipid to a segment of the gut at relatively high concentration, a continuous infusion will deliver lipid at a constant rate, but as a smaller dose. The continuous infusion will therefore result in a steady state condition of lipid uptake, packaging into chylomicrons, and the secretion of chylomicrons into lymph. By testing this approach, we hoped to observe any possible role that apoA-IV plays in mediating TG absorption or secretion that we may have missed after a single bolus lipid infusion.

- As shown in Figure 5, lymph flow rates for both WT and apoA-IV KO mice were relatively constant for the first 2 h of infusion, and then slowed for the remainder of the study period. There were no significant differences in lymph flow between WT and apoA-IV KO mice.

- ApoA-IV KO and WT mice had no significant differences in the lymphatic output of TG, when measured as either a percent of the total hourly infused (Figure 6A) or the total amount of TG transported over the 6 h continuous infusion period (Figure 6B).

- However, 6 h into the continuous infusion, there appears to be a trend towards slightly
lower TG transport in the apoA-IV KO mice, although it does not reach statistical
significance.
We then determined the fate of the infused TG after the continuous infusion
period. Mucosal tissue samples from the duodenum, jejunum, ileum, and colon (M1,
M2, M3, M4, respectively) were isolated from each mouse at the end of the lymph
collection period (Figure 7A). In the apoA-IV KO mice, there was an increase in the
retention of TG in the M1 segment, compared to WT controls, suggesting that without
apoA-IV, chylomicron resident time in the mucosa may be extended, possibly causing
larger and more TG-rich chylomicrons to be formed. Corresponding with the increased
TG accumulation in M1, we did recover an increased amount of infused TG in the
intestinal mucosa of apoA-IV KO mice (Figure 7B).

Does retention of TG in M1 have a physiological consequence?
As described above, only during the continuous infusion of TG did we observe a
subtle difference in intestinal TG transport in apoA-IV KO mice. Given our previously
published data showing that apoA-IV KO mice absorb dietary fat equally to their WT
counterparts (as measured by both fecal fat and by oral fat tolerance)(13), we asked
whether the accumulation of TG in the M1 segment of the intestine during continuous
infusion would result in a physiological effect. Specifically, could this accumulation of TG
in M1 of the apoA-IV KO mice be caused by less efficient esterification of the absorbed
lipid to form TG?
As shown in Figure 8, intestinal segments were isolated following a 6 h continuous duodenal infusion of TG (without lymph collection), followed by lipid extraction and thin layer chromatography (TLC) analysis. We would expect to see that the accumulated TG in the apoA-IV KO intestine might significantly alter the distribution of the labeled fatty acids into the different lipid classes in the intestinal mucosa, which might in turn affect TG secretion. Instead, we observed no differences between the WT and apoA-IV KO mice in the percent of labeled fatty acid found as triglyceride, diacylglyceride, monoacylglyceride, or fatty acid. This suggests, in addition to the lymphatic TG output data presented above, that the accumulation of TG in the M1 segment of the apoA-IV KO intestine is not a result of defective re-esterification of partial glycerides and fatty acids to form TG.

**Continuous Dose (6 µmol/h)**

Although we did not observe a significant difference in TG absorption in response to the loss of apoA-IV after either the bolus or continuous dose experiments, we did want to test the hypothesis that apoA-IV might become more important for the packaging of additional lipid per chylomicron particle, under conditions of large intestinal lipid loads, as suggested by Lu et al (16). Therefore, as shown in Figure 9, we tested TG absorption in apoA-IV KO mice after a 6 µmol/h continuous lipid infusion, for an extended time (8 h). We chose to test a continuous infusion because the trend towards slightly lower TG transport in the apoA-IV KO mice occurred during a continuous infusion, and therefore we thought this experimental design would give us
the best opportunity to measure subtle differences. Despite the higher dose and longer
infusion period, we found no effect of apoA-IV KO on intestinal TG absorption (Figure
9A), with nearly identical TG output into lymph between apoA-IV KO mice and their WT
counterparts. There was no difference in the fate of the infused TG along the length of
the intestine in either the lumen or mucosa (data not shown). As shown in Figure 9B,
however, this higher lipid infusion most certainly stresses the intestine, as we see a
drastically lower absorption of TG in both WT and apoA-IV KO mice. The loss of apoA-IV
does not exacerbate this effect on TG absorption. This supports our hypothesis that
apoA-IV is not required for TG absorption in the intestine, even when the absorptive
system is stressed by a high lipid load.
Apolipoprotein A-IV is synthesized by the intestine and secreted during dietary fat absorption into lymph in chylomicrons. Once in the blood, apoA-IV is a moderator of glucose homeostasis, lipoprotein oxidation, and atherosclerosis; we have recently demonstrated that loss of apoA-IV increases chylomicron size and delays their clearance from the blood (13). There is still uncertainty, however, about the precise role apoA-IV plays inside the intestine, and specifically whether apoA-IV influences the transport of dietary fat across the intestine into the lymph. ApoA-IV KO mice have a lowered plasma lipid levels, but do not have a gross defect in dietary lipid absorption (13, 32). However, using a cell culture model, it has recently been demonstrated that apoA-IV may be necessary for the normal transport of lipid across the intestine (21) and that the overproduction of apoA-IV is associated with the transport of larger chylomicron particles in the IPEC cells derived from pigs (15).

Here, we have used the lymph fistula mouse model to determine how and if apoA-IV influences TG movement across the intestine. This is of significant physiological importance since the movement of TG across the intestinal mucosa into the lymph is the major route of absorption for dietary TG. The in vivo lymph fistula mouse model with a duodenal infusion tube avoids variables such as changes in food intake and stomach emptying; however, it allows direct determination of the efficiency of digestion, uptake, and lymphatic transport of TG. Our model also is void of the confounding effects of anesthesia, peripheral metabolism, or animal-animal variability in feeding or digestive behavior. An important advantage of this in vivo lymph fistula model is that we are able
to collect lymph in the presence of an intact circulatory system, as well as neural and
hormonal influences of both the periphery and the distal gut. Therefore, it is unlikely
that ex vivo compensatory mechanisms are employed by the gut that might impact TG
trafficking.

The question of how apoA-IV influences TG absorption in the intestine is
interesting and warrants re-examination. The concept that apoA-IV is important for
chylomicron formation was first introduced by Weinberg and colleagues, and suggests
that apoA-IV is important in stabilizing the surface of a forming pre-chylomicron particle
inside the enterocyte (28, 29). Therefore, the presence of apoA-IV allows pre-
chylomicrons to grow with the incorporation of TG molecules. This concept was
certainly well supported by the elegant studies of Black and colleagues using the
intestinal pig epithelial cells (15), and in recent work by Weinberg and Shelness (31).
They demonstrated that the production of large amounts of apoA-IV by these cells
result in the copious secretion of large chylomicron particles. Recent work by Weinberg
and Simon have made additional inroads into the role of apoA-IV and intestinal TG
transport (21). They suggest that apoA-IV plays a role in regional lipid absorption,
associated with changes in gene expression. We have shown previously, however, that
there are no gross differences in lipid absorption in apoA-IV deficient mice (13).

The discrepancy between our and Simon and Weinberg’s findings (21) is the
difference in model system. We would argue that the subtle regional changes in lipid
absorption seen in their cell culture model, while certainly interesting, may have missed
taking into account the physiology of the entire intestine. The entire length of the small
intestine is where lipid transport and absorption occurs. By examining independent \textit{ex vivo} regions, changes in transport efficiency may be just as likely to be due to the loss of communication and function of the distal intestine (and possibly activation of the ileal brake), as it is likely that it is simply \textit{in vitro} compensatory mechanisms. In this report, we ask the question, if apoA-IV is truly \textit{physiologically} important to lipid transport, shouldn’t there be a difference in lipid transport when we examine the mesenteric lymph (a representation of the entire intestine’s lipid output) under different experimental conditions? To address these seemingly disparate findings, we used three different approaches in our well-established \textit{in vivo} lymph fistula mouse model; these were a high and low lipid dose, infusion of either a bolus or continuous lipid dose, and extending the time of lymph collection.

Under bolus dose conditions, the loss of apoA-IV did not have an effect on the amount of TG recovered in lymph, nor did it affect accumulation of TG in the intestinal mucosa. This agrees with the earlier finding that apoA-IV KO mice do not have a defect in dietary lipid absorption (13). This suggests that after a bolus dose of lipid in the duodenum, apoA-IV may not be required for the absorption and secretion of that lipid in chylomicron particles. We did consider, however, that the bolus infusion could potentially overwhelm the intestinal transport system thus masking subtle changes in TG absorption in the apoA-IV KO mice. Therefore, we next examined lipid absorption during a continuous infusion. We found no apparent difference in TG output into lymph in the apoA-IV KO mice, but did observe accumulation of TG in the intestinal mucosa. This accumulation was not caused by a defect in the re-esterification of partial
glycerides and fatty acids to form TG. Rather, the accumulation was probably caused by a lack of incorporation of TG into pre-chylomicrons in the apoA-IV KO animals. Despite this, the apoA-IV KO mice did not exhibit a decrease in the lymphatic transport of TG in chylomicrons.

An additional role suggested for apoA-IV is that it may allow packaging of more core lipid into chylomicron particles by serving as a chaperone for nascent chylomicron particle trafficking through the cellular lipidation pathways (31). Both the data presented here and previously from our lab (13) support the conclusion that apoA-IV is not essential for chylomicron assembly and secretion at normal intestinal lipid loads. However, during conditions when the intestine is under stress from high lipid loads (such as during the neonatal period when the mammalian intestine is exposed to high-fat breast milk) (15)(33), might apoA-IV be necessary? We tested this directly (Figure 9) by increasing both the lipid dose (from 4 μmol/h to 6 μmol/h) and the time of infusion (from 6 h to 8 h). Despite the higher dose and longer infusion period, we found no effect of apoA-IV KO on intestinal TG absorption in the adult animals. This higher lipid infusion most certainly stresses the intestine, as we see a drastically lower absorption of TG (in both WT and apoA-IV KO mice), but the loss of apoA-IV does not exacerbate this effect. This supports our hypothesis that apoA-IV is not required for TG absorption in the intestine.

In terms of the discrepancy between our findings and those of Simon and Weinberg (21), we believe that variations in methodology may be responsible for the differences in data. They acknowledge that when the lymph vessel from the entire
intestine is cannulated and sampled that there is no difference in overall TG absorption and secretion. It should however be noted that they studied their animals immediately after surgery and so both their apoA-IV KO and WT animals may not have had sufficient time to recover from the surgery, a variable that has been carefully controlled in our study (the animals were allowed to recover from the surgery overnight). Both Morgan and Borgstrom (17) and Tso and Simmonds (23) have discussed the importance of allowing the animals to recover after surgery in employing the lymph fistula model. Nonetheless, Simon and Weinberg (21) in their study found that it is only when individual segments of intestine are assayed that subtle changes in TG transport are observed. We concede that losing apoA-IV may cause subtle regional variations in TG transport, but whether this is of physiological significance remains unclear. Using the lymph fistula model, we are able to sample lymph that represents an integration of the lacteals from throughout the intestine (M1-M4) – and represents most of the intestinal lymph that enters the general circulation via the left subclavian vein. Therefore, irrespective of whether there are subtle changes regionally, there is no change in the overall lymphatic output of TG in any of our experimental approaches.

Additionally, the differences seen in the cell culture model may indeed recapitulate regional physiology, but they may also be due to an in vitro model. In our view, there are several reasons why the cell culture model is so poor: Firstly, in the everted gut sac culture model there is no blood flow, which plays a less critical role for lipid uptake, but is absolutely critical for the formation and secretion of chylomicrons into the lamina propria and then subsequent emptying into the lacteals. Therefore, the
everted gut sac is an excellent model for studying lipid uptake, but may not be appropriate for studying secretion. Secondly, the cell culture model lacks neuronal communication with the distal gut, which might mean that important physiological signals (such as activation of the ileal brake) are lost in culture. Lastly, the everted gut sac preparation also lacks the hormonal and metabolic influences that the normal gastrointestinal tract experiences.

A question that remains in light of this data is why apoA-IV is so acutely regulated by dietary fat, yet is not required for TG absorption? In addition, why would the loss of apoA-IV have only subtle implications for intestinal TG transport? It seems likely, based upon our data, that apoA-IV is not acting to stabilize the chylomicron TG, nor is it necessary for the packaging of TG into chylomicrons (or absorption in general). One suggestion would be that since a majority of apoA-IV does dissociate from the chylomicrons following their metabolism in the circulation, that perhaps apoA-IV plays a more important role in the metabolism of chylomicrons. We reported previously that the lack of apoA-IV retards the clearance of chylomicrons (13). We suggest that the lack of an effect of apoA-IV on lipid absorption and secretion does not diminish in anyway the importance of the protein biologically (which is as much as 3% of the protein made by enterocytes), since we know that the loss of apoA-IV significantly impairs glucose homeostasis and peripheral lipid clearance (13, 27). Rather, it is tempting to speculate that apoA-IV secreted on chylomicrons serves as an intestinal signal to the periphery of incoming nutrients. Therefore, its endocrine-type actions on the pancreas (enhancing
insulin secretion) serve to bridge lipid metabolism in the gut with whole-body glucose metabolism.

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**DISCLOSURES**

The authors have no disclosures or conflicts of interest to report.


FIGURE CAPTIONS

Figure 1. Lymph flow rate after a [3H]-TG bolus intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). Lymph was collected hourly and analyzed. There were no statistically significant differences between WT and KO mice, as determined by 2-way repeated measures ANOVA (n=6-8). Values are means ± SE.

Figure 2. (A) [3H]-TG and (B) TG mass transport into the lymph during a bolus intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). Lymph was collected hourly and analyzed. There were no statistically significant differences between WT and KO mice, as determined by 2-way repeated measures ANOVA (n=6-8). Values are means ± SE.

Figure 3. (A) Distribution of [3H]-TG in the 4 segments of the intestine. At the end of the study, small intestines were harvested and divided into 4 equal length segments, from proximal to distal: M1, M2, M3, and M4. The amounts of radioactivity in these segments were determined by scintillation counter. (B) The recovery of [3H]-TG radioactivity in the lymph, stomach, intestinal lumen, intestinal mucosa, and colon were determined at the end of the 6 hours by scintillation counter. For both (A) and (B), mice were equipped with lymph and duodenal cannulas, and received a bolus intraduodenal infusion of a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). There were no statistically significant differences between WT and KO mice, as determined by unpaired t-tests (n=6-8). Values are means ± SE.

Figure 4. Total recovery of infused [3H]-TG at the end of the 6 hour collection period. The recovery of [3H]-TG radioactivity in the lymph, stomach, intestinal lumen, intestinal mucosa, and colon were determined at the end of the 6 hours by scintillation counter. Mice were equipped with lymph and duodenal cannulas, and received a bolus intraduodenal infusion of a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). There were no statistically significant differences between WT and KO mice, as determined by unpaired t-tests (n=6-8). Values are means ± SE.

Figure 5. Lymph flow rate after a [3H]-TG continuous intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 6 hours. Lymph was collected hourly and analyzed. There were no statistically significant differences between WT and KO mice, as determined by 2-way repeated measures ANOVA (n=6-8). Values are means ± SE.

Figure 6. (A) [3H]-TG and (B) Total TG recovery into the lymph during a continuous intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas,
and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 6 hours. Lymph was collected hourly and analyzed. No statistically significant differences were found between the WT and KO, as determined by (A) analysis of 2-way repeated measures ANOVA, and (B) t-test (n=6-8). Values are means ± SE.

Figure 7. (A) Distribution of $[^{3}H]$-TG in the 4 segments of the intestine. At the end of the study, small intestines were harvested and divided into 4 equal length segments, from proximal to distal: M1, M2, M3, and M4. The amounts of radioactivity in these segments were determined by scintillation counter. (B) The recovery of $[^{3}H]$-TG radioactivity in the, lymph, stomach, intestinal lumen, intestinal mucosa, and colon were determined at the end of the 6 hours by scintillation counter. For both (A) and (B), mice were equipped with lymph and duodenal cannulas, and received a continuous intraduodenal infusion of a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 6 hours. *P < .05, statistically significant difference between WT and KO, as determined by t-test (n=6-8). Values are means ± SE.

Figure 8. Thin-layer chromatography analysis of $[^{3}H]$-labeled lipid fraction of the small intestine. Mice were equipped with a duodenal cannula, but no lymph cannula, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 6 hours. At the end of the study, the small intestines were divided into 4 equal segments (A) M1, (B) M2, (C) M3, (D) M4. The extracted lipid fraction was separated by thin-layer chromatography into TG (triacylglycerol), DAG (diacylglycerol), MONO (monoacylglycerol), and FA (fatty acids). *P < .05, statistically significant difference between WT and KO as determined by t-test (n=6-8). Values are means ± SE.

Figure 9. (A) $[^{3}H]$-TG recovery into the lymph during a continuous intraduodenal lipid infusion of 6 μmol/h triolein. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 8 hours. Lymph was collected hourly and analyzed. No statistically significant differences were found between the WT and KO, as determined by (A) analysis of 2-way repeated measures ANOVA, and (B) t-test (n=4). Values are means ± SE.
Figure 1. Lymph flow rate after a [³H]-TG bolus intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). Lymph was collected hourly and analyzed. There were no statistically significant differences between WT and KO mice, as determined by 2-way repeated measures ANOVA (n=6-8). Values are means ± SE.
**Figure 2.**

(A) Lymph $[^{3}\text{H}]$-TG transport

(B) Lymph TG mass transport

**Figure 2.** (A) $[^{3}\text{H}]$-TG and (B) TG mass transport into the lymph during a bolus intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). Lymph was collected hourly and analyzed. There were no statistically significant differences between WT and KO mice, as determined by 2-way repeated measures ANOVA (n=6-8). Values are means ± SE.
**Figure 3.**

(A) Distribution of [³H]-TG in the 4 segments of the intestine. At the end of the study, small intestines were harvested and divided into 4 equal length segments, from proximal to distal: M1, M2, M3, and M4. The amounts of radioactivity in these segments were determined by scintillation counter. (B) The recovery of [³H]-TG radioactivity in the, lymph, stomach, intestinal lumen, intestinal mucosa, and colon were determined at the end of the 6 hours by scintillation counter. For both (A) and (B), mice were equipped with lymph and duodenal cannulas, and received a bolus intraduodenal infusion of a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). There were no statistically significant differences between WT and KO mice, as determined by unpaired t-tests (n=6-8). Values are means ± SE.
Figure 4. Total recovery of \[^{3}H\]-TG at the end of the 6 hour collection period. The recovery of \[^{3}H\]-TG radioactivity in the, lymph, stomach, intestinal lumen, intestinal mucosa, and colon were determined at the end of the 6 hours by scintillation counter. Mice were equipped with lymph and duodenal cannulas, and received a bolus intraduodenal infusion of a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG). There were no statistically significant differences between WT and KO mice, as determined by unpaired t-tests (n=6-8). Values are means ± SE.
Figure 5. Lymph flow rate after a $[^3]$H-TG continuous intraduodenal lipid infusion. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 6 hours. Lymph was collected hourly and analyzed. There were no statistically significant differences between WT and KO mice, as determined by 2-way repeated measures ANOVA (n=6-8). Values are means ± SE.
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Figure 9. (A) [$^3$H]-TG recovery into the lymph during a continuous intraduodenal lipid infusion of 6 mmol/h triolein. Mice were equipped with lymph and duodenal cannulas, and were intraduodenally infused with a lipid emulsion containing labeled TG (the label was on the FA moieties of the TG) for 8 hours. Lymph was collected hourly and analyzed. No statistically significant differences were found between the WT and KO, as determined by (A) analysis of 2-way repeated measures ANOVA, and (B) t-test (n=4). Values are means ± SE.