Effect of increasing lipid loads on the ability of the endoplasmic reticulum to transport lipid to the Golgi

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Abstract **We have previously shown (Mansbach, C. M. and P. J. Nevin, 1998. *J. Lipid Res.* 39: 963–968) that after the development of a mass steady state with respect to triacylglycerol absorption in rats, the introduction of radiolabeled trioleoylglycerol, while maintaining the input rate of trioleoylglycerol constant at 135 μmol/h, was followed by a slow (60 min) achievement of a radiolabel steady state in the intestinal endoplasmic reticulum (ER). We hypothesized that this was due to the large input load and that the time to steady state would be shorter at lower lipid loads. Rats were infused intraduodenally with 22.5, 45, 90, or 135 μmol trioleoylglycerol/h for 6 h to obtain a mass steady state in the intestine. [^{3}H]trioleoylglycerol was added to the infusate and the ER and Golgi were isolated from the proximal intestine after 5–60 min of radiolabel infusion. The time required to reach a radiolabel steady state in the ER lengthened from 10 min at the 22.5 μmol/h infusion rate to 60 min at the 135 μmol/h rate. Similar data were obtained for the Golgi. Incubation of the ER with lipase reduced the ER–triacylglycerol amount by 43% and increased its specific activity by 73%. The amount of [^{3}H]TG-dpm in the ER was not reduced unless taurocholate, 10 mM, and colipase were added. We conclude that as the rate of triacylglycerol infusion is increased, TG movement from the ER to the Golgi progressively lengthens until finally all the triacylglycerol infused cannot be transported. A portion of this triacylglycerol is disposed on the cytoplasmic face of the ER and thus able to be attacked by lipase whereas another fraction is sequestered in the ER lumen and immune to lipase attack unless the ER membrane is solubilized.**—Mansbach, C. M., and R. Dowell. **Effect of increasing lipid loads on the ability of the endoplasmic reticulum to transport lipid to the Golgi. *J. Lipid Res.* 2000.** 41: **605–612.**

Supplementary key words lipid absorption • chylomicron • intestine • endoplasmic reticulum • Golgi • lipase

The intestine is equipped to handle large amounts of absorbed dietary lipids by using two different strategies. These are necessary because the intestine has little control over the rate of entry of the products of lumenal triacylglycerol (TG) hydrolysis, fatty acid (FA) and monoacylglycerol (MG) both of which are potentially toxic to the intestinal cell, especially free FA (FFA) in high concentrations. Thus they must be neutralized.

The first strategy used by the intestine is to bind the incoming FFAs and MGs to an abundant cytosolic protein, the fatty acid binding protein (FABP). In the proximal intestine, where normal lipid absorption occurs, two FABPs are expressed, liver FABP (L-FABP) (1) and intestinal FABP (I-FABP) (2). L-FABP has been proposed as a storage mechanism for FFA (3) and could perform a similar function for MG. However, this mechanism of removing FFA from the cytosol is limited by the amount of L-FABP present and is likely to become quickly overwhelmed by the mass of FFA fluxing into the enterocytes. Thus a supplemental strategy is required.

The second strategy used by the intestine is the extremely rapid re-synthesis of TG from absorbed FFA and MG such that most of the FFA present in the enterocytes is esterified to TG (4). This effectively places the absorbed FFA in an inert form with respect to its potential solubilizing effect on cellular membranes. In sum, the effect of both mechanisms is to quickly and effectively remove unbound FA from the intestinal cytosol and thus limit the membrane disruption potential of FFA.

The TG formed in the enterocyte as the end product of lipid absorption is transported out of the cell in the intestine's unique TG-rich lipoprotein, the chylomicron. However, the ability of the intestine to export TG as chylomicrons is limited (5) and under physiological circumstances some TG is removed by the portal route either as TG or FFA (6). The rate-limiting step in the chylomicron export pathway has been suggested to be between the ER and the Golgi (4). This thesis is supported by prior morphologic data (7, 8), physiological data (9, 10), and genetic data (11). More specifically, the rate-limiting step has been postulated to be the formation of a vesicle that transports the developing chylomicron from the ER to the Golgi which has been termed the pre-chylomicron transport vesicle

Abbreviations: ER, endoplasmic reticulum; TG, triacylglycerol; FA, fatty acid; FFA, free fatty acid; MG, monoacylglycerol; FABP, fatty acid binding protein; PCTV, pre-chylomicron transport vesicle; TO, trioleoyl; ID, intraduodenal; MGAT, monoacylglycerol acyltransferase.

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(PCTV) (12, 13). The rate at which TG can be moved to the Golgi by the PCTV parallels the ability of the intestine to export chylomicrons into the lymph (12).

The present investigations were designed to examine the ability of the ER to respond to a graded increase of the TG input load. If the ability to get TG from the ER to the Golgi is the limiting factor in TG export, then as the intestine is exposed to greater amounts of lipid we should see the ER's capacity to move ER-TG to the Golgi become progressively overloaded. Furthermore, consideration needs to be given to the ability of TG to cross the ER membrane. The solubility of TG is suggested to be limited to 3% of the membrane lipids (14) raising the possibility that TG, formed on the cytosolic face of the ER (15), is synthesized more rapidly than it can be translocated across the ER membrane. This provides a possible rationale for the movement of TG into a storage pool and would support prior observations that TG entering either the chylomicron or portal venous pathways bifurcate at the level of the ER (4).

METHODS

Animal preparation and TG specific activity of the ER and Golgi as a function of both time of radiolabel infusion and amount of TG infused

Non-fasting Sprague-Dawley rats, 250–350 g, had a PE 50 (PE 50, Clay Adams, Parsippanny, NJ) cannula placed in the duodenum through a right sub-costal incision and in the jugular vein as previously described (4). The tip of the intraduodenal (ID) cannula was at the level of the entry of the common bile duct. After surgery, the rats were placed in a restraining cage and infused with 0.15 m NaCl containing 5 mm KCl, and 5% (w:v) glucose overnight at 3 ml/h. The jugular vein cannula was plugged by a wire. The following morning, the infusion was changed to a sonicated emulsion containing 5, 10, 20, or 30 mm trioleoylglycerol (TO, 99% TO, Sigma Chemical Co., St. Louis, MO), 10 mm taurocholate, 0.15 m NaCl, and 10 mm Tris-HCl, pH 7.0, which was infused at 4.5 ml/h. This gave ID infusion rates for TO of 22.5, 45, 90, or 135 μmol/h, which were infused for 6 h to develop a mass steady state in the intestinal mucosa with respect to neutral lipids (16). At the conclusion of the 6-h TO infusion period, the infusate was changed only by adding [^{3}H]TO (^{3}H-oleoyl-TO, New England Nuclear, Boston, MA, all oleoyl groups were labeled) as a tracer to final specific activities of 152,000 dpm/μmol, 55,700 dpm/μmol, 27,000 dpm/μmol, and 23,000 dpm/μmol, respectively. The TO mass infused remained the same as previously. To rapidly exchange non-radiolabeled TO for [^{3}H]TO so that an accurate start time of the radiolabel infusion would be known, the radiolabeled infusate was added by injecting first 1 ml air into the duodenal cannula, then injecting 1 ml of the appropriate [^{3}H]TO infusate through the cannula (injection time ≈ 2 sec). The [^{3}H]TO infusion was then re-started at 4.5 ml/h for the specified periods of time, from 5 to 30 min for the lower two levels of TO infusion and from 5 to 60 min for the higher two infusion rates. The radiolabel was given constantly until the time of killing. No food other than the TO infusion was allowed during the experiment. When 90 and 135 μmol/h TO were infused, we have previously shown that 92–99% of the infusate is absorbed from the lumen and distributed to the proximal half of the intestine (5, 17). We assume that the percent of lipid infused remaining in the lumen is similar at the two lower infusion rates. In sum, groups of rats were infused at four levels of TO input, from 22.5 to 135 μmol/h. At the two lower levels of TO infusion (22.5 and 45 μmol TO/h), radiolabeled TO was infused for 4 different time periods from 5 to 30 min. At the two higher levels of TO infusion (90 and 135 μmol/h), TO was infused for 6 different time periods from 5 to 60 min.

At the conclusion of the indicated radiolabel infusion period, the rat was killed by the injection of pentobarbital (50 mg) through the jugular vein cannula so that a rapid response could be obtained. The proximal half of the intestine was quickly (20 sec) removed to a beaker of 0.15 m NaCl at 4°C and the lumen was flushed with iced 0.15 m NaCl. The intestine was placed on an iced glass plate and opened so that the mucosal surface was up. The surface was cleansed with iced 0.2% Triton X-100 from a squirt bottle to remove adherent [^{3}H]TO (16), and then with iced 0.15 m NaCl to remove the Triton. Finally, the mucosa was wiped with tissue and scraped using a glass microscope slide. The mucosa was scraped lightly such that only the mid portion of the villus to the villus tips was removed, as previously shown by histological examination of the remaining tissue (R. Dowell and C. M. Mansbach, unreported observations). The mucosal scrapings were homogenized in 15 ml of 0.15 m NaCl using a glass–Teflon homogenizer in an ice bucket. An ER–Golgi fraction was prepared as described previously (12) by obtaining a post-mitochondrial supernatant by centrifugation of the homogenate for 140,000 g·min in a Sorvall centrifuge (DuPont Instruments, Wilmington, DE) and the supernatant was centrifuged for 9×10^6 g·min in a 60 Ti rotor in an L8-M centrifuge (Beckman Instruments, Fullerton, CA) at 4°C.

The ER–Golgi pellet was gently re-suspended in buffer A (0.25 m sucrose, 30 mm HEPES, 2.5 mm Mg acetate, 30 mm KCl, pH 7.2) and the density of the solution was increased to 1.22 m sucrose as judged by an Abbe refractometer. Three ml of this suspension was overlaid with 1.15 m, 0.86 m, and 0.25 m sucrose (2.6 ml each) in buffer A and centrifuged in a SW 41 rotor for 14.4×10^6 g·min in the L-8M centrifuge at 4°C. The ER was found in the 1.22 m sucrose layer and in the pellet. It was pelleted by re-centrifuging it in 10 mm HEPES, pH 7.0, for 8.9×10^6 g·min in an SW 41 rotor in the L-8M centrifuge at 4°C. The pellet from this final step was used as ER. The Golgi was taken to be the membranes between the 1.15 m/0.86 m sucrose and the 0.86 m/0.25 m sucrose layers (12). These layers were harvested with Pasteur pipettes, combined, and used as Golgi without further purification. Under these conditions, the ER has 8-fold greater specific activity of NADPH-cytochrome C reductase activity than the whole homogenate and the Golgi has 16-fold greater specific activity of galactosyl transferase than the whole homogenate (13).

The ER and Golgi fractions were extracted of their TG content by a differential organic extraction technique (18) shown previously to give comparable results to TG isolated by thin-layer chromatography (4). TG mass was measured by processing 100 μl of the TG-containing extract for gas–liquid chromatography (GLC) (9). The methylated FFAs were dissolved in methylene chloride, and 1 μl was injected onto the GLC column (Model 5880, Hewlett-Packard, Avondale, PA equipped with a DB 23 column from J & W Scientific, Folsom, CA). The remainder of the lipids were dried under N_2 and their radioactivity was determined (Packard Tricarb model 1500 liquid scintillation spectrometer, Packard Instrument Co., Downers Grove, IL). In this manner, TG mass and radioactivity were determined on the same extract enhancing the accuracy of determining the specific activity (dpm/μmol).

Effect of lipase on ER-TG specific activity

Rats were prepared as above except that only the highest dose (135 μmol/h) [^{3}H]TO was infused ID for 6 h, except where indicated. At this time, a mass and a radiolabel steady state are ex-

pected in the intestinal mucosa (16). Purified ER was isolated from the proximal half of the intestine as above and frozen at −80°C until used. Lipase (porcine lipase type VI-S from Sigma Chemical Co.) was assayed using [^{3}H]TO as substrate and found to release 6.5 nmol oleate per μg lipase/min at 37°C. To determine whether the specific activity of the ER-[^{3}H]TG changed on incubation with lipase, 180 nmol ER-TG, average specific activity 28 dpm/nmol, was incubated at 37°C with 20 μg of lipase for 15 min. At the conclusion of the incubation, the reaction was stopped by the addition of the TG extraction mixture (18) and the [^{3}H]TG was isolated in the top organic phase. One hundred μl was prepared for GLC and 1 μl of the methylated FFAs in methylene chloride was separated by GLC. The remainder of the methylene chloride was dried under N_2 and the radioactivity of the sample was determined by liquid scintillation spectroscopy. The TG-mass and dpm found were used to calculate the specific activity of each sample. Control incubations consisted of ER prior to incubation and ER that underwent incubation without the addition of lipase.

It was assumed in these first incubations that because the [^{3}H]TG did not diminish after exposure to lipase, the ER membrane excluded lipase from the ER-lumen. To determine whether the lipase could hydrolyze the ER lumenal TG if it could gain access to it, an additional series of experiments were performed which paralleled the first but which included a tube containing 10 mm taurocholate, to disrupt the ER membrane, 20 μg lipase, and 5 μg colipase (Sigma Chemical Co.) resulting in a molar ratio of lipase to colipase of ≈1:1. In this series of experiments, [^{3}H]TG dpm and TG mass were determined by liquid scintillation and GLC, respectively. Additional control experiments utilizing ER from rats infused with 135 μmol [^{3}H]TO were performed in which the ER was incubated with lipase plus colipase and lipase plus 10 mm taurocholate. After the incubation, TG was isolated and its specific activity was determined as above.

In a another series of experiments, rats were infused with 22.5 μmol/h [^{3}H]TO for 6 h and the ER was isolated and purified as before. The ER (30 nmol ER-TG) was incubated with lipase, 2 μg, for 15 min at 37°C and the reaction was stopped, TG was extracted, and its specific activity was determined as above. Samples of ER-TG were obtained prior to incubation with lipase and of ER-TG that was incubated without lipase.

Materials and statistical analysis

All chemicals were from Sigma Chemical Co. unless otherwise specified. Differences between means were analyzed by ANOVA using Bonferroni post-test multiple comparisons.

RESULTS

In these experiments, rats were prepared by a constant ID infusion of TO at varying loads for 6 h prior to the start of the experiment. This resulted in a progressive increase in the amount of TG associated with the ER as shown in **Table 1**.

The experiment commenced with the introduction of [^{3}H]TO into the infusate while maintaining the same rate of TO mass infusion as for the previous 6 h. As expected, the longer the radiolabeled TO was infused, the greater the [^{3}H]TG specific activity of the ER-TG at each level of TO infusion (**Fig. 1**) until a radiolabel steady state was reached (22.5, 45, 90 μmol/h) or the experiment was terminated at 60 min (135 μmol/h). At the lowest infusion rates (22.5 and 45 μmol/h), the greatest ER-TG specific activity reached was 26–28% of the specific activity of the infusate. At the 90 μmol/h infusion rate, the highest ER-TG specific activity reached was 40%, and at the largest infusion rate, 135 μmol/h, it was 42% of infusate values (Fig. 1).

TABLE 1. Effect of increasing trioleoylglycerol infusion rates on ER triacylglycerol

μmol ID TO/h[a]	μg ER TG/mg ER protein[b]
22.5	15.3 ± 2.3[c]
45	27.5 ± 4.1
90	36.5 ± 0.58
135	74.7 ± 8.7[d]

[a] μmol trioleoylglycerol infused intraduodenally each hour.
[b] Data are the mean ± SEM for n = 4 rats.
[c] 22.5 μmol/h vs. 45, 90, 135 μmol/h, $P < 0.05$.
[d] 135 μmol/h vs. 90, 45, 22.5 μmol/h, $P < 0.01$.

When the mass of ID TO was increased from 22.5 to 135 μmol/h, there were dramatic effects on the length of time that it took to reach a radiolabel steady state in the ER after the onset of the [^{3}H]TO ID infusion (Fig. 1). When the ID infusion rate was the lowest, 22.5 μmol/h, a steady state for radiolabeled TG was essentially reached in 10 min. For the 45 μmol/h TO infusion rate, 15 min was required. Times greater than 30 min were not performed for these two infusion rates because of the attainment of steady state conditions. However, for the 90 μmol/h infusion rate, it took 45 min to reach a radiolabel steady state. For the highest rate of TO infusion, 135 μmol/h, a steady

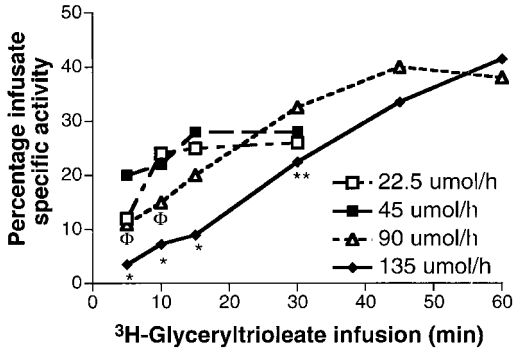

Fig. 1. The percentage of infusate TG-specific activity represented by ER-TG specific activity as a function of time of intraduodenal [^{3}H]TO infusion at increasing infusion rates. Rats were constantly infused intraduodenally with TO in the amounts indicated in the legend. After 6 h of infusion, the infusion was supplemented by [^{3}H]TO such that the infused TO mass remained essentially the same. The [^{3}H]TO infusion was continued for the times indicated, and the ER from the proximal one-half intestine was obtained. The ER-TG was extracted and its mass and radioactivity were obtained (see Methods). The specific activity of the ER-TG was compared to that of the infusate and the result, expressed as a percentage, is displayed on the ordinate. The data are the mean of 5 experiments with the SEM <10%; * indicates $P < 0.05$, 135 μmol/h vs. 22.5, 45, 90 μmol/h; ** indicates $P < 0.05$, 135 μmol/h vs. 90 μmol/h; ϕ indicates $P < 0.05$, 22.5 and 90 vs. 45 and 135 μmol/h at 5 min of infusion only and 90 vs. 22.5, 45, and 135 μmol/h at 10 min.

state was not obtained, but in prior experiments (4) using the same infusion protocol, a steady state was obtained at 60 min. In sum, these data suggest that at low TO infusion rates, the replacement of TG, already resident in the ER from the constant TO infusion, by newly synthesized [^{3}H]TG from dietary [^{3}H]oleate or [^{3}H]oleoyl-MG was rapid. As the infusion rate of TO increased, the rate of replacement of TG with [^{3}H]TG progressively slowed. As the intestinal mucosa was at mass steady state during the entire experiment (5, 16), these data also imply that the percentage of the total TG present in the ER that is exported to the Golgi per min is much greater at the lower TO infusion rates than at the higher ones, i.e., the turnover rate of the total ER-TG is more rapid at low infusion rates than at higher ones. For example, using the data in Fig. 1 it can be calculated that the $t_{1/2}$ for ER-TG is 5 min for the 22.5 μmol/h infusion rate and 30 min for the 135 μmol/h infusion rate.

Another important observation regarding the data in Fig. 1 is that the specific activity of the [^{3}H]TG in the ER is always much less than that of the infusate at the radiolabel steady state. Under the conditions of our experiments, i.e., mass steady state, these data indicate that under all our infusion rates, the majority of the ER-TG-FA comes from unlabeled (endogenous) sources. Although we do not show here that we have reached steady state with regard to the 135 μmol/h infusion rate, we have previously shown (4) this occurs at 60 min at approximately the same percentage of infusate specific activity as shown here for 60 min of infusion.

In **Fig. 2** are shown similar data for the Golgi. These data essentially recapitulate that found for the ER with some small differences. It should be noted that at the lowest infusion rate, 22.5 TO/h, a radiolabel steady state is reached extremely quickly, even at 5 min of infusion. As

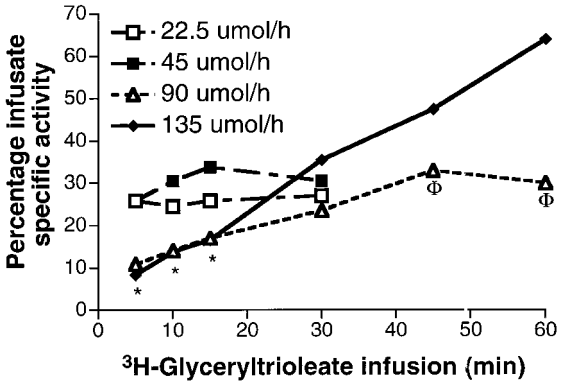

Fig. 2. The percentage of infusate TG-specific activity represented by Golgi-TG specific activity as a function of time of intraduodenal [^{3}H]TO infusion at increasing infusion rates. Rats were infused with TO (for amounts, see legend) and subsequently with [^{3}H]TO as in Fig. 1. Golgi were isolated from the proximal half of the intestine and the specific activity of the Golgi-TG was determined (see Methods). The specific activity of the Golgi-TG was compared to that of the infusate and the result, expressed as a percentage, is displayed on the ordinate. The data are the mean of 5 experiments with the SEM <10%; * indicates $P < 0.05$, 90 and 135 vs. 22.5 and 45 μmol/h; ϕ indicates $P < 0.05$, 90 vs. 135 μmol/h.

was the case with the greater infusion rates in the ER, at the 90 μmol/h infusion rate, a steady state was reached in 45 min and with the 135 μmol/h infusion rate, a steady state was not demonstrated, even at 60 min of infusion. This was expected as our previous work showed that it took 60 min for a radiolabel steady state to occur at this infusion rate (4).

If the time that it takes to develop a radioactive steady state in the ER and Golgi is plotted as a function of the rate of ID TO infusion (**Fig. 3**), a curve similar to that expected for a dose response curve is generated. We have shown previously that the ER and Golgi reach a radiolabel steady state at 60 min of infusion when TO was infused at 135 μmol/h (4), the value used in the figure. Because the ER and Golgi-TG are at mass steady state during the development of the radiolabel steady state, the curve reflects the rate at which the entire pool of ER (Golgi)-TG is turned over at each input rate. As shown in the figure, the curves suggest that as the amount of TO infused is increased, there is a progressive overload of TG in the ER requiring transport to the Golgi. The data further suggest that at an infusion rate of 135 μmol/h, the ER has reached its capacity to handle additional TG as the curve begins to flatten at this point. This supposition is supported by the data presented in **Fig. 4**.

The TG specific activities in the Golgi are a reflection of the specific activities in the ER as the ER is the site of TG synthesis. Therefore the block in TG transport would appear to be at the level of the ER at the exit step of TG from the ER, or more specifically, the formation of the PCTV. The finding that the ER and Golgi curves are nearly parallel suggest that either the transit step from ER to Golgi is very fast and/or that the turnover rate of TG in the Golgi is fast. The data cannot distinguish between the two possibilities. Either of these two mechanisms is the likely cause of the Golgi reaching a steady state at 5 min of TO infusion, slightly ahead of the ER.

Figure 4 shows the specific activity of the Golgi divided by the specific activity of the ER during the ID [^{3}H]TO infusion. For the lowest three infusion rates, this fraction varied between 0.7 and 1.2 after 10 min of infusion. By

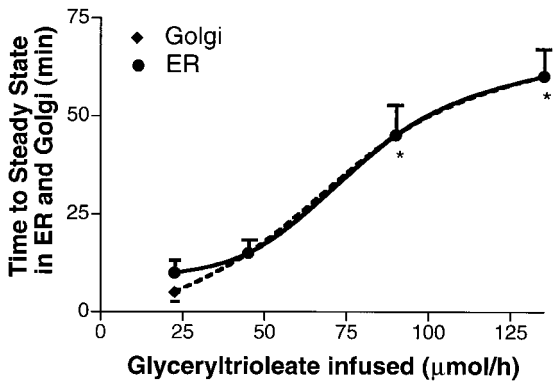

Fig. 3. The time required to obtain a radiolabel steady state in the ER and Golgi of the proximal intestine is plotted as a function of the amount of TO infused intraduodenally in rats; * indicates $P < 0.05$, 22.5 and 45 μmol/h vs. 90 and 135 μmol/h.

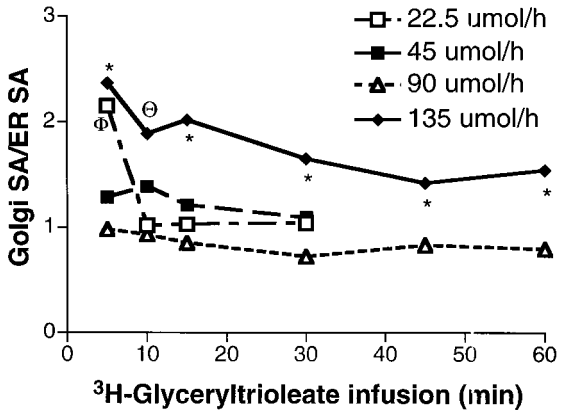

Fig. 4. Golgi specific activity as compared to ER specific activity at each level of TO infusion (see legend) as a function of the time of radiolabel infusion. At a ratio of 1, the ER and Golgi TG specific activities are equal; * indicates $P < 0.02$, 135 vs. 22.5, 45, and 90 μmol/h excluding 22.5 μmol/h at 5 min; ϕ indicates $P < 0.02$, 22.5 vs. 45 and 90 μmol/h; θ indicates $P < 0.05$ 135 vs. 22.5, 45, and 90 μmol/h.

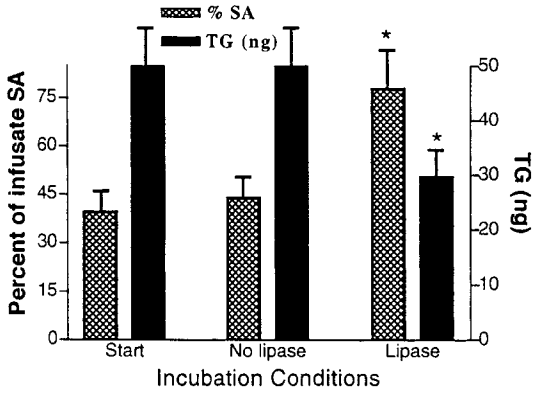

Fig. 5. The percentage of infusate specific activity (solid bars) of ER-TG and the ER-TG mass (hatched bars) in the ER isolated from the proximal half intestine after 6 h of [^{3}H]TO infusion intraduodenally. The infusate specific activity was 68 dpm/nmol. The conditions of the experiment were: "Start", indicating the mass and percent TG specific activity at the beginning of the experiment, "No lipase", indicating the percent TG specific activity and TG mass after 15 min incubation at 37°C with no lipase added, and "Lipase", indicating the percent TG specific activity and mass after 15 min incubation at 37°C with lipase added. The data are the mean ± SEM; n = 4; * $P < 0.01$.

contrast, at the 135 μmol/h infusion rate, the ratio was significantly greater at each time point and varied between 1.4 and 2. These data suggest at the lower infusion rates (22.5–90 μmol/h) that the ER-TG that is transported to the Golgi is representative of the entire TG pool present in the ER. At 135 μmol/h infusion rate, by contrast, a disproportionate amount of ID infused [^{3}H]TG-oleate is transported to the Golgi from the ER, confirming our previous observations (4). Inasmuch as the average Golgi/ER ratio at the high infusion rate is 1.5 (30–60 min of infusion), the data suggest that 33% excess unlabeled (endogenous) TG-FA of the total pool of ER-TG remains with the ER and is not transported to the Golgi (1− specific activity of the ER/specific activity of the Golgi as representative of the specific activity of the TG in the ER lumen (4)). One explanation for the lack of movement of a fraction of the TG from the ER to the Golgi is that some of the TG, whose TG-FA is primarily from unlabeled (endogenous) sources, does not translocate to the lumenal side of the ER but remains on its cytosolic surface. To test this hypothesis, we performed the next series of experiments.

We supposed that if TG did remain on the cytosolic face of the ER membrane, it might be susceptible to hydrolysis by the cytosolic intestinal, alkaline active lipase (19), whereas the TG within the ER lumen would not, as it would be protected from lipase attack by the ER membrane (20). Because we are proposing that at the highest infusion rate the ER-TG on the cytosolic face contains more unlabeled (endogenous) TG-FA than the TG in the ER lumen, we prepared rats by infusing them with [^{3}H]TO at 135 μmol/h to mass and radiolabel steady state, then isolating the intestinal ER. Next, we incubated the [^{3}H]TG-ER with lipase in the expectation that the specific activity of the ER-TG would increase after the incubation if the lipase hydrolyzed the TG with a lower specific activity from unlabeled (endogenous) TG-FA on the ER cytosolic surface, leaving untouched the sequestered TG having a higher specific activity (from infused [^{3}H]TO). The results of experiments to answer this question are shown in **Fig. 5**. As can be seen, there was little change in either the amount of TG in the ER or in its specific activity when the [^{3}H]TG-ER was incubated without lipase as compared to the results prior to incubation at 37°C. By contrast, after incubation with active lipase, there was a 78% increase in ER-TG specific activity and a 43% reduction in ER-TG mass. These data substantiate our hypothesis concerning the ability of lipase to attack and hydrolyze TG that is not protected within the ER membrane.

Because the data presented in Fig. 5, which are suggestive of two pools of ER-TG, were obtained using a TO infusion rate of 135 μmol/h, the question arose as to the effect of lipase attack on [^{3}H]TG-ER under conditions in which one pool of TG is expected, i.e., ER isolated from rats infused with TO at 22.5 μmol/h. To answer this question, [^{3}H]TG-ER was prepared from rats infused for 6 h with [^{3}H]TO at 22.5 μmol/h and incubated with lipase in a manner analogous to the data presented in Fig. 5. Under these conditions, no change in ER-TG specific activity occurred on incubation with or without lipase (68 ± 9 dpm/nmol and 66 ± 8 dpm/nmol, respectively). No reduction in TG mass was found on incubation with lipase as compared to when the ER was incubated without lipase (27 ± 4 μmol vs. 30 ± 4 μmol, respectively).

As we propose that the [^{3}H]TG-ER is mostly within the ER lumen and that the ER membrane blocks access of the lipase to the [^{3}H]TG in its interior, disruption of the membrane by taurocholate should enable prompt lipolytic attack on this TG. As shown in **Fig. 6** there was no decrease in the total [^{3}H]TG dpm with or without lipase as compared to the TG-dpm present before the start of the

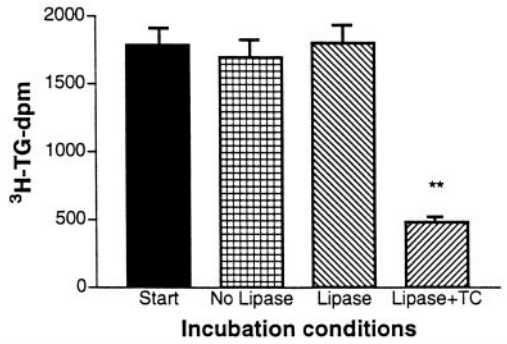

Fig. 6. The total [^{3}H]TG-ER before incubation (Start) and after incubation at 37°C with no lipase, lipase, or lipase, co-lipase, plus 10 mm taurocholate (see Methods). ER was isolated from the proximal half intestine after 6 h of intraduodenal infusion with [^{3}H]TO, 135 μmol/h. The ER was incubated at 37°C for 15 min either with no lipase, lipase, or lipase plus co-lipase and taurocholate. TG (20.4 ng) was hydrolyzed in the presence of lipase, taurocholate, and colipase. The data are the mean ± SEM; n = 4; ** $P < 0.01$.

experiment in the absence of taurocholate. However, when the ER membrane was perforated by exposure to 10 mm taurocholate, rapid hydrolysis of the [^{3}H]TG presumably within the ER lumen occurred, resulting in a 73% reduction in TG-dpm or 131 nmol TG. The post lipase plus colipase plus taurocholate data confirm that the ER membrane provides a barrier to lipase attack of the TG within the lumen of the ER and that when the barrier is disrupted, lipase gains access to this sequestered TG and rapidly hydrolyzes it. These data also suggest that the ER was closed during the incubation with lipase in the absence of taurocholate as there was no loss of ER-TG-dpm unless taurocholate was added to the incubation.

To be able to more clearly interpret the data in Fig. 6, experiments were performed in an analogous manner except that the [^{3}H]TG-ER was incubated with colipase and lipase (molar ratio 1:1) in the absence of taurocholate. Under these conditions, a 79 ± 9% increase in ER-TG specific activity (from 31 ± 4 dpm/nmol to 54 ± 6 dpm/nmol) and a 49 ± 6% reduction in ER-TG mass (from 120 ± 14 nmol TG to 61 ± 8 nmol TG) was found as compared to incubation in the absence of lipase and colipase. These data support those shown in Fig. 5 and confirm that lipolytic activity is active under these conditions. However, when the same [^{3}H]TG-ER preparation was incubated with lipase and 10 mm taurocholate but no colipase, only a 14 ± 3% increase in ER-TG specific activity and a 18 ± 3% reduction in TG mass were found as compared to control incubations in the absence of lipase and taurocholate. The reduction in lipolytic activity in these experiments is due to the absence of colipase which greatly reduces lipase activity in the presence of taurocholate above its critical micellar concentration (21). The modest lipolytic activity present is likely due to a minor contamination by colipase of the lipase used. This was suggested by a protein of an M_r appropriate for colipase on SDS-PAGE of the lipase received from the supplier.

DISCUSSION

The data presented here were designed to show the effect of increasing the input load of TG into the intestine on intestinal ER, the presumptive site of the limiting step in normal lipid absorption (7, 8, 11, 12). These experiments were stimulated by our previous work (4) in which we noted that only on prolonged (60 min) ID infusion of [^{3}H]TO did the ER reach a radiolabel steady state. In those experiments, the radiolabel was introduced after a mass steady state had already been achieved, an infusion protocol similar to that used here. We found, confirmed by our current data at the 135 μmol/h infusion rate, that the specific activity of the ER-TG was low by comparison to that which we found in the Golgi and, in a separate study, chylomicrons (16). These data suggest that unlabeled (endogenous) acyl groups are synthesized to TG at the level of the ER but are not transported to the Golgi and subsequently into the lymph as chylomicrons. This raises questions about the ultimate disposition of these endogenous acyl groups.

In our previous work, using a TO infusion rate of 135 μmol/h (4), we suggested that the slow increase in ER-TG specific activity was due to the large amount of TG resident in the ER at the onset of the [^{3}H]TO infusion. The large ER-TG mass indicates that the pre-steady state increase in TG mass was due to more TG being synthesized by the ER than was being transported out of the ER to the Golgi so that a new, but much greater, steady state level of TG was eventually reached. If this thesis were true, then we reasoned that at lower TG input loads, the amount of ER-TG should be less as was found (Table 1), and therefore a radiolabel steady state in the ER should be more quickly achieved after the onset of the [^{3}H]TO infusion. As shown in Fig. 1, this was indeed the case.

An additional interpretation of Fig. 1 is that as the TG input load is increased, a progressive deterioration of ER function occurs with respect to TG transport from the ER to the Golgi. At the lower two infusion levels, 22.5 and 45 μmol/h, a radiolabel steady state is quickly achieved, suggesting rapid movement of ER-TG to the Golgi. However, at the 90 μmol/h input rate, a steady state did not occur until 45 min of infusion, implying slower ER-TG transport. In all three cases, the radiolabeled TG entered a common pool in the ER which was eventually transported to the Golgi as indicated by the similar TG specific activity in both the ER and Golgi. At the highest rate of infusion, 135 μmol/h, not only was the attainment of the steady state further delayed as compared to the 90 μmol/h input rate, but the radiolabeled TG did not enter a common TG pool. This can be shown by the fact that the TG in the Golgi had a higher specific activity than that of the ER, indicating that a TG-pool containing exogenous TG is preferentially transported from the ER to the Golgi as we have previously suggested (4).

The specific activity increases in the ER and Golgi as shown in Figs. 1 and 2 reflect the rate of replacement of non-radiolabeled TG in both organelles with [^{3}H]TG synthesized from the newly instituted infusion of [^{3}H]TO into

the duodenum. As TG is synthesized in the ER, the appearance of [^{3}H]TG in the Golgi must reflect the specific activity of the TG in the ER lumen over time. As shown in Fig. 3, the rate at which all the TG in both organelles is replaced with newly synthesized TG is the same at each level of TO infusion except at 5 min. These data suggest that the delay in transport between the ER and Golgi is at the level of the formation of the PCTV as it buds from the ER membrane rather than either the transit of the PCTV across the intercellular space from the ER to the Golgi or its fusion with the *cis* Golgi. Once the PCTV is formed, it would appear to rapidly traverse the ER to Golgi space as suggested by the Golgi reaching a radiolabel steady state at the same time as the ER with the exception of the 22.5 μmol/h infusion rate when the Golgi reached a steady state prior to the ER.

The low specific activity TG, which dilutes the [^{3}H]TG-ER pool generated from infused [^{3}H]TO at the 135 μmol/h TO infusion rate, must be from non-radiolabeled or endogenous acyl group sources. Further, these endogenously sourced lipids must not easily translocate to the ER lumen to eventually become a precursor of chylomicron-TG. If true, then the endogenous TG should remain on the cytosolic face of the ER and be accessible to the intestinal, alkaline active, lipase present in the cytosol (19). To test this hypothesis we incubated ER with lipase (Fig. 5) and found that only one pool of ER-TG was hydrolyzed, the TG pool composed of endogenous acyl groups. This was indicated by lack of hydrolysis of [^{3}H]TG representing the TG sequestered in the ER lumen. As the non-radiolabeled TG, representing the TG on the cytosolic face of the ER, was hydrolyzed, a rise in the specific activity of the remaining ER-TG to the level found in the Golgi was observed. A further implication of this series of experiments is that [^{3}H]TG from dietary sources is protected from lipolytic attack by translocating to the lumenal side of the ER membrane. This is supported by the finding that when the ER membrane was disrupted by taurocholate, the [^{3}H]TG was rapidly hydrolyzed (Fig. 6). By contrast, when the input load of TG is small, 22.5 μmol/h TO, the TG pool is homogeneous and nearly all the TG is able to translocate to the ER lumen as shown by the inability of added lipase to hydrolyze it. In sum, the nonhomogeneity of the ER-TG pool and the inability of part of the TG pool to traverse the ER membrane appears to be a function of large lipid input loads into the intestine.

At an input load of 135 μmol TO/h, the question must be raised as to whether the rate of synthesis of TG exceeds the rate at which TG is able to translocate across the ER membrane. This potential is raised by the fact that TG has a finite solubility in membranes of 3% (22). The driving force for TG synthesis by the intestine is that the enterocyte cannot control the input rate of FFA from the lumen. If it does not make absorbed FFAs physicochemically inert, the enterocyte runs the risk of the FFAs disrupting its membranes. To guard against this, the enterocyte has L-FABP which acts as a FFA reservoir (3) and a very high rate of FFA esterification to TG. If the total TG synthetic rate exceeds its rate of translocation, TG would pile up on the cytosolic face of the ER and, if too much TG mass occurs, the TG would oil out into the cytoplasm. This could explain the origin of the storage pool of TG in the enterocyte.

The TG remaining on the cytosolic face of the ER has two potential fates in vivo. The first is to be hydrolyzed with the resulting FFAs transported to the liver via the portal vein (6, 23, 24). The second is for the TG to enter a storage pool in the intestine from which at least a portion goes to the liver as TG (6). This storage pool in the intestine, unlike the case in the liver, does not donate TG for chylomicron formation. This can be shown by metabolically labeling the pool with [^{3}H]oleate from the circulation and finding that when lipid is infused ID, the storage pool TG remains heavily radiolabeled, but chylomicron-TG appearing in the lymph is not (25).

The TG storage pools of the intestine and liver likely perform different functions. In the intestine the majority of the storage pool passes into the portal vein either as TG or as FFA from TG hydrolysis by the cytosolic lipase (6). Further, chylomicron-TG is derived primarily from dietary TG, utilizing MG as the glyceride-glycerol precursor (16, 17, 26). There is direct delivery of newly synthesized TG to the developing chylomicron (4, 16, 26). Even when chylomicron output is blocked by the non-ionic detergent, Pluronic L-81 (L81), the TG that is blocked in the ER from further progression (10), when released into the lymph after the removal of the L81, shows little evidence of hydrolysis (17). By contrast, the liver utilizes its TG storage pool as the major source of TG-FA for VLDL-TG (27). TG, which is synthesized from multiple FA sources in the liver, first may enter the storage pool as a consequence of lacking MGAT expression in the adult liver (28) leaving its glyceride glycerol to be derived from glycerol-3-phosphate. Once synthesized, the TG enters the liver storage pool awaiting its potential disposition to VLDL formation.

In sum we have shown that as the ID infusion rate of TO is increased, there is a progressive impairment of TG movement from the ER to the Golgi. This is first manifested as a lengthening of the time taken to achieve a TG radiolabel steady state on a background of a TG mass steady state and subsequently, at greater amounts of lipid infusion, as an inability to translocate all the TG synthesized to the ER lumen and subsequently to the Golgi. We also establish, for the first time, that a portion of newly synthesized TG remains on the cytosolic face of the ER where it becomes a potential substrate for intestinal lipase. As colipase is not required for lipase activity in the absence of bile salts, lipase should be active against this ER-TG.

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