Intestinal triacylglycerol storage pool size changes under differing physiological conditions

Peter Nevin, David Koelsch, and Charles M. Mansbach II[1]

Department of Medicine, Division of Gastroenterology, The University of Tennessee, Memphis, Memphis, TN 38163, and Veterans Administration Medical Center, Memphis, TN 38104

Abstract: The intestinal mucosal triacylglycerol storage pool consists of triacylglycerol that is predominantly transported from the intestine via the portal vein rather than in chylomicrons (*Am. J. Physiol.* 1991. **261:** G530–G538). Here we examined the size of the storage pool under varying physiological conditions. Four groups of rats were infused intraduodenally for 4 h. Group A was fasted; group B was infused with trioleoylglycerol, 135 μmol/h; group C was infused with trioleoylglycerol, 135 μmol/h plus phosphatidylcholine, 9 μmol/h; and group D was bile-diverted and infused with trioleoylglycerol, 135 μmol/h. The amount of triacylglycerol in the mucosa increased from groups A to D (A > B > C > D) but the storage pool triacylglycerol was least in groups A and C and greatest in groups B and D. The percentage of trioleoylglycerol in mucosal triacylglycerol was greater in groups B and D than in group A and greater in all groups than the percentage of oleate in the total fatty acids. We conclude that the triacylglycerol storage pool size varies inversely with the efficiency of lymphatic lipid output, which is greatest in rats infused with trioleoylglycerol plus phosphatidylcholine (group C) and least in bile-diverted rats infused with trioleoylglycerol (group D).–**P. Nevin, D. Koelsch, and C. Mansbach II.** Intestinal triacylglycerol storage pool size changes under differing physiological conditions. *J. Lipid Res.* 1995. **36:** 2405–2412.

Supplementary key words lipid absorption • intestine • bile diversion

The intestine rapidly absorbs lipids from the lumen and transports them quickly into the lymph with an appearance time of 12 min for fatty acids (FA) (1), suggesting that lipid processing is efficient. This is further supported by the finding that lipid absorption is a nearly complete process, with large loads, up to 500 g of fat, being absorbed per day in humans (2). The export step from the intestine into the lymph is not as efficient under all experimental conditions, however. For example, recovery rates in the lymph of 54–55% of intraduodenally infused lipid have been reported from our own (3) and other laboratories (4). The lipid export rate can be markedly improved by adding phosphatidylcholine (PC) to an intraduodenal lipid infusion (3). Alternatively, the quantity of lipid that fluxes into the lymph can be decreased by diverting bile from the intestine (5). In summary, these data suggest that physiological manipulations can markedly alter the ability of the intestine to export absorbed lipid into the lymph.

Exogenous lipid that does not exit the intestine via the lymph is cleared from the intestine (6) and has been shown to pass into the portal vein (7). This suggests the possibility that lipid entering the portal transport pathway is distributed into a different triacylglycerol (TG) pool than is the lipid to be transported in the lymph. The existence of two TG pools in the intestine was first proposed on the basis of finding that when radiolabeled trioleoylglycerol (TO) was infused intraduodenally until a steady state was obtained, the mucosal specific activity was much lower than the specific activity of the chylomicron TG derived from it (8). The TG pool that does not provide lipid for the lymphatic pathway (pool B) (7) has been isolated (9) and metabolic entry into the pool was obtained by intravenously infusing radiolabeled oleic acid (7).

Steady-state kinetic data show that the turnover rate of the TG precursor pool for chylomicron formation (pool A) (7) is the same despite a 2-fold increase in the output rate of TG into the lymph when intraduodenal TO infusion is compared to an intraduodenal TO plus phosphatidylcholine (PC) infusion (3). Thus, pool A must have increased 2-fold in order to provide enough TG to meet the requirements for TG lymphatic transport when TO + PC was infused intraduodenally as compared to when TO alone was infused. These data suggest the possibility that the size of mucosal TG pool B may also be

Abbreviations: TG, triacylglycerol; TO, trioleoylglycerol; FA, fatty acid; PC, phosphatidylcholine; VLDL, very low density lipoprotein; E600, diethyl-*p*-nitrophenylphosphate.

[1]To whom correspondence should be addressed.

altered in the opposite direction, i.e., when lymphatic TG transport nearly equals the input rate, it decreases, and it increases when TG lymphatic output decreases. The present studies were undertaken to test this potential using rat models in which lymphatic TG output rates are known to vary widely (3). A second objective of this study was to determine whether the proportion of TG acyl groups comprised of oleate was different in pool B than in the mucosa as a whole.

MATERIALS AND METHODS

Animal preparation

Male Sprague-Dawley rats (250–350 g) were maintained on Purina Rat Chow (Ralston Purina, St. Louis, MO) until used in the study. Four groups of rats participated in the experiments. All of the groups received duodenal cannulas (PE-50, Clay Adams, Parsippany, NJ) 1 day prior to the experiments (7). All groups of rats were infused intraduodenally with 0.15 M NaCl, 0.3 M KCl, and 5% glucose at 3 ml/h (Harvard infusion pump model 22, Harvard Apparatus, Millis, MA) overnight. The next day, group A's infusion was changed to 0.15 M NaCl. Group B's infusion was changed to 30 mM TO (99% pure, Sigma Chemical Co., St. Louis, MO), 10 mM taurocholate (Sigma Chemical Co.), 0.15 M NaCl, and 10 mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.4). Group C was given the same TO infusion supplemented with 2 mM PC (Sigma Chemical Co., the acyl constituents were: 38% palmitate, 8.3% stearate, 34% oleate, 9% linoleate, 11% arachidonate). Group D had the bile ducts cannulated (PE 10, Clay Adams) as well as duodenal cannulas implanted, and was given the same TO infusion as group B on the following day. No radiolabel was administered. All infusions were at 4.5 ml/h for 5 h.

After the 5-h infusions were completed, all of the groups were treated identically. The rats were given an overdose of pentobarbital, and the proximal one-half of the small intestine was removed, flushed with ice-cold 0.15 M NaCl, and placed in iced saline. The proximal gut was then cut open longitudinally on an iced glass plate. The mucosa was cleaned by rinsing with 0.15 M NaCl and wiping twice with tissue paper. The mucosa was removed by scraping with microscope slides and placed in 4 times the volume of buffer A [(w/v): 0.25 M sucrose, 0.1 M NaH_2PO_4, 10 mM $MgCl_2$, 10% dextran, 8.3 mM E600 (diethyl-*p*-nitrophenylphosphate) (Sigma), pH 6.8]. The mucosa was homogenized with a glass-Teflon homogenizer (Thomas Scientific, Swedesboro, NJ) and poured through two layers of cheese cloth. One ml was obtained for lipid extraction (10). The remaining homogenate was centrifuged at 7.5×10^4 *g* • min (Sorvall RC5C, Sorvall Instruments, Wilmington, DE) in a SS-34 rotor at 4°C. The pellet was washed with 10 ml buffer A and recentrifuged at 7.5×10^4 *g* min. Next, the pellet was placed in buffer A, 1:4 (v/v) and stirred on ice for 0.5 h. A sample of the slurry was taken for lipid extraction (10). A portion (1.5 ml) of the slurry was placed over a discontinuous sucrose gradient comprised of 4 ml 1.15 M sucrose and 4.5 ml 0.86 M sucrose. One ml of 0.1 M sucrose was added to the top of the gradients. All sucrose solutions were made up in buffer A. The gradients were centrifuged (Beckman L-8 M, Beckman Instruments, Fullerton, CA) in a SW 41 rotor at 2.4×10^7 *g* • min at 4°C. Four regions were harvested for lipid extraction: the floating fat at the top (region 1), the 0.25/0.86 (region 2), the 0.86/1.15 (region 3), and the pellet (region 4).

Analytical procedures

Thin-layer chromatography (TLC) was performed using silica gel G layers and a solvent system of hexanes–diethyl ether–methanol–acetic acid 80:20:6:2 (v/v). In a separate lane from the samples, authentic standards (tri-, di-, and monooleoylglycerols and oleic acid) were co-chromatographed. The standard lane was cut from the plate and the TG and FA bands were identified by charring using 50% H_2SO_4 (11). The TG and FA bands from the lanes containing the samples were separately scraped from the plate using their R_f on the standard lane as a guide. The acyl groups were methylated using a one-stage digestion/methylation (TG) or methylation (FA) procedure followed by gas–liquid chromatography on a Hewlett-Packard model 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) (11) to separate the FA esters. The integrated value of each methyl ester was compared to an internal C-17 standard. The data were used for quantitation and percentile composition. The FA and TG bands were separately analyzed (12).

Statistics

Student's *t*-test was used to test differences between two groups. When more than two groups were present, ANOVA was used with post Bonferroni corrections (InStat, GraphPad, San Diego, CA).

RESULTS

E600 was added to the homogenization medium because in its absence we noted that during the sucrose density centrifugations, the TG originally present became extensively hydrolyzed. This was thought to be due to the presence of an alkaline active lipase in the intestinal mucosa that we have shown to be a cytosolic

enzyme (13). The addition of E600 resulted in 96 ± 2% of the acyl groups (TG-FA plus FA) remaining as TG in the mucosa and 98 ± 3% remaining as TG in the storage pool. Thus, there was no evidence of continued TG hydrolysis during the sucrose density centrifugation in the presence of E600.

The effect of the various pre-treatments on the amount of TG in the intestinal mucosa is shown in **Fig. 1.** As expected, the amount of mucosal TG was least in the fasting rats and similar to previous observations from our laboratory (6). On infusing TO intraduodenally, the amount of TG in the mucosa increased nearly 6-fold. There was a further increment in mucosal TG content when PC was included in the intraduodenal TO infusion (45%) but statistical significance was not achieved. The largest amount of mucosal TG was seen in bile-diverted rats in which TG was infused intraduodendally, confirming calculated values from our previous work (6). This increase was statistically significant.

Of particular interest were the data related to the amount of TG in the pellet that we have previously identified to contain the TG storage pool (9) shown in the right-hand panel of Fig. 1. Only a very small amount of TG was present in the low-speed pellet of the fasting mucosa but the quantity of TG increased 8-fold in the rats infused with TO intraduodenally. The amount of TG in the low-speed pellet increased even more in the bile-diverted group (10-fold over fasting values). By contrast, the amount of lipid in the low-speed pellet from rats infused intraduodenally with TO plus PC was quite low and not different from fasting values. Thus, not only were there major alterations in the total mucosal TG mass on physiological manipulation, even greater changes were found in the TG content of the low-speed pellet.

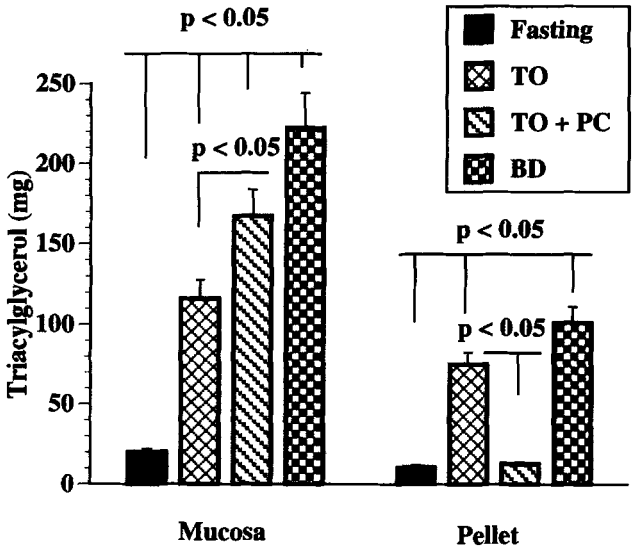

Fig. 1. The mass of triacylglycerol in the mucosa and low-speed pellet in fasted rats that were given trioleoylglycerol, 135 μmol/h (TO), or 135 μmol/h TO plus 9 μmol/h phosphatidylcholine (TO + PC), or bile-diverted and given 135 μmol/h TO (BD) as indicated in the legend to the figure. All infusions were given intraduodenally for 5 h. Triacylglycerol mass was measured by GLC as indicated in Methods. Statistically significant differences are shown by the *P* values whose bars connect the groups between which significant differences were found. The data are the mean ± SEM (n = 5).

The percentage of total mucosal TG in the pellet also varied considerably. Under fasting conditions, 50% of the mucosal TG was in the low-speed pellet. When TO alone was infused (group B rats), 60% of the lipid was in the pellet. Only 6% of the mucosal TG was in the low-speed pellet when PC was included in the intraduodenal infusion (group C rats). In the bile duct-diverted group (group D rats), 45% of the mucosal TG was in the low-speed pellet.

We next wished to determine the composition of the specific TG-FA in the mucosa. Shown in **Fig. 2A** are the fasting values. Palmitate, oleate, and linoleate made up the majority of the mucosal TG-FAs in the fasting state, confirming the previous work of Breckenridge and Kuksis (14) in fasting rats. When TO was infused intraduodenally (Fig. 2B), as expected, oleate became the major TG-FA present (85%). Similar values for the percentage of TG-oleate were found in the TO + PC infused rat (Fig. 2C), 88% oleate, and in the bile-diverted group (Fig. 2D), 82% oleate.

Also shown in Fig. 2 are the acyl group constituents of the mucosal FAs. These clearly differed from the mucosal TG-acyl group composition under all experimental conditions. As shown, the proportion of mucosal FAs that were oleate was smaller than the corresponding mucosal TG-FAs that were oleate ($P < 0.05$ or less for each comparison). The greatest concentration of FA-oleate was in the TO-infused rats but only 55% of the FA were oleate as compared to 85% of the TG-FA. The least concentration of FA-oleate was in the fasting rats, in which case 24% of the total FA was oleate as compared to TG-FA whose composition was 32% oleate. Similar data (not shown) were found in the low-speed pellet in comparing the proportion of TG-FA that was oleate to oleate in the FA pool.

Because there was such a large variation in the amount of TG in the low-speed pellet depending on the infusion conditions, we next sought to determine whether there were alterations in the buoyant densities of TG in the low-speed pellet (9). **Figure 3** shows the percentage distribution of TG in a discontinuous sucrose density gradient in the four groups of rats. In the fasting state, the TG is spread out throughout the gradient with the least dense portion of the gradient containing nearly half of the TG. The two groups of rats with the greatest amount of low-speed pellet TG (B and D) both had a

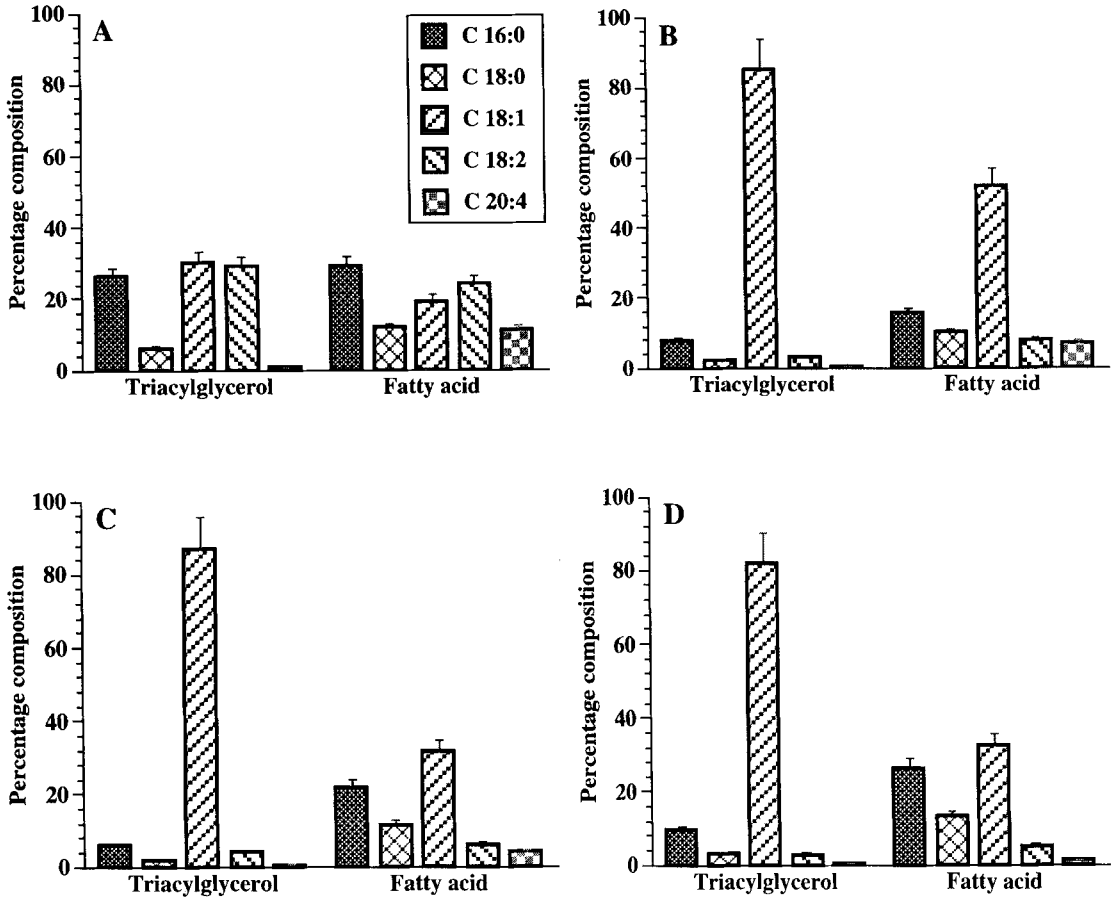

Fig. 2. The percentage composition of the triacylglycerol-acyl groups and fatty acids in the whole mucosa of rats treated as in Fig. 1. The five major fatty acids found are shown in the legend to the figure; A = group A, B = group B, C = group C and D = group D rats (see Methods). Statistically significant differences between the percentage of triacylglycerol-oleate and fatty acid-oleate are given in the text. The data are the mean ± SEM (n = 5).

similar pattern with 60% of the TG present in the least dense fraction and then a continued fall in the amount of TG through the denser portions of the gradient. Group C rats had a third pattern with only 20% of the TG in the least dense fraction, 72% in the second densest, with nearly none in the most dense fractions. The percentage of TG-FA that was oleate did not differ significantly throughout the gradient in any group (data not shown).

The mucosal TG-FA composition in Figs. 2B–2D was compatible with our previous reports (7, 11) in the intraduodenally TO-infused rat and showed a predominance of oleate as expected. Because of our previous findings that the low-speed pellet was associated with mucosal TG pool B (15) and because chylomicrons are mainly formed from exogenous TG, we expected to find that the TG-oleate composition of the low-speed pellet would be lower than the mucosa as a whole. This was the case during fasting and in the group C rats (**Fig. 4**) whose low-speed pellet TG masses were the lowest (Fig. 1) of any of the four groups studied. Where the TG mass of the low-speed pellet was larger in the group B and D rats, the percentage of TG-oleate in the pellet was similar to the mucosa as a whole.

DISCUSSION

During active lipid absorption in the rat, mucosal TG can be divided into at least two pools. The first pool, which we have termed pool A (7), is the pool that subserves chylomicron formation with eventual delivery of the TG into the lymph. It uses primarily *sn*-2-monoacylglycerol derived from dietary TG as its glyceride-glycerol precursor. Its turnover rate is quick ($t_{1/2}$ = 0.6/h) (3) compared to that found in liver (16) and its acyl groups reflect those of the dietary TG (7, 17). By contrast, the second TG pool, pool B, would appear not to be transported from the intestinal cell into the lymph but rather exits the mucosa via the portal vein (18). Its TG turnover rate is not known but is presumed to be slower than pool A (3). Its glyceride-glycerol is

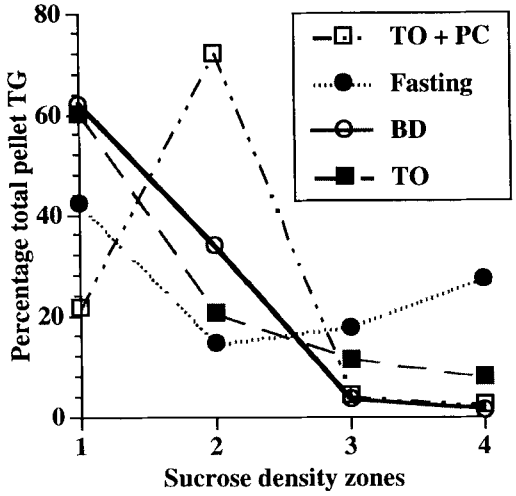

Fig. 3. The percentage distribution of triacylglycerol (TG) in the low-speed pellet from the intestine of rats treated as in Fig. 1. Treatment groups are indicated in the legend; TO + PC are group C rats, BD are group D rats, TO only are group B rats. Sucrose density gradient 1 = floating lipid at the top of the gradient, 2 = 0.25 M/0.86 M, 3 = 0.86 M/1.15 M sucrose interfaces, 4 = the pellet. The data are the mean of five experiments.

predominantly synthesized de novo (8). The TG-FA of pool B is thought to be enriched with endogenous FA as circulating FA can gain entry into pool B but little enters pool A (7). An additional contributor to pool B has recently been shown to be chylomicron remnants, whose acyl groups differ from that of their parent chylomicrons (12) and thus would also enrich pool B with non-dietary TG-FA.

The present study was designed to determine whether the TG-FA of pool B would be different than that of pool A. We also wished to know whether the size of the storage pool changed under conditions in which the partitioning of TG transport into the lymph was altered.

Previous studies from our laboratory have shown that the TG of pool B can be isolated in the low-speed pellet of mucosal homogenates during active lipid absorption (9). A possible identifying feature of pool B could be that its TG-FA differed from that of the whole mucosa and/or pool A. We found, however, that pool B had a radioactive specific activity that was equal to that of the total mucosa under steady state conditions of tri[^{3}H]oleoylglycerol intraduodenal infusion (15), conditions similar to those of the group B rats in the present study. These data would suggest that the low-speed pellet and the mucosa would contain equal percentages of TG-oleate, a finding confirmed in the present studies in the group B and D rats. During fasting or when PC was included in the TO infusion, however, the percentage of TG-oleate in pool B was less than the whole mucosa. In the case of the fasting model, oleate was not infused and therefore large amounts of TG-oleate were not expected. In both the group A and C rats, the low-speed TG pellet was very small which may have been a factor in its TG-FA alteration.

As shown in the present report, the TG storage pool varied greatly under differing physiological conditions; it enlarged when TG lymphatic transport rates were low and contracted when lymphatic TG transport increased. This relationship is shown in **Fig. 5** where the fraction of absorbed TG transported into the lymph is plotted as a function of the size of TG pool B. A linear inverse association is seen. The validity of the plot is enhanced by the finding that when pool B is calculated to be nonexistent, nearly all the mucosal TG would be transported into the lymph. In this figure, the data for the proportional size of the storage pool comes from the present report. The percentage of mucosal TG partitioned into the lymph comes from prior studies by our laboratory which have shown that an intraduodenal TO infusion is associated with only 54% of the absorbed TG being transported into the lymph, whereas when PC was included in the TO infusion, the proportion of lipid transported into the lymph increased to 85% (3). In bile-fistulated rats infused with TO, only 37% of the absorbed TG appeared in the lymph (6). The rates of TO and PC infusion, 135 μmol/h and 9 μmol/h, respectively, were the same as those used in the present studies. In sum, these data show that the size of TG pool B is dependent on the proportion of mucosal TG that is

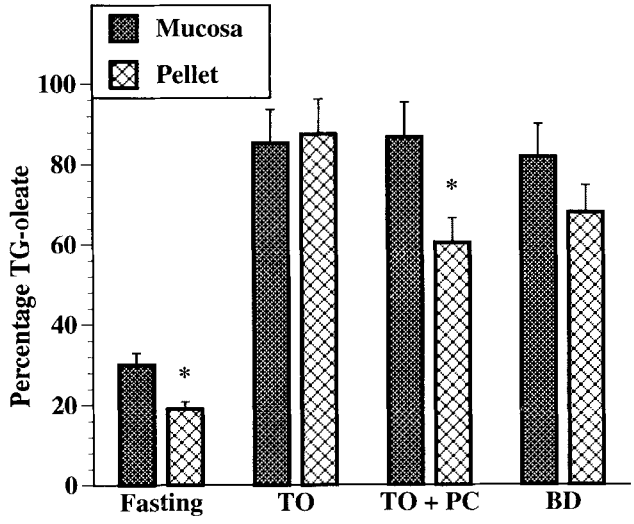

Fig. 4. The percentage triacylglycerol (TG)-oleate in the mucosa and low-speed pellet in the intestinal mucosa of rats treated as in Fig. 1. The low-speed pellet is defined in Methods. Groups of rats, A to D, were treated as in Fig. 1 (TO = trioleoylglycerol; TO + PC = trioleoylglycerol plus phosphatidylcholine; BD = bile-diverted, trioleoylglycerol infused). Statistically significant differences ($P < 0.05$) between pairs are shown by the asterisks above the bars. The data are the mean ± SEM (n = 5).

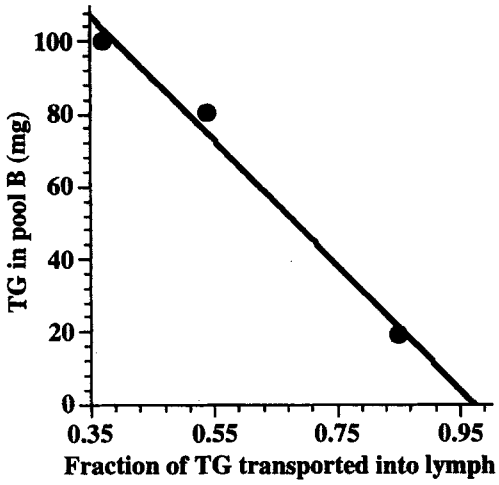

Fig. 5. The fraction of total mucosal triacylglycerol transported into the lymph as a function of the size of the mucosal triacylglycerol storage pool (pool B). Data for the proportion of mucosal triacylglycerol transported at 100 mg triacylglycerol in pool B is from Mansbach, et al. (6). The regression line was drawn by the least squares method ($y = -172x + 167$; $R^2 = 0.986$).

transported into the lymph. When most of the mucosal TG is able to be transported into the lymph, the amount of TG in pool B is small. By contrast, when lymphatic TG delivery is poor, then the fractional amount of mucosal TG in pool B is great.

With respect to the group C rats, supplemented with PC, it can be seen from Fig. 1 that 92% of the TG present in the mucosa is in pool A and available for transport out of the cell as chylomicrons. This should be contrasted with the data in the group B rats in which only 36% of the mucosal TG is in pool A ($P < 0.01$ as compared to group C rats). As the turnover rate of the TG in pool A at the steady state, i.e., chylomicron output, is the same under both physiological conditions (3, 5) it is evident that the output rate for TG into the lymph in the group C rats should be greater than twice that of the group B rats. This is what was found (3, 5) supporting the data presented in Fig. 1.

Although the TG-FA composition was previously noted to be modestly different from the FA pool in fasting rats (14, 19), an unexpected finding in the present studies was that these differences were even more evident in the TO-infused rats (groups B and C) (Fig. 2). This was particularly noticeable in the bile duct-diverted rats and the TO + PC-infused rats. In these rats the percentage of mucosal FA-oleate was ~32% of the total FA present, considerably less than the percentage of TG-FA that was TG-oleate, which was 82–87% ($P < 0.01$). In part, these mucosal, non-oleate FA could come from circulating FA (7) that we have shown can enter the mucosal cells and subsequently pool B. Another source that could contribute non-oleate acyl groups is the chylomicron remnants whose TG-FA differ from that of their parent chylomicron TG-FA (12, 20). Recent preliminary data from our laboratory suggest that the intestine takes up (12, 21) and metabolizes remnant particles (21). The intestine expresses the B-100/E receptor (22, 23) so that remnant uptake could be receptor-mediated via the apolipoprotein E that is known to be on the surface of the remnants (24). In this event, the remnant TG could be hydrolyzed by the intestinal alkaline lipase (13) to produce FA and monoacylglycerol (13) whose composition is enriched in endogenous FA. These endogenous FA have been shown to be transported from the intestine via the portal vein (18). One purpose of these endogenous FA could be to provide acyl groups of varying composition for the synthesis of phospholipids.

A potential consequence of the FAs that are fluxed into the portal vein is that the liver incorporates them, in part, into TG and subsequently exports them as very low density lipoprotein (VLDL)-TG. The VLDL produced in this manner may be a partial explanation of the increase in plasma TG seen as a result of cholestyramine administration (25). Cholestyramine has been shown to bind phospholipids in the intestinal lumen (26) and thus would likely lead to an increase in the distribution of lipid into pool B as suggested by Fig. 1. The lack of PC is suggested to be the cause of the potential increase in pool B lipids in the group D rats as pool B is small in the group C rats and taurocholate was included in the intraduodenal infusion in both groups. Support for this thesis comes from studies that show a reduced concentration of phospholipids in the mucosa of rats fed cholestyramine (27).

As the rapidity with which individual long chain FAs acylate partial glycerides is similar (28) and most FAs are esterified to TG even when the activity of esterification enzymes is reduced (28, 29), increases or decreases in the acylation rate for any specific FA cannot account for differences in FA and TG-FA composition. A potential explanation may be the route of entry of the FA. FAs entering by the apical pole of the cell have been shown to be rapidly esterified to TG (30). Similarly, FAs entering the basolateral portion of the enterocyte can also be extensively esterified to TG (7). The proportion by which esterification occurs on entry from the basolateral surface is dependent on the method of FA presentation, however. When the FA is given intravenously as a bolus bound to albumin, the majority of the mucosal FA are esterified to phospholipids, not TG, and considerable oxidation occurs (31). By contrast, when the FA is given intravenously until a steady state is reached, the great majority of the mucosal FA are esterified to TG (7).

These data are confirmed by experiments in Caco-2 cells in which the majority of the radiolabeled FA entering the cell from either the apical or the basolateral pole becomes esterified to TG (32, 33).

There are two likely explanations for the alteration in the composition of the mucosal FA pool as compared to the TG-FA pool. First, it is known that circulating FA can flux into the enterocyte and contribute to the FA pool (7). The proportion of the FA that are oleate is likely to be in the same proportion as that found to be in the circulation, 28% during the intraduodenal infusion of TO, 135 μmol/h (18). Second, chylomicron remnants also enter the basolateral portion of the enterocyte and their TG-FA composition also differs from that of their parent chylomicrons (12, 20). These TG undergo hydrolysis (M. Soued, C. M. Mansbach, II, unreported observations) and thus would release FA whose oleate composition differs from that of TO.

Recent work by Yang, Kuksis, and Myher (34) suggests that TG synthesized from phosphatidic acid, i.e., from de novo synthesized glyceride-glycerol, is first hydrolyzed to *sn*-2-monoacylglycerol and then re-acylated to TG. As its glyceride-glycerol is synthesized de novo, it is likely that TG synthesized via the phosphatidic acid pathway would enter pool B to a greater extent than TG synthesized from dietary-derived glyceride-glycerol. It is clear, however, than TG synthesized from FA in the absence of dietary monoacylglycerol can be adequately transported into the lymph as chylomicron TG (35).

This study was supported by National Institutes of Health Grant DK-38760 and in part by Veterans Administration Medical Research Funds.

Manuscript received 31 May 1995 and in revised form 24 July 1995.

REFERENCES

1. Tso, P., J. A. Barrowman, and D. N. Granger. 1986. Importance of interstitial matrix hydration in intestinal chylomicron transport. *Am. J. Physiol.* **250:** G497–G500.
2. Kasper, H. 1970. Fecal fat excretion, diarrhea and subjective complaints with highly dosed oral fat intake. *Digestion.* **3:** 321–322.
3. Mansbach, C. M., II, and A. Arnold. 1986. Steady-state kinetic analysis of triacylglycerol delivery into mesenteric lymph. *Am. J. Physiol.* **251:** G263–G269.
4. Tso, P., J. A. Balint, and J. B. Rodgers. 1980. Effect of hydrophobic surfactant (Pluronic L-81) on lymphatic lipid transport in the rat. *Am. J. Physiol.* **239:** G348–G353.
5. Tso, P., M. Kendrick, J. A. Balint, and W. J. Simmonds. 1981. Role of biliary phosphatidylcholine in the absorption and transport of dietary triolein in the rat. *Gastroenterology.* **80:** 60–65.
6. Mansbach, C. M., II, A. Arnold, and M. A. Cox. 1985. Factors influencing triacylglycerol delivery into mesenteric lymph. *Am. J. Physiol.* **249:** G642–G648.
7. Mansbach, C. M., II, and R. F. Dowell. 1992. Uptake and metabolism of circulating fatty acids by rat intestine. *Am. J. Physiol.* **261:** G927–G933.
8. Mansbach, C. M., II, and S. Partharsarathy. 1982. A re-examination of the fate of glyceride glycerol in neutral lipid absorption and transport. *J. Lipid Res.* **23:** 1009–1019.
9. Tipton, A. D., S. Frase, and C. M. Mansbach, II. 1989. The isolation and characterization of a mucosal triacylglycerol pool undergoing hydrolysis. *Am. J. Physiol.* **257:** G871–G878.
10. Folch, J., M. Lees, and G. H. Sloan Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
11. Mansbach, C. M., II, and P. Nevin. 1994. Effect of Brefeldin A on lymphatic triacylglycerol transport in the rat. *Am. J. Physiol.* **266:** G292–G302.
12. Mansbach, C. M., II, and R. F. Dowell. 1995. The role of the intestine in chylomicron remnant clearance. *Am. J. Physiol.* **269:** G144–G152.
13. Rao, R. H., and C. M. Mansbach, II. 1993. Alkaline lipase in rat intestinal mucosa: physiological parameters. *Arch. Biochem. Biophys.* **304:** 483–489.
14. Breckenridge, W. C., and A. Kuksis. 1975. Triacylglycerol biosynthesis in everted sacs of rat intestinal mucosa. *Can. J. Biochem.* **53:** 1184–1195.
15. Parlier, R. D., S. Frase, and C. M. Mansbach, II. 1989. The intraenterocyte distribution of absorbed lipid and effects of phosphatidylcholine. *Am. J. Physiol.* **256:** G349–G355.
16. Zech, L. A., S. M. Grundy, D. Steinberg, and M. Berman. 1979. Kinetic model for production and metabolism of very low density lipoprotein triglycerides. *J. Clin. Invest.* **63:** 1262–1273.
17. Mattson, F. H., and R. A. Volpenhein. 1964. The digestion and absorption of triglycerides. *J. Biol. Chem.* **239:** 2772–2777.
18. Mansbach, C. M., II, R. F. Dowell, and D. Pritchett. 1991. Portal transport of absorbed lipids in the rat. *Am. J. Physiol.* **261:** G530–G538.
19. Breckenridge, W. C., and A. Kuksis. 1975. Diacylglycerol biosynthesis in everted sacs of rat intestinal mucosa. *Can. J. Biochem.* **53:** 1170–1183.
20. Nilsson, A., and B. Landin. 1988. Metabolism of chylomicron arachidonic and linoleic acid in the rat. *Biochim. Biophys. Acta.* **959:** 288–295.
21. Soued, M., and C. M. Mansbach, II. 1994. Isolated enterocytes take up and metabolize chylomicron remnants. *Gastroenterology.* **106:** A633.
22. Stange, E. F., and J. Dietschy. 1983. Cholesterol synthesis and low density lipoprotein uptake are regulated independently in rat small intestinal epithelium. *Proc. Natl. Acad. Sci. USA.* **80:** 5739–5743.
23. Fong, L., E. Bonney, J. C. Kosek, and A. D. Cooper. 1989. Immunohistochemical localization of low density lipoprotein receptors in adrenal gland, liver, and intestine. *J. Clin. Invest.* **84:** 847–856.
24. Green, P. H. R., and R. M. Glickman. 1981. Intestinal lipoprotein metabolism. *J. Lipid Res.* **22:** 1153–1173.
25. Betteridge, D. J., D. Bhatnager, R. F. Bing, P. N. Durrington, G. R. Evans, H. Flax, R. H. Jay, N. Lewis-Barned, J. Mann, and D. R. Matthews. 1992. Treatment of familial hypercholesterolaemia. United Kingdom lipid clinics study of pravastatin and cholestyramine. *Br. Med. J.* **304:** 1335–1338.
26. Gallaher, D., and B. O. Schneeman. 1986. Intestinal interaction of bile acids, phospholipids, dietary fibers, and

cholestyramine. *Am. J. Physiol.* **250:** G420–G426.
27. Cassidy, M. M., F. G. Lightfoot, L. Grau, S. Satchitanandum, and G. V. Vahouny. 1985. Lipid accumulation in jejunal and colonic mucosa following chronic cholestyramine (Questran) feeding. *Dig. Dis. Sci.* **30:** 468–476.
28. Karmen, A., M. Whyte, and D. S. Goodman. 1963. Fatty acid esterification and chylomicron formation during fat absorption. I. Triglycerides and cholesterol esters. *J. Lipid Res.* **4:** 312–321.
29. Bennett Clark, S., T. E. Ekkers, A. Singh, J. A. Balint, P. R. Holt, and J. B. Rodgers, Jr. 1973. Fat absorption in essential fatty acid deficiency: a model experimental approach to studies of the mechanism of fat malabsorption of unknown etiology. *J. Lipid Res.* **14:** 581–588.
30. Jersild, R. A., Jr. 1966. A time sequence study of fat absorption in the rat jejunum. *Am. J. Anat.* **118:** 135–161.
31. Gangl, A., and R. K. Ockner. 1975. Intestinal metabolism of plasma free fatty acids. Intracellular compartmentation and mechanisms of control. *J. Clin. Invest.* **55:** 803–813.
32. Trotter, P. J., and J. Storch. 1991. Fatty acid uptake and metabolism in a human intestinal cell line (Caco-2): comparison of apical and basolateral incubation. *J. Lipid Res.* **32:** 293–304.
33. Levin, M. S., V. D. Talkad, J. I. Gordon, and W. F. Stenson. 1992. Trafficking of exogenous fatty acids within Caco-2 cells. *J. Lipid Res.* **33:** 9–19.
34. Yang, L. Y., A. Kuksis, and J. J. Myher. 1995. Biosynthesis of chylomicron triacylglycerols by rats fed glyceryl or alkyl esters of menhaden oil fatty acids. *J. Lipid Res.* **36:** 1046–1057.
35. Tso, P., L. M., and B. Borgstrom. 1987. Factors regulating the formation of chylomicrons and very low density lipoproteins by the rat small intestine. *Biochim. Biophys. Acta.* **922:** 304–313.