Metabolic adaptations to dietary fat malabsorption in chylomicron-deficient mice

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A mouse model of chylomicron deficiency was recently developed; these mice express a human apolipoprotein (apo) B transgene in the liver but do not synthesize any apoB in the intestine. Despite severe intestinal fat malabsorption, the mice maintain normal concentrations of plasma lipids and liver-derived apoB 100-containing lipoproteins. We investigated the metabolic mechanisms by which plasma lipid levels are kept normal. *De novo* lipogenesis (DNL) and cholesterogenesis were measured by mass isotopomer distribution analysis (MIDA). Plasma non-esterified fatty acid (NEFA) fluxes and hepatic re-esterification of labelled plasma NEFA were also measured. Hepatic and plasma triacylglycerol (TG) concentrations and plasma NEFA fluxes were not different between chylomicron-deficient mice and controls. The contribution from DNL to the hepatic TG pool was only modestly higher in chylomicron-deficient mice [$12 \pm 2.1\,\%$ ($n = 7$) compared with $3.7 \pm 1.0\,\%$ ($n = 9$); means $\pm$ S.E.M.], whereas cholesterogenesis was markedly elevated. The fractional contribution from plasma NEFA to hepatic TG was greatly elevated in the chylomicron-deficient animals ($62\,\%$ compared with $23\,\%$). Accordingly, $73\,\%$ of hepatic TG was neither from DNL nor from plasma NEFA in controls, presumably reflecting prior contribution from chylomicron remnants, compared with only $26\,\%$ in the chylomicron-deficient group. The long-term contribution from DNL to adipose fat stores reached approximately the same steady-state values ($\approx 30\,\%$) in the two groups. Body fat accumulation was much lower in chylomicron-deficient animals; thus, whole-body absolute DNL was significantly lower. We conclude that plasma and hepatic TG pools and hepatic secretion of apoB-containing particles are maintained at normal levels in chylomicron-deficient mice, not by *de novo* fatty acid synthesis, but by more avid re-esterification of plasma NEFA, replacing the normally predominant contribution from chylomicrons, and that some dietary fat can be absorbed by apoB-independent mechanisms.

Key words: apolipoprotein, *de novo* lipogenesis, fatty acid turnover, lipoprotein, triacylglycerol.

INTRODUCTION

The formation of chylomicrons is essential for the absorption of dietary triacylglycerol (TG), fat-soluble vitamins and cholesterol. Chylomicrons normally carry dietary fats and fat-soluble vitamins from the intestine to the general circulation, delivering lipids both to the liver and to peripheral tissues. Chylomicron-deficient mice have recently been developed by crossing apolipoprotein (apo) B knock-out mice with human apoB transgenic mice [1] in which the transgene is expressed in the liver but not in the intestine. The chylomicron-deficient mice lack all apoB expression (i.e. both mouse and human) in the intestine, which prevents the assembly and secretion of TG-rich lipoproteins from the intestine. These mice accumulate massive amounts of cytosolic fat droplets within the absorptive enterocytes of the intestine and manifest severe intestinal fat malabsorption and retarded growth [1].

Although chylomicron-deficient mice cannot effectively absorb and transport dietary fats, they maintain normal plasma TG, cholesterol and apoB-100 concentrations and are capable of depositing some subcutaneous adipose tissue [1]. The metabolic mechanism by which their plasma lipids are maintained at normal levels has not been established. We have hypothesized that plasma lipids are maintained by an increased rate of endogenous fatty acid and cholesterol synthesis [i.e. *de novo* lipogenesis (DNL) and *de novo* cholesterogenesis], stimulated by the absorption of a high-carbohydrate, low-fat, low-cholesterol diet [2–5].

Recent advances in isotope measurement techniques [6–9] and methods for catheterizing mice [10] allow the metabolic response to genetic modifications to be studied directly in individual mice. In this study, we examined the metabolic mechanisms by which normal concentrations of plasma lipids and apoB-containing lipoproteins are maintained in the absence of chylomicron formation and normal fat absorption.

MATERIALS AND METHODS

Genetically modified mice

Heterozygous apoB knock-out mice ($Apob^{+/-}$) were crossed with hemizygous human apoB transgenic mice ($HuBTg^{+/0}$) to generate human apoB transgenic mice that were heterozygous for the *Apob* knock-out mutation ($HuBTg^{+/0}$,$Apob^{+/-}$) [1]. The human

Abbreviations used: apo, apolipoprotein; DNL, *de novo* lipogenesis; MIDA mass isotopomer distribution analysis; NE-FA, non-esterified fatty acid; TG, triacylglycerol; CE, cholesterol ester; TMS, trimethylsilyl; SREBP, sterol-response-element-binding protein.

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apoB transgenic mice used in these breeding experiments express the human apoB transgene at high levels in the liver, but human apoB expression is absent in the intestine. These HuBTg$^{+/0}$ *Apob*$^{+/-}$ mice were then intercrossed to generate human apoB transgenic mice that were homozygous for the apoB knockout mutation. These mice (genotypes HuBTg$^{+/+}$,*Apob*$^{-/-}$ or HuBTg$^{+/0}$,*Apob*$^{-/-}$), theoretically constituting 3/16 of the offspring from this mating, express human apoB in the liver but lack all apoB synthesis (mouse or human) in the intestine [1]. Genotyping was performed by Southern blot analysis of genomic DNA prepared from tail biopsies [11,12]. Mice deficient in intestinal apoB expression (chylomicron-deficient mice) had a higher mortality rate in the pre-weaning period [1] and therefore were allowed to wean 1 week later than their littermates to help ensure survival. After weaning, the mice were fed standard mouse chow *ad libitum*.

In this study, 14 of 566 offspring (2.5%) from HuBTg$^{+/0}$, apoB$^{+/-}$ intercrosses had either the HuBTg$^{+/+}$,*Apob*$^{-/-}$ or HuBTg$^{+/0}$,*Apob*$^{-/-}$ genotype and survived for more than 1 month after birth. These chylomicron-deficient mice were used for the infusion and feeding studies described here. Control mice were littermates that expressed human apoB in liver as well as mouse apoB (i.e. HuBTg$^{+/0}$*Apob*$^{+/+}$ or HuBTg$^{+/+}$*Apob*$^{+/+}$).

Isotope infusion studies

Chylomicron-deficient mice and littermate controls underwent surgical implantation of a jugular venous catheter 48 h before the start of the infusion [10]. Mice were placed in a plexiglas restraint and an infusion of [1-$^{13}C_1$]acetate (15 mg·min^{-1}·kg^{-1}) and [1,2,3,4-$^{13}C_4$]palmitate (70 μg·min^{-1}·kg^{-1}) complexed with serum albumin was administered for 3 h. Stable isotopes were purchased from Isotech (Miamisburg, OH, U.S.A.) or Cambridge Isotope Labs (Andover, MA, USA). After infusion, the mice were anaesthetized with ketamine and exsanguinated from the retro-orbital sinus. Liver and adipose tissue samples were harvested, and the carcasses were frozen for body composition analysis.

Long-term (dietary) labelling studies

For long-term labelling studies, the mice were fed powdered standard mouse chow (Purina 5015) containing [1-$^{13}C_1$]acetate (20 mg/g chow) *ad libitum* for up to 30 days. The chow was ground and mixed with sodium [1-^{13}C]acetate dissolved in absolute ethanol, and the ethanol was then evaporated at room temperature. Animals ate the diet in normal quantities and grew normally. Biopsies of subcutaneous adipose were taken from the lower abdomen at 7, 14, 21, and 30 days. At the end of the study, animals were killed, and samples of blood, liver and adipose tissues were taken and frozen.

GC–MS

Plasma, liver and adipose tissue samples were weighed, and lipid was extracted with heptane and propan-2-ol (30:70, v/v) containing pentadecanoic acid and tripentadecanoylglycerol (tripentadecanoin) as internal standards. Non-esterified fatty acid (NEFA), TG and cholesterol ester (CE) fractions were separated by TLC (Silica gel G plates, Analtech, Newark, DE, U.S.A.). The developing medium was 100 ml of hexane/ethyl ether/acetic acid (80:20:1). These fractions were derivatized to fatty acid methyl esters with methanolic 3 M HCl for analysis by GC–MS (models 5970 and 5971, Hewlett-Packard, Palo Alto, CA, U.S.A.).

Fatty acid methyl esters were analysed by GC–MS with a 12.0 m DB1 fused-silica column under isothermal conditions at 200 °C [6]. For methyl palmitate, the molecular anion and its isotopes (m/z 270–274 representing M_0–M_4) were quantified under the selected ion-monitoring mode. For trimethylsilyl (TMS)-cholesterol analysis, the same DB1 column was used with split injection under isothermal conditions at 270 °C. The parent M_0 fragment (m/z 368), representing underivatized cholesterol minus [OH], was monitored, as were M_1–M_4 (m/z 369–372).

Measurement of DNL and cholesterol synthesis

DNL and cholesterol synthesis were calculated by mass isotopomer distribution analysis (MIDA [6]. Briefly, MIDA is based on the mathematics of combinatorial probabilities; the central principle is that the pattern of labelled subunits present in a polymer reflects the isotopic enrichment of the precursor pool used for its synthesis [6,7]. The mass isotopomer pattern reveals the proportion of labelled and unlabelled monomeric subunits in the biosynthetic precursor pool, according to the binomial or multinomial expansion, as described in detail elsewhere [6–9].

For DNL, the ratio of molecular species containing a single [^{13}C]acetate subunit to those containing two [^{13}C]acetate subunits reveals the precursor pool (acetyl-CoA) enrichment, and thus fractional DNL [6]. For cholesterol synthesis, the enrichments of mass isotopmers M_0, M_1, M_2, M_3 and M_4 were determined; the ratio of M_3/M_1-M_4 was used [8].

Measurement of NEFA flux and re-esterification

Both NEFA flux and re-esterification were measured using a constant infusion of [1,2,3,4-$^{13}C_4$]palmitate bound to human serum albumin [13]. The dilution by endogenous palmitate at steady state reveals the rate of appearance (Ra) in plasma:

$$\text{Ra palmitate} = \left(\frac{\text{Rate of infusion of } ^{13}\text{C-palmitate}}{\text{Fraction labelled palmitate}}\right) - (\text{Rate of infusion of } ^{13}\text{C-palmitate})$$

The enrichments of palmitate in plasma and liver TG were analysed in the same manner. The fraction of serum or hepatic TG derived from plasma NEFA was calculated by comparing TG palmitate enrichments with plasma palmitate enrichments, providing an index of plasma NEFA re-esterification.

Fatty acid compositional analysis

TG, NEFA and CE fractions from plasma and adipose tissue were transesterified to their methyl esters. Samples were then analysed by GC with flame-ionization detection [14]. Individual fatty acids were identified by retention time compared with standards, and their relative molar proportions were calculated. FA composition of diet was analysed in the same manner, by extraction followed by TLC and GC analysis.

Body composition analysis

The body composition of the mice was determined by a modification of the technique developed by Leshner and Litwin [15]. The whole, frozen, eviscerated carcass was weighed, ground thoroughly in a Waring blender and dried to a constant weight in a mechanical convection oven at 60 °C. Four samples (1 g each) of this ground material were used for lipid extraction, first with 6 ml of chloroform and methanol (2:1) and then with 4 ml of chloroform. The extracted lipid layer was dried in an oven at 60 °C and weighed. The fat content of the whole carcass was calculated by dividing the average of the four lipid extractions by the weight of the eviscerated carcass.

Statistical analyses

The statistical significance of differences between chylomicron-deficient mice and controls was determined by the unpaired Student's *t*-test.

RESULTS

Body composition

Chylomicron-deficient mice had considerably less adipose mass than age-matched controls (Table 1). The percentage of body fat was $2.5 \pm 0.6\,\%$ compared with $6.1 \pm 0.8\,\%$ ($P < 0.01$) in 2-month-old mice and $3.4 \pm 0.2\,\%$ compared with $27.9 \pm 2.8\,\%$ ($P < 0.001$) in 7-month-old mice.

DNL

The short-term fractional contribution from DNL to TG-palmitate in plasma and liver, measured after a 3 h intravenous infusion of [1-$^{13}C_1$]acetate, was modestly higher in chylomicron-deficient mice than in controls (Table 2) [$6.0 \pm 0.6\,\%$ compared with $3.1 \pm 0.5\,\%$ (mean $\pm$ S.E.M) ($P = 0.002$) for plasma TG and $12 \pm 2.1\,\%$ ($n = 7$) compared with $3.7 \pm 1.0\,\%$ ($n = 9$) ($P = 0.002$) for liver TG]. Precursor pool enrichments for TG-palmitate were not significantly different between groups (isotopically-labelled fraction for hepatic acetyl-CoA, 0.09 ± 0.04 in controls compared with 0.13 ± 0.07 in chylomicron-deficient mice).

The long-term contribution of DNL to body fat stores was evaluated in mice fed chow containing labelled acetate for up to 30 days. The contribution to adipose TG-palmitate from DNL rose faster in chylomicron-deficient mice, reflecting the smaller adipose tissue pool size, but reached approximately the same values as in controls (30–35 % contribution, Table 2). Moreover, after correction for the lower adipose mass in chylomicron-deficient mice, the absolute rates of accumulation of *de novo* synthesized palmitate in the whole-body were significantly lower in chylomicron-deficient mice (Table 3).

Table 1 Percentage body fat in 2–3 month-old and 7–8 month-old chylomicron-deficient mice and age-matched controls

Extractable fat as a percentage of wet weight of the eviscerated carcass. Mean $\pm$ S.E.M. (*n*), * $P < 0.01$ compared with controls

	Body fat (%)	
	2–3 Months old	7–8 Months old
Chylomicron-deficient mice	2.5 ± 0.6* (3)	3.4 ± 0.2* (5)
Control mice	6.1 ± 0.8 (6)	27.9 ± 2.8 (2)

Table 2 Fractional DNL in plasma, liver and adipose TG

Percentage of palmitate synthesized *de novo*, calculated by MIDA. Short-term study values were after a 3 h intravenous infusion of [1-$^{13}C_1$]acetate. Long-term study values based on subcutaneous adipose samples taken after 7, 14, 21 or 30 days of [1-$^{13}C_1$]acetate fed in chow. Means $\pm$ S.E.M. (*n*). * $P < 0.01$ compared with controls.

	Fraction of palmitate synthesized *de novo* (%)					
	Short-term study		Long-term study			
	Plasma TG	Liver TG	7 Days	14 Days	21 Days	30 Days
Chylomicron-deficient mice	6.0 ± 0.6* (7)	12 ± 2.1* (7)	33 ± 5.8* (3)	23 ± 4.3* (3)	24 ± 1.5* (2)	28 ± 4.2* (2)
Control mice	3.1 ± 0.5 (9)	3.7 ± 1.0 (9)	18 ± 6.4 (2)	24 ± 1.3 (3)	31 ± 2.1 (3)	36 ± 1.0 (2)

Table 3 Absolute accumulation of *de novo* synthesized palmitate in the whole body

Accumulation of *de novo* synthesized palmitate in adipose tissue TG was calculated, after feeding [1-$^{13}C_1$]acetate, by the following equation: accumulation = (fraction of palmitate DNL in subcutaneous adipose) $\times$ (mg total body fat) $\times$ (percentage of fatty acids which are palmitate). Means $\pm$ S.E.M. (*n*). * $P < 0.01$ compared with controls.

	Absolute whole-body palmitate DNL (mg)			
	7 Days	14 Days	21 Days	30 Days
Chylomicron-deficient mice	63 ± 11 (3)	45 ± 8* (3)	45 ± 2* (2)	54 ± 7* (3)
Control mice	76 ± 26 (2)	99 ± 5 (3)	128 ± 9 (3)	149 ± 4 (2)

Table 4 Fraction of TG derived from plasma NEFA in liver and plasma

Ratio of the enrichment of [1,2,3,4-$^{13}C_4$]palmitate in plasma or liver TG to the enrichment of [1,2,3,4-$^{13}C_4$]palmitate in plasma NEFA. Means $\pm$ S.E.M. (*n*). * $P < 0.01$ compared with controls.

	Fraction derived from plasma NEFA	
	Plasma TG	Liver TG
Chylomicron-deficient mice	0.28 ± 0.04* (7)	0.62 ± 0.09* (5)
Control mice	0.11 ± 0.02 (7)	0.23 ± 0.08 (7)

Plasma NEFA flux and hepatic re-esterification

Plasma NEFA fluxes were not significantly different between the two groups, despite the lower adipose TG mass in the chylomicron-deficient mice [rate of palmitate appearance in plasma ($mg \cdot min^{-1} \cdot kg^{-1}$), 2.3 ± 1.3 in controls compared with 3.3 ± 2.0 in chylomicron-deficient mice]. There were, however, significant differences in hepatic re-esterification of plasma NEFA between groups, as measured by the contribution from plasma [1,2,3,4-$^{13}C_4$]palmitate to TG in liver and plasma (Table 4). The contribution from M_4-labelled plasma NEFA to M_4-labelled hepatic TG was much higher in chylomicron-deficient mice ($62 \pm 9\,\%$ compared with $23 \pm 8\,\%$, $P < 0.03$), as was the contribution

Table 5 Cholesterogenesis

Fraction of plasma cholesterol synthesized *de novo* after a short (3 h infusion of [1-$^{13}C_1$]acetate infusion) or long (10 day chow feeding of [1-$^{13}C_1$]acetate) labelling period. Means ± S.E.M. (*n*). * $P < 0.01$ compared with controls.

	Cholesterogenesis (%)	
	Short-term synthesis	Long-term synthesis
Chylomicron-deficient mice	4.1 ± 2.6 (6)	54.9 ± 0.1* (2)
Control mice	Undetectable (6)	29.4 ± 0.1 (2)

from plasma M_4-labelled FFA to plasma M_4-labelled TG (28 ± 4 % compared with 11 ± 2 %, $P < 0.01$).

Fatty acid composition of tissues and diet

The relative proportions of the various fatty acids in adipose tissue and plasma were measured. Chylomicron-deficient mice had a higher proportion of non-essential fatty acids (oleate, 57 ± 2 % compared with 47 ± 4 %; palmitoleate, 9 ± 4 % compared with 6 ± 1 %, $P < 0.01$) and a lower proportion of linoleate (18:2) (7 ± 3 % compared with 19 ± 2 %, $P < 0.01$) than control mice, in visceral adipose TG. There were no differences between groups observed for myristate (2 ± 1 % compared with 1 ± 1 %), palmitate (21 ± 2 % compared with 22 ± 2 %), stearate (2 ± 1 % compared with 3 ± 1 %) or linolenate (1 ± 1 % compared with 1 ± 1 %). Similar relationships were seen in plasma TG and NEFA as well as in subcutaneous adipose and liver TG. Analysis of dietary FA composition revealed 36 % oleate, 24 % linoleate, 23 % palmitate, 10 % stearate, 3 % palmitoleate, 2 % linolenate and 1 % myristate.

Cholesterol synthesis

Plasma CE showed no ^{13}C label in control mice after a 3 h infusion of [1-^{13}C]acetate. In chylomicron-deficient mice, however, 4.1 ± 2.6 % of cholesterol in CE came from *de novo* synthesis after a 3 h infusion (Table 5).

Long-term cholesterogenesis was evaluated in mice fed chow containing labelled acetate for 10 days. The contribution from cholesterogenesis to plasma CE was considerably higher in chylomicron-deficient mice than in controls (Table 5) (54.9 ± 0.1 % compared with 29.4 ± 0.1 %, $P = 0.0001$).

Serum NEFA and TG concentration

Serum NEFA concentrations were not significantly different between the two groups: 611 ± 297 and 730 ± 240 nmol/ml in chylomicron-deficient mice ($n = 6$) and controls ($n = 8$) respectively. The liver TG content was also similar [12 ± 3 μmol/g ($n = 7$) and 13 ± 5 μmol/g ($n = 6$) respectively].

DISCUSSION

We initially hypothesized that the normal plasma lipid and lipoprotein levels observed in chylomicron-deficient mice [1] would be maintained by increased rates of endogenous fatty acid and cholesterol synthesis. This study shows that chylomicron-deficient mice maintained normal levels of lipids and apoB-containing lipoproteins, not by increasing synthesis of fatty acids by liver or adipocytes, but instead by more avid re-esterification of plasma NEFA. Moreover, most of the body fat that accumulated in chylomicron-deficient mice did not ultimately derive from DNL, suggesting that some dietary fat can be absorbed by apoB-independent mechanisms.

Role of DNL

The contribution of DNL to the hepatic TG pool was only slightly higher in chylomicron-deficient mice and remained a quantitatively minor pathway after short-term isotope infusions. This finding of relatively normal rates of hepatic DNL is noteworthy in view of the higher ratio of non-essential fatty acids (e.g. palmitoleate and oleate) to essential fatty acids (e.g. linoleate) in the visceral adipose stores of chylomicron-deficient mice. In some human studies, this ratio in adipose tissue relative to diet has been used as an indirect measure of DNL [16].

To determine if the modest increase in short-term DNL underestimated the true long-term contribution to body fat stores, we fed the mice chow containing labelled acetate. In this manner, the isotope was delivered over a long enough period to allow turnover of the pool of adipose TG. The contribution of DNL to adipose rose to its plateau value faster in chylomicron-deficient mice, but reached approximately the same fractional value (30–35 %) as in controls. These results indicate that the adipose tissue TG in chylomicron-deficient mice were no more the result of DNL than in control mice. The rapid increase in adipose-TG labelling probably occurred because the chylomicron-deficient mice have a smaller, more rapidly turning over adipose tissue pool and therefore reached a steady-state more rapidly. Moreover, the absolute accumulation rate of newly synthesized fat in adipose tissue was significantly lower in chylomicron-deficient mice, after correction for their reduced adipose tissue mass (Table 2). Since most of the adipose fat in chylomicron-deficient mice was not ultimately derived from DNL, we conclude that the majority must have arisen from the apoB-independent absorption of dietary fat.

Quantitative estimates of the amount of dietary fat absorption required for deposition of adipose tissue in the chylomicron-deficient animals are instructive. The total accumulation of non-DNL-derived fatty acids in the whole mouse can be estimated if the measured steady-state contribution from DNL to palmitate (about 30 %) is assumed to apply for all non-essential fatty acids in adipose tissue. Based on this calculation, a total of 227 mg of non-DNL body fat is estimated to accumulate in these mice over 6 months. Thus, about 1.25 mg/day of dietary fat absorption represents a minimum value required to explain the accumulated body fat. These animals ate roughly 800 mg of fat per day (7 g chow × 11 % fat by weight). Less than 1 % absorption efficiency for dietary fatty acids is therefore required to account for the body fat accumulation observed in the chylomicron-deficient mice. Even if 90 % of absorbed fat were oxidized, and therefore did not get stored in adipose tissue, only a 10 % absorption efficiency would be required to account for the fat accumulation observed here.

Of interest in this context, a recent study by Hamilton et al. [17] documented the existence of rare lipid-staining particles in the Golgi apparatus in the intestinal enterocytes of chylomicron-deficient mice. It was not clear whether those Golgi lipid particles contained apoB, but the presence of lipid-staining particles in that subcellular compartment suggested that some fats could be absorbed in these mice. Based on the observation of plasma α-tocopherol levels that were approx. 10 % of normal values [1], this route might occur at about 10 % absorption efficiency of wild-type mice. This absorption efficiency would be sufficient to account for the estimated non-DNL body fat accumulation (as discussed above). There have also been reports of significant

amounts of portal absorption of long-chain fatty acids [18–20]. It is tempting to speculate that some chylomicron-independent fat absorption may also occur in humans and may partially explain the generally mild symptoms of fat malabsorption in humans with abetalipoproteinaemia [21].

The explanation for the higher proportions of non-essential fatty acids in tissues of the chylomicron-deficient mice compared with controls is uncertain, since it is not due to increased DNL. It may reflect preferential utilization of essential fatty acids by tissues under these conditions of fat malabsorption [22]. Another possible explanation for the increased oleate to linoleate ratios could be increased activity of stearyl desaturase, an enzyme that is induced by sterol-response-element-binding protein (SREBP) [23]. It is likely that SREBP levels are elevated in chylomicron-deficient mice, as evidenced by the higher rates of endogenous cholesterogenesis (Table 2).

Metabolic sources of hepatic TG

If the majority of hepatic TG is not derived from the DNL pathway and dietary fat absorption is markedly impaired, what is the source of the hepatic TG pool? To address this question, we measured the contribution from plasma NEFA (which is derived from lipolysis under the fasting condition of this study) to hepatic TG. Although there were no significant differences between the groups in the rate of appearance of NEFA in plasma or in plasma NEFA concentrations, 62 % of TG in the liver came directly from plasma NEFA in chylomicron-deficient mice compared with only 23 % in controls, after a 3 h infusion of labelled FFA. These results indicate rapid turnover of TG in the liver, and show that the liver TG was synthesized primarily from plasma NEFA entering the liver as a result of lipolysis, in the absence of dietary chylomicron input. Together, DNL plus re-esterification of plasma FFA accounted for all but 26 % of the hepatic TG pool in the chylomicron-deficient mice. In contrast, the majority of hepatic TG in control mice came from unlabelled sources after a 3 h isotope infusion (73 % of palmitate was not accounted for by either DNL or re-esterification of plasma NEFA), which probably reflects prior input into the hepatic cytosolic TG storage pool from chylomicron remnants [24].

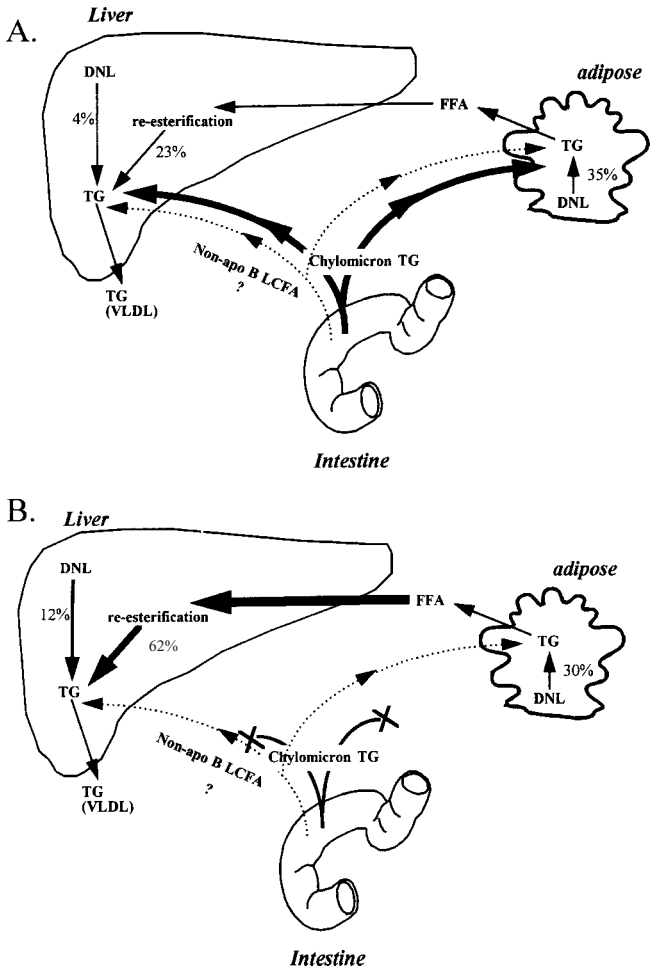

Figure 1 Schematic model of the adaptations of fatty acid metabolism to dietary fat malabsorption

Thickness of arrows reflect relative quantitative flux. Percentage values represent contribution from pathway. Normal (**A**) are compared with chylomicron-deficient (**B**) mice.

Implications for normal TG metabolism

These observations are of interest for the normal regulation of TG metabolism. Despite markedly reduced dietary fat absorption, plasma and hepatic TG pool sizes and plasma apoB concentrations were maintained at normal levels, whereas adipose TG pool size was not restored to normal. Hepatic lipids are thought to be important in protecting apoB from degradation within the endoplasmic reticulum, thereby maintaining the secretion of apoB-containing particles [25]. Our results suggest that a sufficient amount of hepatic lipid is present for maintaining lipoprotein secretion by liver, even when chylomicron production is absent. Because very-low-density lipoprotein and low-density lipoprotein also carry cholesterol, phospholipids and other lipid components (e.g. fat-soluble vitamins) to tissues, in addition to serving in the transport and delivery of TG, maintenance of hepatic secretion of apoB-containing particles might be important, even in the setting of fat malabsorption. Of note, the hepatic TG pools were not maintained through induction of DNL, since hepatic DNL remained a minor pathway under these conditions. Instead, the primary source of hepatic TG was re-esterification of plasma NEFA derived from lipolysis. The reduced total body adipose stores apparently did not interfere with the release of NEFA from adipocytes or delivery of NEFA to the liver. Despite similar levels of plasma NEFA flux, chylomicron-deficient mice exhibited a 3-fold greater accumulation of re-esterified NEFA in hepatic TG compared with control mice. Because control mice all expressed human apoB in the liver, the presence of human apoB cannot by itself explain differences in hepatic re-esterification rates between chylomicron-deficient and control animals. Interestingly, endogenous cholesterol synthesis was induced significantly in the chylomicron-deficient mice; this finding is consistent with the previous observation of increased plasma mevalonate concentrations in these mice [1].

These results suggest that the hepatic TG pool size is prevented from falling below levels required for secretion of apoB-containing particles and that, in the absence of the normal source of hepatic TG (dietary fat packaged as chylomicrons), adipose-derived NEFA, rather than *de novo* synthesized fatty acids, make up the difference. It would be of great interest to determine whether hepatic and plasma TG concentrations would fall if the contribution from plasma NEFA were blocked, or whether hepatic DNL would be induced and thereby maintain secretion of apoB-containing particles.

In summary, although chylomicrons normally contribute the majority of hepatic TG (Figure 1), the hepatic TG pool is efficiently maintained by adipose lipolysis in the absence of chylomicrons, even when the adipose pool size is reduced substantially. Enhanced utilization of plasma NEFA by the liver permits hepatic TG pools and apoB-containing particle secretion to be maintained at normal levels without a large increase in DNL. Accordingly, the metabolic consequence of absorbing a low-fat fuel mixture in mice is not to synthesize more fat in liver or adipocytes but to increase the delivery of lipolytic NEFA to the liver and thereby maintain normal rates of hepatic lipoprotein assembly and secretion.

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