

# Intestinal absorption of essential fatty acids under physiological and essential fatty acid-deficient conditions

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**Abstract** The adequate supply of essential fatty acids (EFA) to the body depends upon sufficient dietary intake and subsequent efficient intestinal absorption. Lipid malabsorption is not only a leading cause of EFA deficiency (EFAD), but also occurs secondarily to EFAD. Understanding the relationship between EFAD and lipid malabsorption may be helpful in the development and optimization of oral treatment strategies. Sequential steps involved in EFA absorption, including lipolysis, solubilization by bile, uptake into the enterocyte, and chylomicron secretion into lymph are reviewed, both under physiological and EFAD conditions. EFAD in itself affects the deficiency state by impairment of EFA absorption due to its effects on bile formation and on chylomicron secretion. These processes may be interrelated as decreased phosphatidylcholine secretion into the bile (a consequence of EFAD) is known to result in decreased chylomicron assembly and secretion. Possible treatments of EFAD include increasing dietary amounts of triacylglycerols and/or specifically tailoring lipids (structured triacylglycerols, EFA-rich phosphatidylcholines, EFA-ethyl esters). It is foreseen that insights into the relationship between lipid malabsorption and EFAD will refine rational approaches to prevent and treat EFAD in specific patient groups.—**Minich, D. M., R. J. Vonk, and H. J. Verkade.** Intestinal absorption of essential fatty acids under physiological and essential fatty acid-deficient conditions. *J. Lipid Res.* 1997. **38**: 1709–1721.

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Due to their inability to be synthesized *de novo* by humans, linoleic (18:2n-6) and linolenic (18:3n-3) acids have been referred to as essential fatty acids (EFA). Once 18:2n-6 and 18:3n-3 are adequately ingested and absorbed, they can be converted to long chain polyunsaturated fatty acids (LCPUFA) such as arachidonic (20:4n-6), eicosapentaenoic (20:5n-3), and docosahexaenoic (22:6n-3) acids. EFA deficiency (EFAD) can result in biochemical and clinical changes such as relatively high levels of nonessential fatty acids

in tissue lipids, alopecia, brittle nails, scaly dermatitis, impaired water balance, infertility and nerve dysfunction (1–4). EFAD has been suggested to be implicated in behavioral and learning disorders (5–7), immunological impairment (8–10) and cardiovascular and neoplastic diseases (11–14).

Theoretically, EFAD can be related to a number of factors including extent of demand for EFA, quantity of endogenous EFA stores, dietary EFA levels and ratios, and efficiency of lipid absorption. In addition to the many studies focusing on sufficient dietary amounts of EFA for formula-fed infants, a recent interest has been generated concerning the occurrence of EFAD in patient groups with lipid malabsorption in common. A high incidence of EFAD has been reported in (pre-term) infants (15–18) and patients with cholestasis (19–25) or chronic gastrointestinal disease such as Crohn's disease (26–28). Because the levels of EFA in the body are dependent on their adequate dietary supply and efficient intestinal absorption, it is worthwhile to investigate the pathophysiological mechanism(s) behind lipid malabsorption that can lead to compromised EFA status and eventual EFAD.

While it is well known that lipid malabsorption can result in EFAD, the observation that EFAD itself impairs lipid absorption has been appreciated only recently (29). In rats with EFAD, lipid absorption has been reported to drop to 80–83%, which is significantly lower than in controls (97%) (29). This review is intended to

Abbreviations: DAG, diacylglycerol; EFA, essential fatty acids; EFAD, essential fatty acid deficiency; FABP, fatty acid binding protein; FFA, free fatty acid; IFABP, intestinal fatty acid binding protein; LFABP, liver fatty acid binding protein; LCPUFA, long-chain polyunsaturated fatty acids; MAG, monoacylglycerol; MCT, medium-chain triacylglycerols; PC, phosphatidylcholine; TAG, triacylglycerol.

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discuss the processes involved in EFA absorption under physiological and EFAD conditions. The available data on possible mechanism(s) of the effect of EFAD on lipid malabsorption are discussed in the present review. Finally, present insights are related to potential novel treatment strategies of EFAD in specific patient groups.

## LIPID MALABSORPTION LEADING TO EFAD

The overall process of lipid absorption can be classified as a chain of events occurring after lipid ingestion, including lipolysis, solubilization, uptake into the enterocyte, reesterification and transport into the lymph or portal blood. The relative importance of each step depends strongly on the dietary fatty acid species and on the membrane structure of the intestine. Under physiological conditions, the efficacy of lipid absorption ranges from 96 to 98% (30, 31). Various excellent reviews have appeared concerning the mechanisms involved in physiological absorption of dietary lipids in general (31–35). However, information specific to the absorption of EFA either under physiological or EFAD conditions is quite limited. Although EFA absorption does not differ in many ways from overall lipid absorption, some characteristic differences apply and are discussed shortly.

### Lipolysis

About 95% of the lipids in the human diet are composed of triacylglycerols (TAG). TAG require lipolysis of the *sn*-1 and *sn*-3 positions to produce two free fatty acids (FFA) and 2-monoacylglycerol (MAG) for efficient solubilization and uptake into the enterocyte (35). Hydrolysis of dietary lipids in humans is catalyzed by lipases from gastric and pancreatic origin, with pancreatic colipase-dependent lipase being functionally the most predominant enzyme under physiological conditions (36). The impact of lipase activity on EFA and LCPUFA absorption, especially of 20:5 $n$ -3 and 22:6 $n$ -3, has been extensively investigated using various lipid formulations (TAG, ethyl ester, FFA) and measuring subsequent appearance in the lymph (37–41). This approach may not correctly quantify differences in lipolysis as lipolysis may not be the only rate-limiting step for appearance into the lymph. Apart from this reservation, various studies are in agreement with the finding that longer chain fatty acids (>C20) are lipolyzed at a slower rate than shorter chain fatty acids ( $\leq$ C18) (38, 42, 43).

To optimize TAG *sn*-configurations with respect to EFA absorption, the influence of the *sn*-position on lipolysis, as measured by subsequent secretion into lymph, was investigated in several animal studies (36,

44, 45). After intraduodenal administration, EFA at the *sn*-2 position, compared with *sn*-1 and *sn*-3, have the highest absolute recovery in the lymph. Compared to all other configurations, intraduodenally administered TAG with 18:2 $n$ -6 in the *sn*-2 position made the most rapid appearance in lymph (46). Similarly, Christensen, Mullertz, and Hoy (36) found a higher percent recovery of 18:2 $n$ -6 in the lymph of pancreatic and biliary diverted rats when administered as *sn*-2 TAG compared to 18:2 $n$ -6 supplied at random positions or as soybean oil. These observations may indicate a resistance towards the enzymatic hydrolysis of the *sn*-1 and *sn*-3 acyl chains by pancreatic colipase-dependent lipase, as whale oil TAG, in which 20:5 $n$ -3 and 22:6 $n$ -3 are predominately esterified at the *sn*-1 and *sn*-3 positions, have been characterized as being lipase resistant (45). Unfortunately, the kinetics of pancreatic lipolysis of EFA and LCPUFA from TAG have not been thoroughly investigated, therefore, it is not possible to adequately compare these fatty acids with regard to lipase activity.

No conclusive human studies have been performed investigating the intestinal lipolysis of EFA-containing TAG in vivo. Yet, this information may be important to consider in EFA administration in the form of structured TAG to various patient groups. Finally, the issue of lipolysis in the role of EFA status is important especially for those patients with low levels of lipase in the intestine and also for pharmacologically induced (i.e., tetrahydrolipstatin) lipid malabsorption (47, 48). Because tetrahydrolipstatin is a nonspecific inhibitor of all lipases, other lipases (i.e., gastric lipase) are unable to compensate for the lack of pancreatic lipase as is seen in conditions such as cystic fibrosis.

### Enterohepatic circulation of bile

The presence of bile in the lumen of the intestine appears to be involved in more than one aspect of the absorption of EFA. It is relevant to note that concentrations of bile constituents and bile flow vary among species (49). One of the important properties of bile is its ability to increase the solubility of lipolytic products (i.e., 2-MAG and FFA) in the aqueous intestinal lumen by the formation of mixed micelles. In the absence of or at low concentrations of bile salts, the absorption of fatty acids occurs to a relatively lower and slower extent (50). It is known that the unsaturated, less hydrophobic structure of unsaturated fatty acids such as EFA relies less upon bile for solubilization in the intestine compared with saturated long chain fatty acids (33–35, 51–53). This feature is illustrated by the dissociation rate constants for 18:2 $n$ -6 from transbilayers in small unilamellar PC vesicles, which are 10 and 5 times greater compared to 18:0 and 18:1 $n$ -9, respectively (54).

In the absence of bile, unsaturated fatty acids are



taken up more efficiently than those that are saturated, though still significantly less efficient than in the presence of bile. Bile-diverted, germ-free rats absorb less saturated compared to unsaturated fatty acids (55% vs. 88%) (55). Many other studies support the difference in absorption between unsaturated and saturated fatty acids, but they generally do not account for metabolism of these labeled compounds, particularly of 18:2n-6, to other fatty acids such as 18:0 by intestinal bacteria. Recovery of fecal 18:0 may be, in fact, "hydrogenated 18:2n-6." Bacterial hydrogenation is commonly overlooked but could lead to overestimated absorption of unsaturated fatty acids and, therefore, to erroneous lipid absorption measurements.

Apart from the role of bile in solubilization, strong indications are available that biliary phospholipids are crucial for proper intestinal chylomicron assembly and secretion of lipid into the lymph, especially during high intestinal lipid loads (56–58). Mice lacking *mdr2* gene product in the bile canalicular membrane, also known as *mdr2* knockout mice, have recently become available for studying the effects of normal bile salt secretion rates accompanied by the virtual absence of phospholipid secretion (59, 60). Under physiologic conditions, phospholipids of lymph chylomicrons are derived predominantly from biliary rather than from dietary origin (61, 62). Studies utilizing interruption of the enterohepatic circulation via cholestyramine feeding (63) or dietary manipulation of biliary composition (64, 65) consistently revealed a substantial accumulation of lipids, including fatty acids (63–65) and retinol (65), in enterocytes coinciding with a reduced intestinal availability of biliary phospholipid. On the other hand, enrichment of diets with PC was associated with increased biliary phospholipid secretion and increased chylomicron transport in rats (66). Available *in vitro* data also indicate that the amount and species of phospholipid affects intestinal lipoprotein secretion. Using *Caco-2* cells, Mathur et al. (67) found that PC above a concentration of 250  $\mu\text{M}$  increased secretion of apoB-containing, TAG-rich lipoproteins. Although other phospholipids such as phosphatidylethanolamine and phosphatidylserine were also capable of stimulating lipid secretion, PC produced the greatest effect (67).

Apart from the obvious roles of biliary PC as an active participant in micelle and chylomicron formation, it may also be relevant that the PC molecule is EFA-rich. This supply of endogenous EFA may be important for a number of reasons relating to EFA absorption. Biliary PC, which comprise approximately 95% of the total biliary phospholipids (61, 68), contain up to 40 mol% EFA and EFA-LCPUFA as measured in humans (69, 70) and rats (71). Daily enterohepatic circulation that provides roughly 12 g of biliary PC (subsequent 95% absorption)

can therefore supply 2.3–3.4 g of 18:2n-6 and 0.5–1.7 g 20:4n-6 (34). An average Western diet of 2367 kcal (9894 kJ) supplies  $\pm$  8.8 g 18:2n-6 and 1.8 g 20:4n-6/day (72), indicating that the intestinal supply of EFA significantly depends on biliary PC secretion. It can be speculated that the relatively hydrophilic conformation of EFA is needed for solubilizing the more insoluble saturated fatty acids in micelle structures. It has also been proposed that the supply of biliary EFA contributes to stereospecific structural requirements of the intestinal cell membrane that are necessary for optimal functioning of membrane transporters and uptake of dietary fatty acids (73). The effect of PC fatty acid composition, particularly of EFA acyl chains, on lipoprotein assembly or secretion has not been thoroughly studied. Although the mechanism has not been elucidated, luminal PC (74), as well as unsaturated fatty acids (75), have been noted to increase the basolateral secretion of lipid-rich, apoB-containing lipoproteins in polarized *CaCo-2* cells, a frequently used *in vitro* model system for intestinal absorption.

In much the same way that bile has an effect upon fatty acid absorption, fatty acids are capable of altering bile production and flow. Numerous animal studies have demonstrated that, compared with other types of lipids, EFA and LCPUFA stimulate bile flow and bile acid output (76–83). Additionally, biliary phospholipid and cholesterol concentrations in rats have been documented to increase after LCPUFA administration (79). Alternatively, these effects were not observed in rats intravenously infused with either medium chain TAG (MCT) or mixtures of MCT and LCPUFA (79). The effects of EFA on bile metabolism vary in intensity. In our own studies, high n-3 diets (fish oil) fed to rats resulted in an increased bile salt size by 28%, increased biliary excretion of cholesterol, and a higher bile flow compared with high n-6 diets (corn oil) (84–86). The increased bile salt pool size coincided with a 30% increase in bile salt synthesis in rats (86). Apart from effects on bile salt biosynthesis, it is tempting to speculate that n-3 fatty acids could play a role in enhancing *mdr2* activity in the canalicular membrane, thereby augmenting bile salt-dependent lipid secretion into bile (49).

#### Uptake into the enterocyte

It is established that even slight changes in dietary fat composition (EFA and nonessential fatty acids) and constant total fat, carbohydrate, or protein levels influence intestinal uptake rates of fatty acids *in vivo* rat models (32, 87, 88). The mechanism by which EFA are taken up by the enterocyte across its apical membrane remains unresolved. Both facilitated (89–92) and passive diffusion processes (91, 93) have been hypothesized for 18:2n-6 translocation across the membrane.

Uptake of EFA and LCPUFA by facilitated membrane translocation has been suggested to involve membrane-bound fatty acid binding protein(s), namely FABP and/or a fatty acid translocase (89, 94, 95). A saturable uptake of long chain (16:0 and 18:1n-9), but not of short chain (8:0) fatty acids has been demonstrated on the apical membrane of Caco-2 cells (96). In studies on isolated hamster intestinal cells, Gore, Hoinard, and Couet (97) found indications that uptake of 18:3n-3 is carrier-mediated and can be competitively inhibited by LCPUFA.

### Intracellular transport of fatty acids in the enterocyte

Approximately 20 years ago, a fatty acid binding protein (FABP) was isolated by Ockner and Manning (98). Since this observation, various members of the FABP family have been identified and characterized, of which two are located in the intestine, namely, the intestinal FABP (IFABP) and liver FABP (LFABP). Based on its expression pattern, predominantly in absorptive areas (jejunal villi) of the intestine, and on its responsiveness in expression to a high-fat diet (98), IFABP is hypothesized to be involved in the intracellular transport of fatty acids in the enterocyte. In vitro studies have indicated that saturated and unsaturated fatty acids (99, 100) can be bound by IFABP with similar high affinity, whereas the binding specificity of LFABP is considerably broader and includes long-chain fatty acids (18:1n-9, 20:4n-6), lysophosphatidylcholine, retinoids, bilirubin, and carcinogens (101, 102). Indirect support for the role of IFABP and LFABP in the intracellular transport of fatty acids can be derived from recent studies by Baier, Bogardus, and Sacchettini (103). A single amino acid substitution (Ala54Thr) in human IFABP is associated with altered binding of fatty acids to IFABP in vitro compared with the wild-type IFABP. A 2-fold increased transport of 16:0 and 18:1n-9 across Caco-2 cells compared to that of the wild-type protein was also observed. These findings suggest fatty acid binding to IFABP may influence intracellular uptake and/or metabolism. It is not determined if or to what extent these FABPs are involved in activation and reesterification of EFA in normal physiology or in EFAD.

### Chylomicron assembly and secretion

EFA and LCPUFA are predominantly reacylated into TAG inside the enterocyte and assembled into chylomicrons to be excreted and transported into the lymph. The main biochemical route for TAG synthesis is the MAG pathway, in which 2-MAG (taken up by the enterocyte) is sequentially reacylated to diacylglycerols (DAG) and to TAG by MAG-acyltransferase and DAG-acyltransferase, respectively (34). The other route of TAG

synthesis, the alpha-glycerolphosphate pathway, involves conversion of glycerol-3-phosphate via phosphatidic acid to DAG and, subsequently, to TAG by various enzymes (34). Under conditions of normal lipid absorption, in which there is an ample supply of 2-MAG and FFA, the 2-MAG pathway predominates relative to the alpha-glycerolphosphate pathway (34, 104).

The newly formed TAG from either pathway are thought to be metabolically distinct, in that TAG made from 2-MAG are secreted more rapidly across the basolateral membrane compared to those originating from the alpha-glycerolphosphate pathway (105). The DAG from each pathway have been suggested to enter into separate intracellular pools (34). This hypothesis is supported by observations that DAG from the alpha-glycerolphosphate pathway is preferentially used for *de novo* synthesis of PC (106). Although it has not been studied extensively, some indirect indications support selectivity of EFA in the routing to TAG synthesis relative to PC synthesis (29, 52, 107). Ockner, Pittman, and Yager (52) compared the absorption of [<sup>14</sup>C]16:0 and [<sup>14</sup>C]18:2n-6 in normal rats and found that the amount of 18:2n-6 esterified into TAG was twice that of 16:0 when expressed as percentages of <sup>14</sup>C in the intestinal cell wall or as percent of <sup>14</sup>C administered.

Lyso-PC, the phospholipase A2-metabolite of PC, can be routed to various metabolic pathways within the enterocyte: it can be reacylated to form PC (108–112), hydrolyzed into fatty acids and glycerol-3-phosphorylcholine (112), or two lyso-PC molecules can react to give PC and glycerol-3-phosphorylcholine (113). The addition of certain lipids to the diet may target the use of specific pathways. 18:2n-6 is preferentially routed into TAG synthesis; however, administration of lyso-PC produces an increase in the percent 18:2n-6 incorporated into the phospholipid fraction (107). Administration of lyso-PC and [<sup>3</sup>H]20:4n-6 to rats resulted in increased incorporation of [<sup>3</sup>H]20:4n-6 into mucosal PC and increased transport of [<sup>3</sup>H]20:4n-6 in the phospholipid fraction of lymph lipoproteins compared with [<sup>3</sup>H]20:4n-6 administration alone (107).

Before being transported into the lymph, TAG are assembled together with phospholipids and apolipoproteins to form a lipoprotein particle. For an in-depth discussion on chylomicron assembly, the reader is referred to the excellent recent review of Hussain et al. (114). In the enterocyte, apolipoprotein B (apoB) is translocated across the endoplasmic reticulum during translation and lipidated via interactions with microsomal transfer protein (34, 115).

Within the group of unsaturated fatty acids, individual differences in the kinetics of lymphatic transport are evident. After an enteral infusion of a lipid emulsion

containing [<sup>3</sup>H]18:2n-6 and [<sup>14</sup>C]20:4n-6 in rats, Nilsson et al. (116) reported that lymph recovery of labeled 20:4n-6 was slower and more extended compared with 18:2n-6. Nilsson and Melin (29) demonstrated that a larger part of administered 20:4n-6 was retained in intestinal phospholipids compared to 18:2n-6. To a quantitatively minor extent, LCPUFA have also been shown to be transported portally. By intraduodenal infusion of labeled FFA into rats and subsequent analysis of their portal blood concentrations, Bernard and Carrier (33) reported evidence that the portal route could account for about 15% of the lymph route of the fatty acids 18:2n-6 and 20:4n-6. However, Mansbach, Dowell, and Pritchett (56, 117) demonstrated that increasing the lipid load and including PC in the infused emulsion promoted lymph transport of enterally infused emulsions containing [<sup>3</sup>H]tri-18:1n-9. The enteral supply of PC, which may be rate-limiting for chylomicron assembly, led to less portal transport (0.5% for the low lipid load and 1.4% for the high lipid load), indicating that portal transport is a relatively minor pathway for long chain unsaturated fatty acids (56).

#### EFAD LEADING TO LIPID MALABSORPTION

In comparison to the amount of studies performed on fatty acid absorption under physiological conditions, the effects of EFAD on lipid malabsorption are studied in less detail. However, the few studies available indicate that a causal relationship exists between EFAD and lipid malabsorption. EFAD-mediated changes in the various processes involved in lipid absorption will be discussed.

##### Lipolysis

Studies on the effects of EFAD indicate that lipolysis does not appear to be affected by EFAD. Using pancreatic extracts from EFAD and control rats, Levy et al. (118) reported no significant difference in lipolytic activity. After oral administration of [<sup>14</sup>C]tri-18:1n-9, Bennett Clark et al. (119) determined similar quantities of <sup>14</sup>C hydrolysis products (fatty acids, MAG, TAG) in intestinal luminal contents of EFAD and control rats, indicating comparable lipase activity. The medium chain TAG, tri-8:0, which requires only lipolysis for absorption, was shown to disappear at a similar rate from luminal contents in EFAD and control groups, again suggesting that lipolytic activity is not affected by EFAD (119).

##### Enterohepatic circulation of bile

Decreased bile flow and bile acid secretion rate have been demonstrated in rat models of EFAD (83, 118,

120–122). Different animal species vary in biliary lipid output. EFAD rats have a decreased phospholipid and cholesterol secretion rate (83, 118, 120–122) whereas EFAD hamsters have significantly increased biliary cholesterol compared to controls (83, 123) and an increased (83) or unchanged (123) biliary phospholipid secretion rate. No values for biliary lipid content have been reported for humans with EFAD.

Upon analysis of acyl species composition of biliary PC, it is noted frequently that biliary PC EFA content is decreased in EFAD (119, 124). Biliary PC content of 18:2n-6 and 20:4n-6 has found to be decreased in EFAD rats to roughly 10% and 26% of that of controls, respectively (119). The ability of other fatty acids to be incorporated into biliary phospholipids during EFAD has been noted (124–127). In EFAD, decreasing levels of 18:2n-6 and 20:4n-6 in phospholipids were compensated for by increased amounts of 18:1n-9 and 16:1n-7 (124).

It has been speculated that EFAD effects on bile flow may be affected by altered prostaglandin biosynthesis. Prostaglandins, the metabolic products of EFA-LCPUFA, have been reported to influence bile flow; however, data are not consistent for all prostaglandin species. Some studies in the *in situ* perfused rat liver suggest prostaglandins F<sub>2</sub>-α and D<sub>2</sub> decrease bile flow in a dose-dependent manner (128, 129), possibly mediated at the level of the canalicular membrane (128). On the other hand, Solomon et al. (130) have found a stimulating effect of the prostaglandins, E<sub>2</sub> and prostacyclin, on bile flow. Although this area requires additional studies, it is reasonable to suppose that changes in prostanoid production and activity of membrane proteins could ultimately affect hepatic bile flow by alteration of lipid membrane contents and function. As mentioned previously, elucidation of the expression of *mdr2*, particularly under EFAD conditions, as well as of other canalicular transport proteins, will help to further address these questions.

##### Uptake into the enterocyte

In studies examining EFAD and lipid absorption, uptake of FFA into the enterocyte appeared not to be affected (4, 119, 131, 132). *In vitro* studies on everted jejunal rings showed similar uptake rates of [<sup>14</sup>C]18:1n-9 into enterocytes of EFAD and control animals (119). In accordance with these results, similar absorption of various radiolabeled FFA was found in *in vivo* studies on EFAD and control rats (119, 131). Conversely, the typically high amounts of EFA recovered in intestinal tissue of EFAD rats after oral bolus administration (119, 131) compared to controls could be compatible with delayed transport through the enterocyte. Available *in-*



dications do not support major differences during EFAD, but at present, it cannot be excluded that the endpoint measurement such as the amount recovered in the enterocyte is not precise enough to detect differences.

### Chylomicron assembly and secretion

As discussed above, indirect indications support the view that neither lipolysis nor mucosal uptake are responsible for the lipid malabsorption encountered in EFAD rats. The predominant mechanisms by which EFAD induces lipid malabsorption seem to involve the effects on bile formation (see above) and the intracellular handling of lipids by the enterocyte. In the intestine of EFAD rats, some pathological intestinal changes have been noted, namely a restricted surface area due to villi shortening and a lack of cellular differentiation (132). Changes in the composition of the intestinal mucosa due to EFAD include a reduction of total lipid (126) and of 18:2n-6 and 20:4n-6 (133, 134). After an oral bolus, [<sup>14</sup>C]18:2n-6 is relatively retained to a longer extent in the intestinal mucosa of EFAD rats compared with control rats (126, 135) Hjelte et al. (131) studied the absorption of [<sup>3</sup>H]20:4n-6 and [<sup>14</sup>C]18:2n-6 in EFAD and control rats. Significantly less [<sup>3</sup>H]20:4n-6 was recovered in various non-intestinal tissues of EFAD rats compared to controls. However, at 2 h after bolus administration, recovery of [<sup>3</sup>H]20:4n-6 and [<sup>14</sup>C]18:2n-6 in the small intestine of EFAD rats was higher than that of controls. One explanation for increased intestinal retention during EFAD is that EFA may be diverted from being oxidized by the enterocyte and, instead, be incorporated into phospholipid. A lower oxidation of [<sup>14</sup>C]18:2n-6 in the intestine has been reported in rats fed low amounts of EFA (126, 135) whereas conversion rates of [<sup>14</sup>C]18:2n-6 to [<sup>14</sup>C]20:4n-6 in the upper small intestine were significantly increased in EFAD rats (131). These observations are compatible with a sparing mechanism of EFA under EFAD conditions. No human studies are available on the intestinal morphology, fatty acid uptake or interconversion during EFAD.

The mechanism by which EFA are retained in phospholipids of EFAD rats is not known, but has been speculated to be related to the pathways of reacylation in the intestine (131). Relatively little information is known regarding how the various phospholipid biosynthetic pathways (i.e., reacylation of lyso-PC, DHAP pathway, acyl exchange, CDP-choline pathway) are affected by EFAD. Because unsaturated fatty acids such as 18:2n-6 and 20:4n-6 are preferred substrates for enzymes such as 1-lyso-PC-acyl-CoA-acyltransferase (131, 136), it can be envisioned that during EFAD, the intracellular pool sizes are decreased, allowing immediate routing of absorbed EFA. Theoretically, the activity of reacylation

enzymes can also be stimulated in EFAD, yet, fatty acid Co-A ligase and microsomal acyl CoA:monoglyceride acyltransferase activities were not affected in EFAD rats when enzyme activity was corrected for body weight (119).

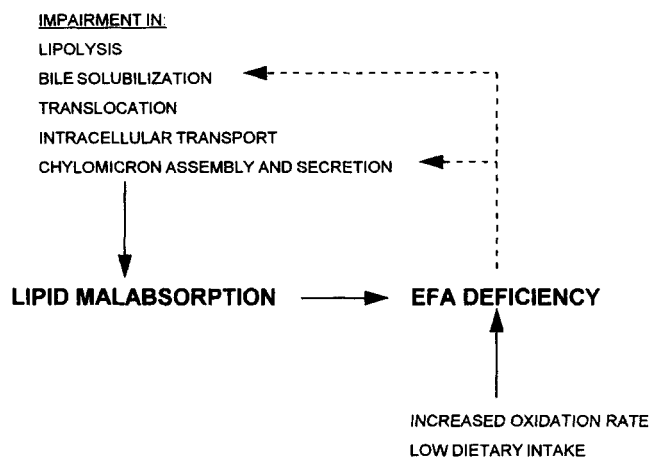
EFAD has been shown to decrease the initial rate of esterification, increase phospholipid retention (126), and decrease TAG output (118, 119, 137, 138) in the enterocyte. In vitro studies using everted gut sacs have demonstrated a 30% decrease in total lipid esterification and secretion by EFAD rats compared with controls (118). This observation was confirmed in rat jejunal rings by Bennett Clark et al. (119), who reported a decreased capacity of EFAD to synthesize TAG from free 18:1n-9. It is not known whether a quantitative increase in portal transport is utilized in EFAD to counteract impaired chylomicron assembly. Regardless of type of diet fed to induce EFAD in rats (fat-free, hydrogenated coconut oil, or hydrogenated cottonseed oil), fasting plasma TAG levels are increased during EFAD (139). Levy et al. (118) reported fasting plasma TAG values in EFAD rats to be more than twice that in controls. However, administration of an oral fat load led to a lower postprandial increase in plasma chylomicron concentrations in EFAD compared to controls, which is in accordance with a decreased efficiency of chylomicron formation.

Compositional analysis of chylomicrons from EFAD mice have revealed greater percentage contribution of MAG plus phospholipid, DAG, cholesterol, and cholesteryl ester, and less of TAG compared to control mice (118). Similarly, after administration of a lipid bolus containing [<sup>3</sup>H]20:4n-6 and [<sup>14</sup>C]18:2n-6, recovery of [<sup>3</sup>H]20:4n-6 in FFA and in TAG was lower in EFAD than in control rats (131). In EFAD rats, more [<sup>14</sup>C]18:2n-6 was incorporated into phospholipid (PC, phosphatidylserine, phosphatidylethanolamine, cardiolipin) rather than in TAG (118, 119, 131). The different metabolic fates of 18:2n-6 and 20:4n-6 indicate that specificity may exist between the various EFA/LCPUFA.

Not only the (decreased) quantity, but also the altered quality (i.e., acyl composition) of biliary phospholipids in EFAD could affect chylomicron assembly in the enterocyte. In vitro studies have shown that both the concentration and the acyl chain composition of PC have an effect on chylomicron assembly, secretion (67, 140), and clearance (140). In depth studies investigating whether impaired chylomicron production in EFAD is (partly) based on alterations in bile phospholipids are lacking.

### Prevention and treatment of EFAD

Available data on EFAD and lipid malabsorption can thus be schematized as in **Fig. 1**: disturbances in one or



**Fig. 1.** Relationship between lipid malabsorption and essential fatty acid (EFA) deficiency.

more of the processes involved in lipid absorption can lead to lipid malabsorption, followed by EFAD. EFAD can then perpetuate lipid malabsorption via its effects on bile solubilization and chylomicron assembly and secretion. With this current information, it may be possible to design optimal methods of treatment of EFAD. Treatment is becoming increasingly important due to EFAD prevalence in several patient groups (19–22, 26, 27). It remains to be addressed whether the dietary amount of EFA needed to treat/prevent EFAD in specific patient groups depends upon the biochemical form of delivery (TAG, phospholipid, ethyl ester). Conventionally, EFAD has been treated by increased dietary TAG, which are thought to compensate for malabsorbed EFA. Enteral administration of vegetable oils, which are high in 18:2n–6 (7–10 en%), have been shown to improve or eliminate EFAD in cystic fibrosis patients (141–144). Similarly, dietary intake of EFA oils has restored plasma EFA levels to normal in children with atopic eczema (145). However, the quantitative approach of increased oral supply of the conventional formulation (i.e., as TAG) may not be solely effective, and may not be effective in the various patient groups (146, 147). In addition to lipid therapy, another treatment alternative may be to orally administer bile acids such as taurocholate or ursodeoxycholic acid (UDCA) to improve micelle formation in the intestinal lumen and to stimulate bile flow. Supplementation of EFA-rich (40 g/100 g) powder plus taurocholate to cholestatic children with EFAD has been successful in normalizing serum 18:2n–6 levels (146). UDCA, a bile acid commonly given to cholestatic patients because of its ability to improve symptoms of fatigue, pruritus, depleted nutritional status, and biochemical liver tests (148–150), is considered less toxic and able to stimulate bile salt cir-

ulation, thereby diluting the more toxic bile acids in the total bile salt pool (150, 151). However, UDCA is a poor micelle-forming bile salt, and data on its efficacy to stimulate EFA absorption are inconclusive (152).


As PC has consistently been reported to be involved in the complete absorption of EFA through assistance in solubilization and in chylomicron packaging, we speculate that a more strategic practical way to ameliorate or alleviate EFAD may involve specific lipid formulations, including PC. Modulation of bile phospholipid quantity and acyl composition by addition of dietary PC could be beneficial in humans as it has shown to be successful in animal studies (61, 153–157). Favorable effects with PC have been noted when orally supplemented in models of liver disease (158). For humans, PC is readily accessible and safe as it is obtained from egg yolk or sometimes from soybean (159). An addition of up to 36 g of phospholipids to the daily diet of healthy humans for 14 days produced no side effects (160). Supplementation in these subjects caused a relative enrichment of 18:2n–6 in bile phospholipids probably due to the fact that it composed 54% of the dietary PC acyl chains. Patients with a disrupted enterohepatic circulation fed 10 g soybean PC for 5–10 days demonstrated a significant increase in the bile phospholipid/cholesterol ratio. (150). Similarly, Holan et al. (161) fed patients with gallstones 4.5 g PC for 3 weeks and consequently observed an increase in 18:2n–6 in bile phospholipid acyl chains.

Animal studies in which PC was orally administered show a directly enhanced effect on lymph transport of lipid (65, 162–164). In humans, duodenal infusion of PC stimulated the production of small TAG-rich lipoproteins (165). In rats treated with hydrophobic surfactants known to inhibit TAG secretion into lymph, Rodgers, Beeler, and Tso (166) found that TAG secretion normalized after an intraduodenal administration of a mixture of tri-18:1n–9 and PC. Similarly, Fukui et al. (167) noted that absorption of a fat-soluble vitamin (d-alpha-tocopherol acetate) was highest when MCT was given in combination with a PC emulsion as compared to PC alone. In particular, as it has been suggested that intact PC is not directly taken up in significant quantities by the enterocyte and that phospholipase A2 is required for its hydrolysis, it may be even better to administer its hydrolysis product, lyso-PC, directly (163, 164). Indeed, [<sup>14</sup>C]20:4n–6 absorption was enhanced in the presence lyso-PC in rats (107).

The alternative formulations should be tailored to the pathophysiological origin of EFAD. Optimally, therapeutic EFA formulations should be efficiently absorbed in a relatively bile-independent and lipolysis-independent fashion. Currently, information is lacking regarding optimal strategies for treatment of EFAD in

specific patient groups, for example whether to supply EFA-rich PC, structured TAG, or EFA-ethyl esters. The present insights into the various processes involved, and the availability of stable isotope techniques, however, allow the ability to address many of these questions in the near future.

### Conclusion

In summary, it is imperative that EFA are properly absorbed from the diet as the human body is unable to synthesize them. Disorders in the individual steps involved in lipid absorption may lead to EFAD. In addition, EFAD in itself affects the deficiency state by impairment of EFA absorption due to its effects on bile formation and on fatty acid reesterification and TAG transport into lymph. The relationship between bile formation and EFA absorption is intimately regulated, thus allowing for numerous perspectives on the development and treatment of EFAD. From one aspect, dietary EFA heavily influences EFA status and ultimately, biliary PC composition. Biliary components, particularly PC that consist mostly of EFA, are essential components for the adequate intestinal solubilization and packaging of fatty acids in chylomicron. As a result, a promising area of research involves the application of (acyl chain) specific PC to patients with lipid malabsorption syndromes. 

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