# Oral treatment of essential fatty acid deficiency with triglycerides or phospholipids in children with end stage liver disease

*Submitted*

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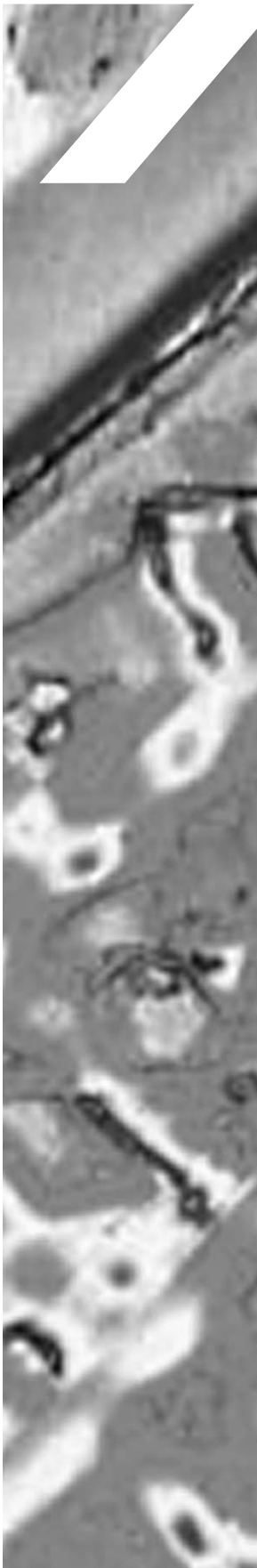

## ABSTRACT

Background: Essential fatty acid (EFA) deficiency is frequently observed in children with end-stage liver disease, mainly due to malabsorption of dietary EFA. Recently, we demonstrated in cholestatic EFA-deficient mice that oral phospholipids (PL) more effectively improve EFA-status than oral triglycerides (TG). In this study, we compared the effects of oral EFA supplementation in the form of PL or TG on EFA status in children with end-stage liver disease.

Methods: Pediatric candidates for liver transplantation were orally supplemented with EFA-rich TG or EFA-rich PL for three months. Red blood cell (RBC) fatty acid composition was determined at baseline and after one, two and three months of EFA supplementation, as were serum liver enzyme and vitamin A and E concentrations. Results were compared to data obtained from non-supplemented patients prior to the study period. Dietary EFA intake was calculated from food diaries at baseline and after three months of EFA supplementation.

Results: Mead acid (C20:3n-9) significantly increased ($p<0.05$), and LA and the total amount of n-6 fatty acids decreased ($p<0.005$) in RBC of the non-supplemented group ($p<0.01$), whereas total n-6 fatty acids remained stable in both the PL and the TG group. The rate of increase in RBC-LA was significantly higher in PL-supplemented, but not in TG-supplemented children, compared with non-supplemented children. No significant differences in RBC alpha-linolenic acid (ALA), arachidonic acid (AA) or docosahexaenoic acid (DHA) were found between TG-, PL-, or non-supplemented patients. Baseline dietary LA intake was well above the Dutch daily recommended intake, and similar in the three groups.

Conclusions: Oral EFA supplementation prevents deterioration of EFA status in children with end-stage liver disease. Present results suggest that EFA supplementation as PL is slightly more effective for improving RBC EFA than as TG.

## INTRODUCTION

Essential fatty acid (EFA) deficiency is a common finding in children with cholestatic liver disease[1;2]. Retrospective analysis of fatty acid profiles determined in our hospital between 1990 and 1996 revealed that almost 80% of children listed for orthotopic liver transplantation for end-stage (cholestatic) liver disease (ESLD) had indications for compromised essential fatty acid status[3]. EFA deficiency during cholestasis is predominantly due to dietary fat malabsorption[4]. Absorption of dietary fat during enteral bile insufficiency may be reduced from 97% to 40% of the amount ingested. It has been demonstrated that EFA deficiency not only can result from dietary fat malabsorption, but reversely, inadequate EFA levels may also impair absorption of dietary fat[5-8].

EFA deficiency in children with end-stage liver disease (ESLD) has been proven difficult to treat by merely increasing dietary EFA ingestion[3;9;10]. In dietary fat, EFA is predominantly present in the form of triglycerides (90%), and only 10% as phospholipid and cholesterol ester. The major steps involved in intestinal lipid absorption are intraluminal hydrolysis, micellar solubilization, translocation across the unstirred water layer and the microvillous enterocyte membrane, and intracellular lipoprotein assembly and secretion[5;6;11]. The relative importance of each of these processes differ for the absorption of triglycerides and phospholipids, partly based on physicochemical differences. Lipids have been categorized into polar and non-polar lipid classes according to the nature of their interactions with water[11;12]. Polar lipids are divided into 3 subclasses. Triglycerides are hydrophobic class 1 polar lipid molecules and are insoluble in the aqueous intestinal lumen, require (partial) hydrolysis and highly depend on bile for solubilization into micelles. After absorption by the enterocyte and subsequent re-esterification, triglycerides are secreted into lymph as core components of chylomicrons. Phospholipids, on the other hand, are class 2 polar lipids, which are more hydrophilic and associate into liquid crystals in the intestinal lumen, which has been suggested to improve luminal fat solubilization during bile deficiency. Phospholipids are the major surface components for chylomicrons, and have been postulated to have a higher post-absorptive bioavailability for the target organs of EFA, liver and brain. In EFA-deficient mice with acute cholestasis, we recently demonstrated that oral EFA-rich phospholipids more effectively improved EFA status than oral EFA-rich triglycerides[13]. Carnielli *et al.* demonstrated in preterm infants that n-3 LCPUFA were more effectively absorbed from infant formulas when supplied as PL than as TG[14]. In this study, PUFA absorption efficacies were measured by fecal balance techniques, and no data on RBC EFA were provided. Obviously, in human studies, obtaining material to analyze brain or liver EFA contents

is not feasible. Yet, considering the favorable outcomes of PL-supplementation on brain and liver PUFA in mice, equal effects of TG and PL supplementation on RBC EFA in humans might still advocate preferential EFA supplementation with PL rather than with TG in cholestatic patients.
In the present study, we compared the efficacy of oral EFA-rich PL and oral EFA-rich TG supplementation on RBC-EFA status in children with ESLD. Non-supplemented ESLD patients formed the control group.

## SUBJECTS AND METHODS

### Patient characteristics

Pediatric liver transplantation in the Netherlands is centralized in Groningen. Patients listed for orthotopic liver transplantation (OLT) visit the University Medical Center Groningen outpatient clinic on a monthly basis for clinical and biochemical evaluation during the waiting period for transplantation. Participants for the study were recruited between January 2000 and January 2004. Since we had previously observed marginal EFA-status in the majority of children with ESLD[3], children were randomly assigned to the TG or the PL supplementation group. Participants consented to ingest an EFA supplement for three months, and were unaware of the type of supplement they were assigned to. A non-supplemented control group was composed of data available from patients, prior to the supplementation study. The study group included 19 pediatric pre-OLT patients (10 TG, 9 PL), 11 male and 8 female, ranging in age from 1 to 16 years.

### Study protocol / Experimental procedures

Venous EDTA-anticoagulated blood samples were collected (3-5 ml) during regular out-patient clinic visits (baseline) and after 1, 2 and 3 months of oral EFA supplementation. EFA status in erythrocytes, plasma vitamin A and E concentrations and serum liver enzyme activities (AST, ALT, gamma-GT, alkaline phosphatase, bilirubin, albumin and coagulation parameters) were determined monthly during the study period, conform our standard procedures for pediatric liver transplant candidates.

Dietary EFA intake (apart from the supplement) was calculated from 2-day consecutive food diaries, at the start and at the end of the 3-month supplementation period, by a clinical dietician using the Netherlands Nutrients Table "NEVO" 2001. A schematic overview of the experimental design is depicted in Figure 1. The Medical Ethics Committee of the University Medical Center Groningen approved the study

protocol, which included obtaining informed consent from the participating children and their parents. Figure 2 summarizes the anthropometric data (age, body weight, male/female) and the underlying liver disease.

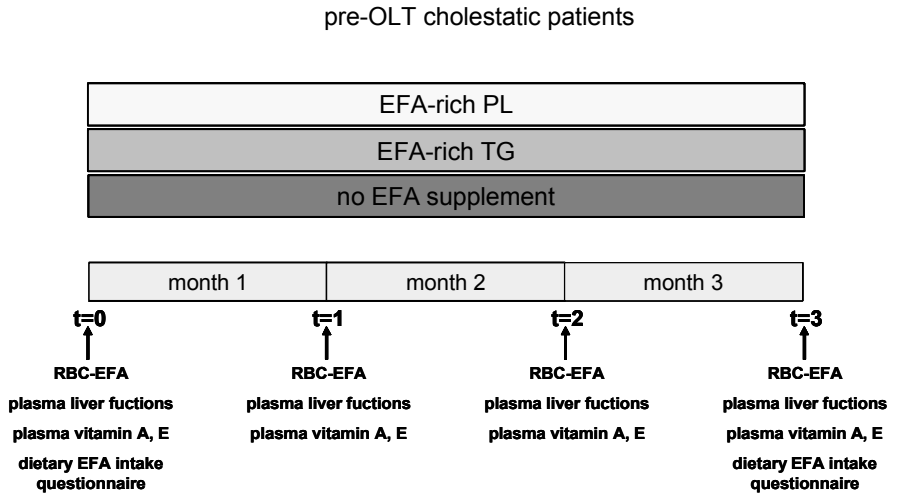


**Figure 1:** Experimental design of EFA supplementation to children with end-stage liver disease. RBC fatty acid profiles, serum liver enzymes and vitamin A and E levels were determined monthly, and dietary EFA intake was calculated from food diaries at baseline and after 3 months of supplementation.

## Experimental EFA supplements

The primary TG and PL were purified from crude soybean oil and had the following fatty acid profile:

| | ***TG-supplement*** | ***PL-supplement*** |
|---|---|---|
| | mol% | mol% |
| linoleic acid (C18:2n-6) | 53.5 | 56.1 |
| alpha-linolenic acid (C18:3n-3) | 7.6 | 8.2 |
| oleic acid (C18:1n-9) | 22.8 | 13.2 |
| palmitic acid (C16:0) | 10.7 | 16.1 |
| stearic acid (C18:0) | 4.0 | 4.5 |
| myristic acid (C14:0) | <0.5 | <0.5 |
| palmitoleic acid (C16:1n-7) | <0.5 | <0.5 |
| arachidic acid (C20:0) | <0.5 | <0.5 |
| arachidonic acid (C20:4n-6) | <0.5 | <0.5 |
| behenic acid (C22:0) | <0.5 | <0.5 |
| eicosapentaenoic acid (C20:5n-3) | <0.5 | <0.5 |
| docosahexaenoic acid (C22:6n-3) | <0.5 | <0.5 |

Both the PL and the TG oils were a generous gift of Unimills BV, the Netherlands.
We aimed to supplement the cholestatic children with 0.125 mg LA per kg body weight per day, which is 25% of the daily recommended dietary intake (RDI) as formulated by the Netherlands Nutrition Council (Nederlandse Voedingsraad). In the TG oil, 4% of molecular mass is accounted for by the glycerol backbone and 96% by fatty acids, of which 54% is linoleic acid (LA). The PL oil (lecithin) was

almost exclusively composed of lecithin (>98%). For the PL oil, the glycerol-phosphate-choline backbone composes 28% of molecular mass, and 56% of the remaining 72% fatty acid mass is linoleic acid (LA). To obtain equimolar daily amounts of linoleic acid (LA) for the two EFA-supplements, the TG-oil was dosed at 0.23 ml per kg bodyweight per day, and the PL-emulsion at 1.5 ml per kg per day, resulting in 0.4 mmol LA per kg bodyweight for each supplement. The purified PL oil (lecithin) was administered in the form of a water-in-oil emulsion, prepared by our University Medical Center Groningen (UMCG) Hospital Pharmacy, based on the high viscosity of the pure PL oil. The PL emulsion contained per liter: 1.0 g methyl-parahydroxybenzoate (conservative), 1.0 g saccharine sodium-2-water (sweetener), 150 g PL oil, 20 g caramellose sodium, 10 g polysorbate 80 (emulsion stabilizer), and 1.0 g peach or banana aroma, ad 1.0 liter distilled water. The EFA supplements were advised to be taken in three daily doses, during or after meals. Based on pilot studies, daily ingestion of 30 ml of supplement was the maximum tolerable amount for most children. Prescription of greater volumes of EFA-supplement frequently resulted in malcompliance, partly due to the moderate palatability of the supplement. Therefore, the prescribed amount of PL-supplement of 1.5 ml per kg per day was restricted to a maximum intake of 3 dd 10 ml for children of 20 kg and more. This maximum LA intale was matched with a maximum daily TG supplementation of 4.6 ml for children over 20 kg. As a result, both TG-and PL-supplemented children on average received 2.2±0.5 g LA per day extra.

Shelf life investigation of both EFA-supplements, including fatty acid profile analyses at baseline and at 2-weekly intervals for 3 months, before and after sterilization (3 hours at 140°C), revealed no alterations in fatty acid composition (data not shown). The pH of both supplements remained stable at 6.0 for 3 months and no micro-biological contamination occurred.

## Analytical techniques

### *Fatty acid analysis*

EDTA-plasma, platelets and erythrocytes (RBC) were separated by centrifugation (10 min at 2500 g). Platelet-poor plasma was stored at -80°C. The buffy coat (WBC) was removed from the RBC pellet, and RBC were washed thrice with physiological saline. To prevent fatty acid oxidation, erythrocyte membrane lipids were hydrolyzed and transmethylated to fatty acid methyl esters in methanol/HCl (5:1 vol:vol) and extracted in hexane the same day[15]. Aliquots of the TG and PL supplements were dissolved in chloroform/methanol (1:2), and lipids were hydrolyzed, methylated and extracted for fatty acid analysis as described above. Heptadecanoic acid (C17:0) was added to all samples as internal standard prior to methylation and extraction

procedures, and BHT was added as antioxidant. Fatty acid methyl esters were separated and quantified by gas liquid chromatography (GLC) on a Hewlett Packard gas chromatograph model 6890, with a 50mx0.2mm Ultra 1 capillary column (Hewlett Packard, Palo Alto, CA) and a FID detector as described previously[8]. Plasma liver enzyme activities and vitamin A and E concentrations were measured using standard clinical laboratory procedures.

### Calculations

Relative concentrations (mol%) of individual fatty acids in RBC membranes and in the EFA supplements were calculated by summation of all fatty acid peak areas and subsequent expression of individual fatty acid peaks as a percentage of this amount. Fatty acid contents were quantified by relating the areas of their chromatogram peaks to that of the internal standard heptadecanoic acid (C17:0). Fatty acid and liver enzyme concentrations were corrected for inter- and intra-individual variations in time between blood sampling, by normalization to 30 days between each measuring point. Occasional missing values from time points of one or two months after starting EFA supplementation were interpolated. Correlations between fatty acid concentrations and age have been described previously[16], but since there was no significant difference in age and bodyweight between the study groups, fatty acid concentrations were not corrected for these parameters. Intake of dietary EFA was calculated from food diaries from the participants by a clinical dietician, using the Netherlands Nutrients Table "NEVO" 2001. Intakes were expressed in g/day, and compared to the RDI for children as determined by the Netherlands Nutrition Council.

### Statistics

All results are presented as means ± S.D. for the number of patients indicated. Data were statistically analyzed using Student t-test, or, for comparison of more than two groups, ANOVA-test with post-hoc Bonferroni correction. Statistical significance of differences between means was accepted at $p<0.05$. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL).

## RESULTS

### Patient characteristics

Figure 2 shows age, bodyweight, sex and underlying hepatic disease of the included ESLD patients. Significant correlations existed between age and weight and baseline RBC-concentrations of linoleic acid (LA), alpha-linolenic acid (ALA), docosahexaenoic acid (DHA) but not arachidonic acid (AA) ($p<0.05$, data not shown).

| | age | weight | |
|---|---|---|---|
| TG1 (m) | 2 | 10 | biliary atresia |
| TG2 (m) | 3 | 14 | biliary atresia |
| TG3 (f) | 1 | 8 | biliary atresia |
| TG4 (m) | 2 | 15 | biliary atresia |
| TG5 (m) | 2 | 11 | biliary atresia |
| TG6 (f) | 9 | 35 | biliary atresia |
| TG7 (f) | 10 | 35 | biliary atresia |
| TG8 (m) | 13 | 53 | auto-immune hepatits |
| TG9 (f) | 13 | 50 | biliary atresia |
| TG10 (m) | 16 | 60 | biliary atresia |
| PL1 (m) | 2 | 10 | biliary atresia |
| PL2 (m) | 16 | 52 | biliary atresia |
| PL3 (f) | 7 | 22 | biliary atresia |
| PL4 (m) | 2 | 15 | biliary atresia |
| PL5 (m) | 13 | 53 | auto-immune hepatits |
| PL6 (f) | 13 | 50 | biliary atresia |
| PL7 (f) | 11 | 27 | PFIC2 |
| PL8 (f) | 6 | 19 | PFIC2 |
| PL9 (m) | 16 | 73 | biliary atresia |
| X1 (m) | 14 | 53 | auto-immune hepatits |
| X2 (m) | 2 | 14 | biliary atresia |
| X3 (m) | 2 | 15 | biliary atresia |
| X4 (m) | 9 | 35 | biliary atresia |
| X5 (f) | 1 | 10 | biliary atresia |
| X6 (f) | 10 | 27 | PFIC2 |
| X7 (f) | 6 | 19 | PFIC2 |
| X8 (m) | 16 | 73 | biliary atresia |
| X9 (f) | 15 | 39 | biliary atresia |
| X10 (m) | 13 | 40 | tyrosemia type 1 |
| X11 (f) | 10 | 26 | nonsyndromatic bile duct paucity |
| X12 (f) | 14 | 50 | cystic fibrosis, cirrhosis |
| X13 (m) | 3 | 16 | biliary atresia |
| X14 (f) | 1 | 9 | biliary atresia |
| X15 (m) | 5 | 18 | congenital intrahepatic cholestasis |
| X16 (m) | 14 | 45 | primary sclerosing cholangitis |
| X17 (f) | 13 | 66 | acute liverfailure, M. Wilson |
| X18 (f) | 1 | 12 | biliary atresia |
| X19 (f) | 3 | 15 | TPN cholestasis |
| X20 (m) | 9 | 31 | primary sclerosing cholangitis |
| X21 (f) | 1 | 5 | cystic intra+extrahepatic bile ducts |
| X22 (m) | 0 | 5 | Alagille syndrome |
| X23 (f) | 0 | 6 | biliary atresia |
| X24 (m) | 11 | 30 | auto-immune hepatits |
| X25 (m) | 1 | 7 | biliary atresia |

**Figure 2**: Age, bodyweight, sex and underlying hepatic disease of ESLD patients not supplemented with EFA or supplemented with EFA as TG or PL.

The underlying cause for end-stage liver disease was biliary atresia in 59% of patients, and cirrhosis by various causes (primary sclerosing cholangitis, progressive familial intrahepatic cholestasis, alpha-1-antitrypsin deficiency, auto-immune hepatitis, cystic fibrosis) in the remaining 41%. In the PL supplemented group, one patient failed to complete the three-month supplementation period due to perceived impalatability of the supplement. In the TG supplemented group, one patient did not complete the supplementation period due to liver transplantation. No complaints of nausea or other gastrointestinal symptoms due to the supplements were reported by any of the patients. The groups did not differ in mean age or bodyweight.

| | Age: | Bodyweight: |
|---|---|---|
| Non-supplemented | 6.8 ± 5.6 | 27.9 ± 19.7 |
| TG | 7.1 ± 5.8 | 28.9 ± 20.1 |
| PL | 9.6 ± 5.5 | 35.5 ± 21.9 |

## Fatty acid composition of erythrocyte membranes

Figure 3 shows the effects of non-supplementation (control) and EFA supplementation as TG or PL on RBC linoleic acid (LA, C18:2n-6), alpha-linolenic acid (ALA, C18:3n-3) and their respective metabolites, arachidonic acid (AA, C20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), during the study period. Significant differences in concentrations of linoleic acid (LA) could not be detected between the three groups at baseline, nor any time point during supplementation, but within each group, remarkable changes were observed. RBC-LA concentration decreased in the non-supplemented group over the 12 week study period (-0.9 mol%, $p<0.05$), and was stable in the TG group (+0.3 mol%, NS). Yet, the increase in LA was significantly higher in the PL supplemented group (+1.2 mol%) than in the non-supplemented group ($p<0.005$). Accordingly, the calculated monthly change in RBC-LA concentration was significantly higher in the PL- compared with the non-supplemented group (+0.45% vs. -0.39%, $p<0.01$; TG group, +0.09 mol%, NS). Concentrations of ALA and DHA slightly increased in both the TG- and PL-supplemented groups, and decreased in the non-supplemented patients, but the differences were not statistically significant. Arachidonic acid (AA) concentrations remained remarkably stable over time within and between the three groups (Fig. 3d).

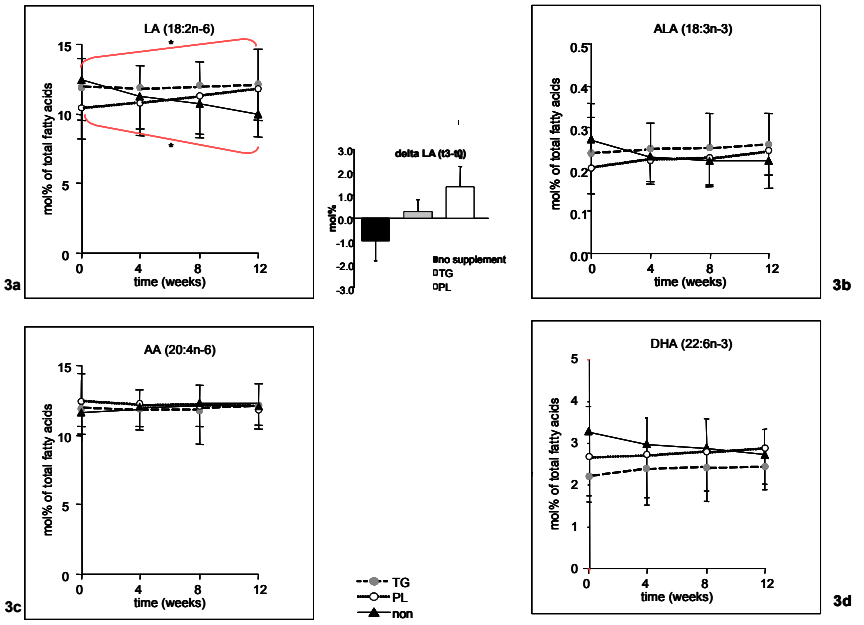


**Figure 3:** Concentrations of linoleic acid, alpha linolenic acid, arachidonic acid and docosahexaenoic acid in red blood cells of children with end-stage liver disease, recieving EFA-rich PL (open circles), EFA-rich TG (grey circles) or no EFA supplement (black triangles) for 3 months. Fatty acid concentrations are in mol% of total fatty acids. Inserted is the increase in RBC LA from baseline until 3-months of suppletion. Data represent means±SD of 9-25 patients per group. *$p<0.05$

No significant correlation existed between baseline RBC-LA level and monthly change in RBC-LA concentration in any of the groups (Figure 4).

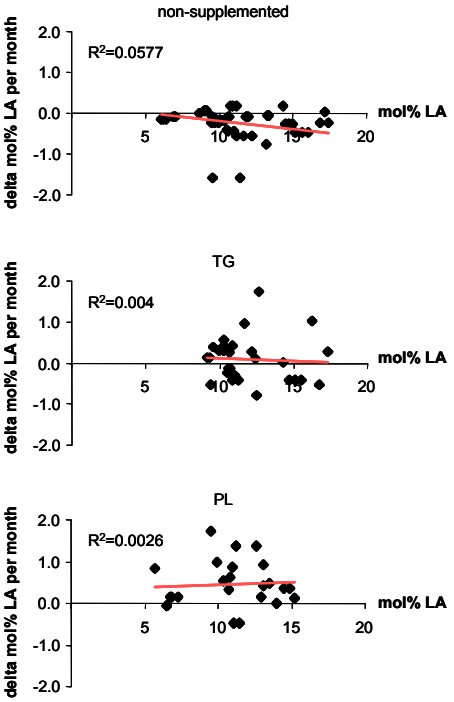


**Figure 4:** Monthly change in RBC-LA was not significantly correlated to baseline RBC LA levels.

Figure 5 shows markers for EFA-deficiency, such as the sum of n-6 fatty acids (n-6 deficiency), C20:3n-9 (also called mead acid, indicating n-6 deficiency) and the ratio between C22:5n-6 and C20:4n-6 (n-3 deficiency). Total n-6 fatty acids significantly decreased and mead acid significantly increased in the non-supplemented group, indicating development of n-6 and n-3 deficiency, whereas in both supplemented groups these parameters stabilized. A mild deficiency of n-3 fatty acids, defined by a C22:5n-6 to C20:4n-6 ratio > 0.068[16], was and remained present in all groups.

## Estimation of desaturase activity

For estimation of hepatic desaturase activities in chronic cholestatic patients, we calculated the ratio of C20:4n-6 to C20:3n-6 (delta-5-desaturase), C22:5n-6 to C24:4n-6 (delta-6-desaturase) and C22:6n-3 to C22:5n-3 (delta-6-desaturase). Based on these ratios, the estimated desaturase activities were not significantly different between supplemented or non-supplemented patients (Figure 6), and did not change over time during the three-month study period (data not shown).

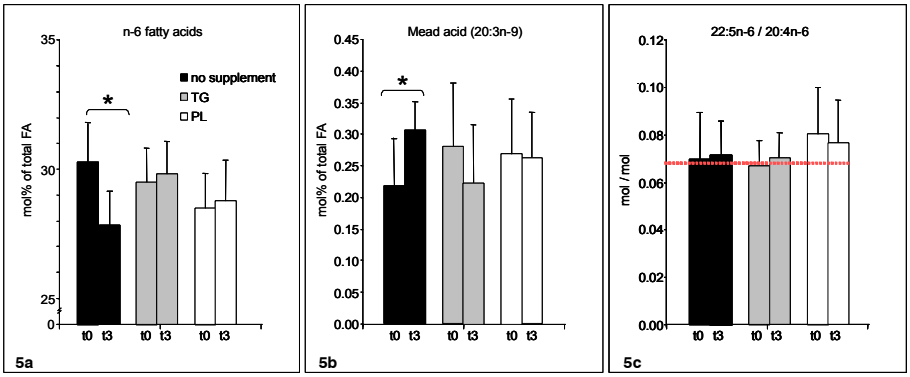


**Figure 5:** Total n-6 fatty acids, Mead acid (C20:3n-9) and the ratio of C22:5n-6 tp C20:4n-6) in red blood cells (RBC) of children with end-stage liver disease fed EFA-rich PL (white bars), EFA-rich TG (grey bars) or no EFA (black bars) for 3 months. Individual fatty acid concentrations are expressed as molar percentages of total fatty acids. Data represent means ± SD of 9-25 patients per group. *p<0.05

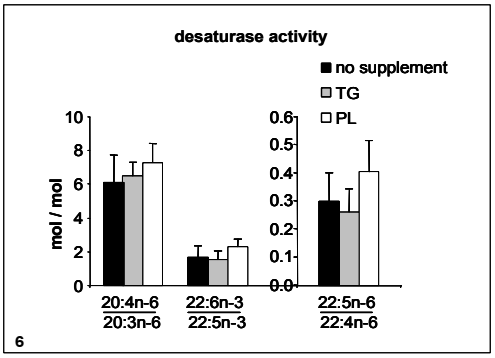


**Figure 6:** Activities of hepatic desaturases in chronic cholestatic patients as estimated by the ratios of C20:4n-6 to C20:3n-6 (delta-5-desaturase), C22:5n-6 to C24:4n-6 (delta-6-desaturase) and C22:6n-3 to C22:5n-3 (delta-6-desaturase). Ratios were not significantly different between supplemented or non-supplemented patients and did not change over time during the 3-month study period

## Dietary fat and LA intake

Basal dietary intake of linoleic acid (LA), saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were not significantly different between PL, TG or non-supplemented cholestatic children (Figure 7). When expressed per kg bodyweight, dietary LA intake was well above the RDI for children (0.5 g/kg), as formulated by the Netherlands Nutrition Council (TG: 0.8±0.5, PL: 0.7±0.5, non-supplemented: 0.8±0.6, NS). Average extra LA intake via the supplement was 2.2±0.5 g per day for each supplementation group (NS), i.e., 0.082 and 0.087 g extra LA per kg bodyweight per day for the TG- and the PL-group, respectively (NS).

## Liver enzymes

Serum markers for cholestasis and liver failure did not differ between supplemented and non-supplemented children at baseline (Figure 8), and did not alter significantly

during the three-month study period (not shown). Similarly, plasma vitamin A and vitamin E concentrations remained at a stable level during the study, and did not differ significantly between the three groups (Figure 9).

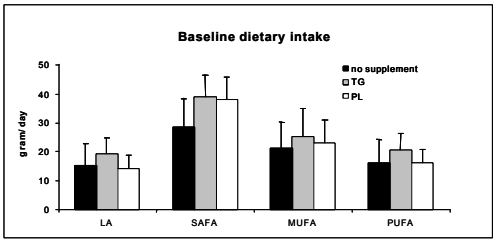


**Figure 7:** Basal dietary intake of linoleic acid (LA), saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), calculated in grams per day from 2-day consecutive food diaries, were not significantly different between PL, TG or non-supplemented cholestatic children.

| | non | TG | PL |
|---|---|---|---|
| AP (U/l) | 570 ± 271 | 729 ± 259 | 753 ± 307 |
| LDH (U/l) | 304 ± 96 | 312 ± 61 | 288 ± 63 |
| ASAT (U/l) | 133 ± 109 | 176 ±256 | 125 ± 73 |
| ALAT (U/l) | 90 ± 63 | 107 ± 91 | 85 ± 48 |
| bili tot (umol/l) | 127 ± 157 | 97 ± 109 | 137 ± 164 |
| alb (g/l) | 34.9 ± 6.9 | 32.2 ± 6.3 | 34.6 ± 7.9 |
| gGT (U/l) | 196 ± 200 | 238 ± 176 | 206 ± 194 |
| PT (sec) | 18 ± 7 | 16 ± 5 | 17 ± 5 |
| APTT (sec) | 37 ± 10 | 34 ± 7 | 36 ± 5 |
| fibrinogeen (g/l) | 2.7 ± 0.8 | 2.7 ± 0.6 | 2.6 ± 0.7 |
| AT (%) | 82 ± 29 | 85 ± 36 | 85 ± 41 |

**Figure 8:** Serum markers for cholestasis and liver failure did not differ between supplemented and non-supplemented children.

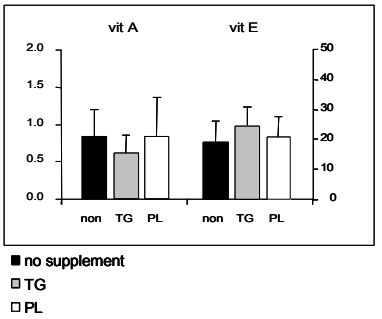


**Figure 9:** Plasma vitamin A and vitamin E concentrations remained at a stable level during the 3-month study period, and did not differ significantly between TG-, PL-, and non-supplemented children.

## DISCUSSION

We compared the efficacy of oral phospholipids (PL) and oral triglycerides (TG) as vehicles for EFA, for prevention and correction of EFA deficiency in children with end-stage liver disease (ESLD). We hypothesized that PL would be more effective than TG for oral EFA supplementation in cholestatic liver disease, since dietary TG are markedly malabsorbed during cholestasis, and intestinal PL absorption is relatively bile-independent. In addition, PL have been postulated to have a higher post-absorptive bioavailability for target organs of EFA and their long-chain poly-unsaturated metabolites, such as brain and liver[14;17;18]. Our results show a subtle superiority of oral PL compared to TG on monthly increase of erythrocyte-LA.

Between 1982 and 2000, 180 liver transplantations were performed in Groningen, in 136 children[29]. A high incidence of EFA deficiency in these children with ESLD has frequently been described, but due to the aspecificity of symptoms and the prolonged subclinical course, EFA deficiency is a biochemical rather than a clinical diagnosis. Still, no consensus exists regarding validated "gold standard" cut-off values that clearly define EFA deficiency in children. Previous studies in our hospital demonstrated that 75-80% of chronic cholestatic children had various indications for EFA deficiency, i.e., n-3 deficiency, n-6 deficiency or both[3]. In the present study we included patients irrespective of the EFA status at base line. One could argue that supplementation would only be indicated for patients with a biochemically marginal or deficient EFA status. However, biochemical analysis of RBC-EFA of included patients indicated marginal EFA status in all groups at baseline. Furthermore, baseline concentration of RBC-LA was not significantly correlated with its monthly increase of decrease (with or without supplementation), which in retrospect seems to validate random inclusion for supplementation.

Theoretically, chronic cholestasis may impair activities of hepatic desaturation and elongation enzymes, which synthesize AA and DHA from their respective precursors LA and ALA. Previous studies in short-term cholestatic rats showed no indications for altered desataration/elongation capacity[4]. In this study in children with chronic cholestasis, ratios between C20:3n-6 and C20:4n-6, marking delta-5 desaturase activity, and between C22:5n-3 and C22:6n-3 as a marker for delta-6 desaturase activity, were not significantly different between TG-, PL-, or non-supplemented cholestatic children. These estimates do not support a major difference in EFA metabolism among the groups. It is not quite clear, however, how these values relate to non-diseased controls. Also, the contribution of dietary LCPUFA intake on these ratios is unclear, preventing strong conclusions on the competence of EFA metabolism in these children. Liver enzymes and plasma fat-soluble vitamin concentrations remained stable during the three-month study period and did not differ significantly between groups.

Characteristic biochemical hallmarks of EFA deficiency[19;20] in RBC fatty acid profiles, such as decreased total n-6 fatty acids and increased C20:3n-9 concentrations, significantly deteriorated over time in the non-supplemented patients (Figure 5). The TG and PL supplements did not differentially affect LA, ALA, DHA or AA levels in RBC membranes after three months of supplementation. Interestingly, however, the PL supplement clearly induced an positive monthly increase in RBC LA, in contrast to the TG supplement, despite the fact that overall LA ingestion in the former tended to

be lower (TG vs. PL, 0.88 vs. 0.79 g/kg bodyweight/day, respectively). Thus, subtle changes in favor of EFA supplementation in the form of PL can be derived from the results, although it needs to be restated that the changes did not materialize into significant differences upon group-wise comparisons over the three-month study period. Several explanations are possible for this paradox. Firstly, considering the long half-life of RBC, the supplementation period may have been too short to observe profound differences between the groups in RBC fatty acids. It can not be excluded that upon prolonged supplementation, the rate of LA increase in the PL-group could have resulted in significantly higher RBC LA contents. Secondly, the dosage of supplemented LA could have been too low. Originally, we aimed to supply the patients with an extra 50% LA of the recommended daily intake (RDI) for children. However, the high viscosity of the PL supplement (soybean lecithin) required emulsification into an oil-in-water solution that resulted in a strong increase in the volume of supplement to be ingested daily. The maximum ingestible volume of supplement for the children was 30 ml per day, and therefore we decreased the dose of extra LA from 50% to 25% of RDI (i.e., 0.125 g LA/kg/day), and restricted the volume of supplement to a daily maximum of 30 ml for children above 20 kg. Average daily dietary LA ingestion (apart from the supplements) of the cholestatic children was well above the RDI of 0.5 g/kg/day, presumably due to clinical dietary counseling. Since baseline dietary LA intake was 0.75±0.35 g/kg/day, and 11 of 19 supplemented patients weighed more than 20 kg, the actual extra supply of LA in the cholestatic patients as TG/PL supplement eventually was 15±7% of dietary intake. Possibly, this amount may have been insufficient to result in a pronounced increase in RBC-EFA. Yet, even at this relatively low supplementation dose, a positive effect of PL-supplementation could be observed. Strandvik *et al.* supplemented cystic fibrosis patients with even smaller doses of LA (30-50 mg/kg/day)[21], which significantly improved serum EFA status in these patients. However, supplementation in this study was intravenous, and the effects were evaluated after one year of supplementation.

Several authors have questioned the concept that the erythrocyte membrane fatty acid composition represents a reliable reflection of overall body EFA status. Due to their long half-life and short-term independence of post-prandial plasma fatty acid concentrations, erythrocytes are regarded as a stable and easily accessible compartment for evaluation of body EFA status. However, studies by Korotkova *et al.*[22] demonstrated that phospholipid fatty acid profiles of erythrocytes, serum, jejunum, ileum and colon were differentially affected by EFA deficiency in rats. Similarly, Rioux *et al*.[23] reported that in piglets, the plasma phospholipid fatty acid profile adequately reflects that of liver and bile, but not of brain phospholipids.

In EFA-deficient cholestatic mice, we previously observed that oral EFA-PL adminis-

tration improved LCPUFA concentrations in brain and liver more efficiently than EFA-TG, but remarkably, EFA concentrations in erythrocytes were equally improved by TG and PL[13]. These results suggest that after absorption, specific targeting occurs of LCPUFA to organs as the central nervous system, liver or gut, at the expense of less critical tissues such as erythrocytes. The brain has been postulated to preferentially absorb LCPUFA as lyso-PL, in contrast to unesterified LCPUFA bound to albumin, or LCPUFA esterified into lipoproteins[24-28]. Since dietary PL are partly absorbed as lyso-PL and partly as intact PL molecules, the highly efficient increase in brain LCPUFA levels in mice after PL supplementation supports the postulated high bioavailability of enteral PL for LCPUFA target organs[14;17;18].

We conclude that oral EFA supplementation in the form of PL is slightly more effective than as TG for prevention or correction of EFA deficiency in children with end-stage liver disease.

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