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Supplementation of Arachidonic Acid Plus Docosahexaenoic Acid in Cirrhotic Patients Awaiting Liver Transplantation: A Preliminary Study

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Abstract

Background—In patients with cirrhotic liver diseases, supplementation of linoleic acid and α -linolenic acid often does not alter the levels of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), suggesting the necessity to directly provide these nutrients.

Methods—In a double-blind, placebo-controlled fashion, 9 cirrhotic patients listed for liver transplantation at Lahey Clinic Center were given daily supplementation with either 10 gel caps containing 500 mg of AA and 1000 mg of DHA (AA/DHA) or 250 mg of linolenic acid (LA) and 125 mg of oleic acid (OA; OA/LA) for 6 weeks. α -Tocopherol at 200 IU was provided daily. No other dietary prescription was made. Plasma fatty acid profiles were determined in triglyceride and phospholipids fractions. Plasma levels of C-reactive protein (CRP), tumor necrosis factor (TNF), interleukin 6 (IL-6), and soluble TNF receptor II (sTNFRII) were also measured.

Results—Four patients receiving OA/LA and 5 patients receiving AA/DHA completed the study without evidence of any adverse effects or intolerance. The supplementation of LA, AA, and DHA effectively raised their levels in either one or both plasma lipid fractions in this limited number of subjects. DHA plus AA also lowered 22:4 ω -6, 22:5 ω -6, and 22:5 ω -3, suggesting that DHA reduced the elongation and desaturation of AA and EPA.

Conclusions—It is feasible to improve the liver disease-associated deficiency of AA or DHA with modest intakes of AA and DHA. Whether this maneuver will affect the systemic inflammatory responsiveness and ultimately clinical outcome will require a large-scale and well-controlled intervention.

An abnormal fatty acid pattern in the plasma phospholipid (PL) fraction has been reported in patients with advanced liver cirrhosis.¹⁻³ In our previous study, we found significantly lower levels of linoleic acid (LA), arachidonic acid (AA), and docosahexaenoic acid (DHA) in the plasma PL of patients with end-stage liver disease.⁴ Insufficient food intake and abnormal intestinal fat absorption were thought to be important factors in the development of essential fatty acid deficiency seen in these patients. Impairment of subsequent desaturation steps of linoleic and α -linolenic acid that regulate very-long-chain polyunsaturated fatty acid

biosynthesis may also contribute to the very-long-chain polyunsaturated fatty acid deficiency found in these patients.

Providing the essential fatty acids, LA and α -linolenic acid (LNA), by parenteral nutrition (PN) can successfully increase the plasma concentrations of LA and LNA to normal in cirrhotic patients.⁵ However, supplementation with these essential fatty acids did not alter the levels of AA, eicosapentaenoic acid (EPA), and DHA in such patients.⁵ Rimola et al⁶ also found similar results with IV administration of LA, which failed to raise the plasma concentrations of AA in patients with liver disease. Cabre et al⁷ reported that AA deficiency was associated with a significant increase in the mortality risk in patients with advanced liver cirrhosis, suggesting that the deficiency of these very-long-chain polyunsaturated fatty acids might be a contributing pathogenic factor in chronic liver diseases.^{8,9} This suggests that supplementation with preformed AA, EPA, or DHA might be necessary to overcome the impaired regulatory steps in the biosynthesis of very-long-chain polyunsaturated fatty acids in patients with cirrhotic liver diseases.

In 1 study, supplementation of fish oil enriched in EPA and DHA to cirrhotic patients significantly increased the levels of EPA and DHA in plasma PL and triglycerides (TG), respectively.¹⁰ Unfortunately, the plasma AA levels were not compared before and after this supplementation. It is well documented that *in vivo* EPA competes with AA for its esterification and membrane incorporation, leading to a reduction of AA concentrations in plasma and tissues,¹¹ which could be undesirable for cirrhotic patients who may already be deficient in AA. Studies have shown that administration of not only LA and LNA but also AA can reverse abnormalities induced by essential fatty acid deficiency abnormalities, such as slow growth, scaly dermatitis, and skin thickness.¹² In cirrhosis, moreover, the portal absorption pathway is particularly efficient for unsaturated fatty acids.^{13,14} Thus, we hypothesize that supplementation of AA plus DHA could uniquely raise levels of both AA and DHA while maintaining EPA levels in the cell membranes in these patients.

The present study sought to examine (1) whether taking 500 mg of AA plus 1000 mg of DHA daily for 6 weeks was feasible in cirrhotic patients, and (2) whether this combination could increase the plasma levels of these 2 fatty acids in these patients. The amount of AA (500 mg/d) is 2.5 times the estimated daily intakes of AA (200 mg/d) for a healthy adult, and the amount of DHA (1000 mg/d) is equivalent to onethird that of a high-fish-oil consumer.^{15,16} The control group was given the gel caps available on market, containing 125 mg of oleic acid (OA, 18:1 ω -9) and 250 mg of LA (18:2 ω -6). The amount of fat was 4 times more in the test group compared with the control group (1500 mg/d vs 375 mg/d). Therefore, the caloric contents of the 2 different fatty supplementations were 13.5 kcal/d and 3.4 kcal/d, respectively.

Plasma TG and PL were compared before and after supplementation. TG composition principally reflects dietary fatty acids postprandially and adipose tissue composition postabsorptively, whereas PL reflects fatty acid metabolism by the liver and is the serum lipid component that most nearly reflects composition of tissue membranes.

MATERIALS AND METHODS

Patient Selection

The study was conducted at Lahey Clinic Medical Center, Burlington, which is the largest center for liver transplantation in the New England area. From August 2002 to January 2004, all the adult patients with end-stage liver disease (ESLD) who were approved and listed for orthotopic liver transplant were eligible to be enrolled into this study. The exclusion criteria were (1) significant encephalopathy that would make it difficult to

participate; (2) hospitalized patients; (3) active gastrointestinal bleeding; (4) inability to sign an informed consent; (5) diabetes; and (6) receiving glucocorticoid therapy. According to these criteria, only 9 patients were included over the period.

Methods

The study protocol was approved by the institutional review board of Lahey Clinic Medical Center. Written informed consent was obtained from each patient.

At enrollment, each patient underwent a full medical history and physical examination, and upper arm anthropometric measures, as well as bioimpedance analysis (BIA) to assess body composition. Patients then were randomly given unmarked bottles containing gel caps for 6 weeks, with either 500 mg of AA plus 1000 mg of DHA (AA/DHA) per gel cap or 125 mg of OA plus 250 mg of LA (OA/LA) per gel cap. Patients and investigators were blinded to the contents of the bottles. Martek Biosciences Inc (Columbia, MD) performed the randomization and labeled each bottle with a number. Only at the completion of the study were the investigators provided the randomization list by Martek Biosciences Inc. All the patients also were given 200 IU of vitamin E/d for 6 weeks.

In this study, dietary intakes and dietary habits were maintained as usual. However, all the patients were asked to record their daily food intakes, number of capsules taken, and side effects noted over the 6 weeks of study.

Fasting blood samples were taken at the beginning and the end of the study for fatty acid analyses and the measurements of C-reactive protein (CRP), tumor necrosis factor (TNF), interleukin 6 (IL-6), and soluble tumor necrosis factor receptor II (sTNFR II), the latter to assess in a preliminary fashion whether there was any impact on the systemic inflammatory response. Blood samples were immediately put on ice. Plasma was separated within 2 hours of the collection. Aliquots were stored at -80°C for later analysis.

Analytical Methods

Lipids from plasma were extracted by liquid-liquid extraction with 6 volumes of chloroform:methanol (2:1, vol/vol), centrifuged at $800 \times g$ for 3 minutes, and the resulting lower phase was aspirated.¹⁷ Before the extraction, an aliquot of 30 μL of a 1 mg/mL solution of diheptadecanoyl phosphatidylcholine and triheptadecanoyl glycerol (Nu-Check Prep, Elysian, MN) in chloroform-methanol (1:1, vol/vol) was added as an internal standard to all samples. The chloroform:methanol extracts were evaporated to dryness under nitrogen. Samples of 0.5 mL of methanol base-BF₃ reagent (Supelco, Bellefonte, PA) were added, and the sample was then placed in a heating block at 100°C for 4 minutes and then removed from the heating block and allowed to cool at room temperature. Then, 0.5 mL of n-hexane was added. After cooling the tubes, 6.5 mL of a NaCl-saturated solution was added and the reaction mixture centrifuged at $800 \times g$ for 2 minutes. The upper hexane layer was aspirated. Fatty acid methyl esters were analyzed by gas chromatography-FID with a Hewlett Packard 5890 A, using a Supelcowax-10, 0.25 mm ID column (Supelco, Bellefonte, PA). Fatty acid methyl ester peaks were identified by comparison of retention times of standard mixtures and then quantified against the internal standards. Results are expressed as % of total fatty acid content (%/nmol) and nmol/mL in the sample.

Plasma CRP was determined using human CRP ELISA kit (Alpha Diagnostic International, San Antonio, TX). Plasma levels of TNF, sTNFR II, and IL-6 were measured using ELISA kits (Biosource, Camarillo, CA).

Statistics

Data were presented as mean $\pm$ SEM. The baseline levels of fatty acids, cytokines, CRP, and the Child-Turcotte-Pugh score and Child-Pugh score were compared by Student's *t*-test between OA/LA (control) and AA/DHA (test) groups. The changes in fatty acids, cytokines, and CRP before and after supplementation were compared by paired Student's *t*-test.

RESULTS

All 9 patients completed the study without evidence of intolerance. Among 9 qualified patients, 5 patients received AA/DHA gel caps and 4 patients received OA/LA gel caps. Clinical characteristics at the baseline are listed in Tables I and II. Despite double-blinded randomization, the OA/LA control group had significantly higher Child-Pugh classification scores. This differential allocation occurred most likely due to the small sample size. No essential fatty acid deficiency was found in any of the 9 patients. The levels of total amount (nmol/mL) of TG and PL in plasma were almost identical between the control and the test groups (data not shown).

Figure 1 shows the percentage changes of OA (18:1 ω -9) and LA (18:2 ω -6) in plasma TG and PL in different groups. In the OA/LA group, the supplementation of 125 mg of OA and 250 mg of LA significantly increased the levels of LA, but not OA, in both TG and PL fractions in plasma. However, the supplementation of 500 mg of AA and 1000 mg of DHA did not alter the levels of LA or OA in plasma TG and PL.

Figure 2 shows the percentage changes in the very-long-chain (≥ 20 carbons) polyunsaturated fatty acids in plasma TG and PL fractions in different groups. In the OA/LA group, there was a slight but nonsignificant increase in AA (20:4 ω -6) in plasma TG after supplementation. No other differences in fatty acids were observed in the plasma PL fraction. AA/DHA supplementation, however, significantly increased the plasma TG levels of AA (20:4 ω -6) and DHA (22:6 ω -3). The same changes were also observed in plasma PL, although the increases of AA did not reach statistical significance. Interestingly, the PL levels of 22:4 ω -6, 22:5 ω -6, and 22:5 ω -3 were significantly decreased by AA/DHA supplementation. There were no significant differences in other fatty acids (data not shown).

The changes in CRP and measured cytokines in plasma are shown in Figure 3. No significant differences were found in the concentrations of CRP, TNF, IL-6, and sTNFRII before and after each supplementation. However, with OA/LA supplementation, there was an increase in cytokine levels (sTNFRII and IL-6) with supplementation, whereas these levels decreased with AA/DHA.

DISCUSSION

This study showed that supplementation of polyunsaturated fatty acids up to 1500 mg daily for 6 weeks was feasible and well tolerated in relatively stable patients with advanced cirrhosis awaiting liver transplantation. Interestingly, supplementation of 125 mg of OA plus 250 mg of LA only slightly increased the percentage amount of LA in total TG in plasma and not at all in plasma PL, which is usually more slowly responsive. Furthermore in essential fatty acid deficiency, providing dietary LA beyond the requirement level has limited impact on AA levels in serum and membrane PL, presumably reflecting the tight control of AA levels under most conditions.¹¹ Supplementation with 500 mg of AA and 1000 mg of DHA significantly increased the percentage amounts of AA and DHA in both TG and PL in plasma, presumably reflecting the greater amount provided and possibly their lesser metabolism through oxidation or conversion. Thus, oral supplementation of AA or DHA at this level for 6 weeks can effectively raise the content of these 2 fatty acids in

plasma or cell membrane, which might translate into physiologic and clinical benefits for these patients.¹⁸

Under normal conditions, DHA (22:6 ω -3) is derived from 18:3 ω -3 *via* the 20-carbon EPA (20:5 ω -3) through 2 elongation and desaturation steps. It is then converted to DHA by additional elongation and desaturation through the intermediary 22-carbon docosapentaenoic acid (DPA, 22:5 ω -3). Similarly 20:4 ω -6, AA, is derived from LA (18:2 ω -6) with the production of the DHA analog, another DPA 22:5 ω -6, and the corresponding intermediary 22:4 ω -6. In this study, the DHA supplementation in AA/DHA group doubled the amount of DHA in both plasma TG and PL (Figure 2). In association with these changes in DHA, the amount of 22:5 ω -3 (DPA) was significantly decreased from 0.68% $\pm$ 0.02% to 0.42% $\pm$ 0.04%/nmol in total plasma PL ($p < .005$). Because DPA (22:5 ω -3) can either be converted from EPA (20:5 ω -3) or retroconverted from DHA (22:6 ω -3), it appears that the accumulated end substrate, DHA, inhibited the further fatty acid conversion from EPA in this group. Indeed, the levels of EPA in this group were maintained. Similarly DHA/AA decreased 22:4 ω -6 and 22:5 ω -6. Because all 3 of these fatty acids were diminished with DHA/AA supplementation, this strongly suggests that DHA inhibits the elongation and desaturation of both AA and EPA. The failure to see these changes in the TG fraction presumably is a consequence of the fact that TG reflect dietary intake and tissue stores, whereas the PL fraction reflects hepatic metabolism of fatty acids.

In this study, several limitations should be noted. The first and most important is the sample size. There were only 4 and 5 patients in each group due to the necessity to find candidates who were likely to complete this preliminary trial. Given the effectiveness of compliance and ability to change essential fatty acid metabolism, it would seem reasonable to expand the protocol in the future. The second is the variability between and within patients in each group, which is to be expected with this clinical condition. Therefore, larger patient numbers will be required to detect changes in biomarkers like cytokine levels and acute phase protein levels and even larger numbers to achieve the likelihood of detecting potential changes in clinical outcome. However, this preliminary study does set the stage for a much larger trial, perhaps with expansion of eligibility. Study design considerations include whether it would be important to increase the dose of OA/LA to make the comparison isocaloric and perhaps detect whether AA levels might respond to this maneuver, although this is unlikely for the reasons discussed earlier. Another consideration would be to determine whether the effects of supplementation are dependent on the degree of liver impairment as reflected in Child-Pugh scores because the degree of impairment of essential fatty acid metabolism is related to residual hepatic function.

CONCLUSION

In conclusion, this small preliminary study provides the feasibility for a larger-scale intervention by AA or DHA supplementation in chronic liver disease in attempts to improve hepatic function and perhaps the outcome in end-stage liver disease with and without liver transplantation.

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REFERENCES

1. Gunnlaugsson O, Berkowitz D. Individual free fatty acids in patients with liver disease. *Am J Dig Dis.* 1977; 22:1005–1009. [PubMed: 920697]

2. Wilcox HG, Dunn GD, Schenker S. Plasma long chain fatty acids and esterified lipids in cirrhosis and hepatic encephalopathy. *Am J Med Sci.* 1978; 276:293–303. [PubMed: 742637]
3. Johnson SB, Gordon E, McClain C, Low G, Holman RT. Abnormal polyunsaturated fatty acid patterns of serum lipids in alcoholism and cirrhosis: arachidonic acid deficiency in cirrhosis. *Proc Natl Acad Sci U S A.* 1985; 85:1815–1818. [PubMed: 3920655]
4. Palombo JD, Lopes SM, Zeisel SH, et al. Abnormal polyunsaturated fatty acid patterns of serum lipids in alcoholism and cirrhosis: arachidonic acid deficiency in cirrhosis. *Gastroenterology.* 1987; 93:1170–1171. [PubMed: 3315823]
5. Duerksen DR, Nehra V, Palombo JD, Ahmad A, Bistrian BR. Essential fatty acid deficiencies in patients with chronic liver disease are not reversed by short-term intravenous lipid supplementation. *Dig Dis Sci.* 1999; 44:1342–1348. [PubMed: 10489916]
6. Rimola A, Gines P, Cuso E, et al. Prostaglandin precursor fatty acids in cirrhosis with ascites: effects of linoleic acid infusion in functional renal failure. *Clin Sci.* 1988; 74:613–619. [PubMed: 3135142]
7. Cabre E, Periago JL, Gonzalez J, et al. Plasma polyunsaturated fatty acids in liver cirrhosis with or without chronic hepatic encephalopathy: a preliminary study. *JPEN J Parenter Enteral Nutr.* 1992; 16:359–363. [PubMed: 1386392]
8. Cabre E, Abad-Lacruz A, Nunez MC, et al. The relationship of plasma polyunsaturated fatty acid deficiency with survival in advanced liver cirrhosis: multivariate analysis. *Am J Gastroenterol.* 1993; 88:718–722. [PubMed: 8480737]
9. Cabre E, Gassull MA. Polyunsaturated fatty acid deficiency in liver diseases: pathophysiological and clinical significance. *Nutrition.* 1996; 12:542–548. [PubMed: 8878152]
10. Miyamoto A, Wakabayashi H, Watanabe A. Abnormality in fatty acid composition of gastric mucosal phospholipids in patients with liver cirrhosis and its correction with polyunsaturated fatty acid-enriched soft oil capsule. *J Gastroenterol Hepatol.* 1997; 12:644–652. [PubMed: 9407327]
11. Bistrian BR. Clinical aspects of essential fatty acid metabolism: Jonathan Rhoads lecture. *JPEN J Parenter Enteral Nutr.* 2003; 27:168–175. [PubMed: 12757109]
12. Lehninger, AL., editor. *Biochemistry.* 2nd ed.. Vol. 281. Worth Publishers, Inc; New York, NY: 1975. p. 669
13. McDonald GB, Saunders DR, Weidman M, Fisher L. Portal venous transport of long-chain fatty acids absorbed from rat intestine. *Am J Physiol.* 1980; 239:G141–G150. [PubMed: 7435568]
14. Cabre E, Hernandez-Perez JM, Fluvia L, Pastor C, Corominas A, Gassull MA. Absorption and transport of dietary long-chain fatty acids in cirrhosis: a stable-isotope-tracing study. *Am J Clin Nutr.* 2005; 81:692–701. [PubMed: 15755841]
15. Nelson GJ, Kelley DS, Emken EA, Phinney SD, Kyle D, Ferretti A. A human dietary arachidonic acid supplementation study conducted in a metabolic research unit: rationale and design. *Lipids.* 1997; 32:415–420. [PubMed: 9113630]
16. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among U.S. men and women. *Circulation.* 2003; 108:155–160. [PubMed: 12821543]
17. Folch J, Lees M, Sloanestanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biochem.* 1957; 226:497–509.
18. Pantaleo P, Marra F, Vizzutti F, et al. Effects of dietary supplementation with arachidonic acid on platelet and renal function in patients with cirrhosis. *Clin Sci.* 2004; 106:27–34. [PubMed: 12877651]

Figure 1.

Changes in plasma triglyceride and phospholipids contents (%/nmol) of 18:1w9 (OA) and 18:2w6 (LA) in the different groups. The solid bars indicate the basal levels; the open bars indicate the levels at the end of the study.

Figure 2. Changes in plasma triglyceride and phospholipids contents (%/nmol) of the longer-chain (≥ 20 carbons) polyunsaturated fatty acids in the different groups. The solid bars indicate the basal levels. The open bars indicate the levels at the end of the study.

Figure 3.

Changes in plasma levels of C-reactive protein (CRP), tumor necrosis factor (TNF), interleukin (IL)-6, and soluble tumor necrosis factor receptor II (sTNFRII) in the different groups. The solid bars indicate the basal levels; the open bars indicate the levels at the end of the study.

Table I
Clinical characteristics of patients

	Control (n = 4)	AA/DHA (n = 5)
Age, y	59.3 ± 6.5	56.6 ± 3.4
BMI, kg/m ²	26.8 ± 1.7	27.1 ± 3.3
Usual body mass, lbs	178.0 ± 7.4	149.4 ± 16.2
Urinary creatinine, g/24 h	1.0 ± 0.1	1.4 ± 0.5
Total bilirubin, mg/dL	2.1 ± 0.8	2.0 ± 0.2
INR	1.2 ± 0.1	1.3 ± 0.1
Albumin, g/dL	2.8 ± 0.2	2.9 ± 0.4
Glucose, mg/dL	104.0 ± 6.6	100.0 ± 2.7
TSF, mm	22.8 ± 8.3	19.0 ± 3.3
MAMC, cm	35.0 ± 4.7	27.7 ± 2.5
Child-Turcotte-Pugh score [*]	9.0 ± 0.5	7.2 ± 0.5
Child-Pugh score	3 B ⁺ and 1 C	3 B, 1 B ⁺ and 1 C

Mean ± SEM. AA, arachidonic acid; DHA, docosahexaenoic acid; BMI, body mass index; INR, international normalized ratio; TSF, triceps skinfold; MAMC, mid-arm muscle circumference.

*
p < .05, control vs AA/DHA by Student's t-test.

Table II
Plasma levels of C-reactive protein (CRP) and selective cytokines at baseline

	Control (n = 4)	AA/DHA (n = 5)
CRP, mg/dL	0.8 ± 0.0	0.3 ± 0.1
TNF, pg/mL	4.2 ± 1.1	2.9 ± 1.0
sTNFRII, ng/mL	24.6 ± 3.4	17.8 ± 8.0
IL-6, pg/mL	32.6 ± 17.8	7.7 ± 4.0

Mean ± SEM. AA, arachidonic acid; DHA, docosahexaenoic acid; TNF, tumor necrosis factor; sTNFRII, soluble tumor necrosis factor receptor II; IL, interleukin.