Polyunsaturated Fatty Acid Pattern in Liver and Erythrocyte Phospholipids from Obese Patients

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Abstract

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Objective: Our aim was to study the fatty acid (FA) composition of liver phospholipids and its relation to that in erythrocyte membranes from patients with obese nonalcoholic fatty liver disease (NAFLD), as an indication of lipid metabolism alterations leading to steatosis.

Research Methods and Procedures: Eight control subjects who underwent antireflux surgery and 12 obese patients with NAFLD who underwent subtotal gastrectomy with a gastro-jejunal anastomosis in Roux-en-Y were studied. The oxidative stress status of patients was assessed by serum F_2-isoprostanes levels (gas chromatography/negative ion chemical ionization tandem mass spectrometry). Analysis of FA composition of liver and erythrocyte phospholipids was carried out by gas-liquid chromatography.

Results: Patients with NAFLD showed serum F_2-isoprostanes levels 84% higher than controls. Compared with controls, liver phospholipids from obese patients exhibited significantly 1) lower levels of 20:4n-6, 22:5n-3, 22:6n-3 [docosahexaenoic acid (DHA)], total long-chain polyunsaturated FA (LCPUFA), and total n-3 LCPUFA, 2) higher 22:5n-6 [docosapentaenoic acid (DPAn-6)] levels and n-6/n-3 LCPUFA ratios, and 3) comparable levels of n-6 LCPUFA. Levels of DHA and DPAn-6 in liver were positively correlated with those in erythrocytes ($r = 0.77$ and $r = 0.90$, respectively; $p < 0.0001$), whereas DHA and DPAn-6 showed a negative association in both tissues ($r = -0.79$, $p < 0.0001$ and $r = -0.58$, $p < 0.01$, respectively), associated with lower DHA/DPAn-6 ratios.

Discussion: Obese patients with NAFLD showed marked alterations in the polyunsaturated fatty acid pattern of the liver. These changes are significantly correlated with those found in erythrocytes, thus suggesting that erythrocyte FA composition could be a reliable indicator of derangements in liver lipid metabolism in obese patients.

Key words: polyunsaturated fatty acids, insulin resistance, oxidative stress, liver disease

Introduction

The knowledge of the mechanisms whereby liver injury such as steatosis and its progression to steatohepatitis occurs is key to understanding the pathophysiology of nonalcoholic fatty liver disease (NAFLD)[1] (1,2). Frequently associated with obesity and type 2 diabetes, NAFLD is related to insulin resistance and oxidative stress as a critical pathogenic factor (1–3). In the setting of obesity associated with insulin resistance, hepatic fat accumulation may be caused by 1) a higher peripheral lipolysis secondary to insulin

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[1] Nonstandard abbreviations: NAFLD, nonalcoholic fatty liver disease; FA, fatty acid; LCPUFA, long-chain polyunsaturated FA; DHA, docosahexaenoic acid; DPAn-6, docosapentaenoic acid; PUFA, polyunsaturated FA; HOMA, homeostasis model assessment; TLC, thin layer chromatography; FAME, fatty acid methyl ester.

resistance leading to increased fluxes of fatty acids (FAs) and glycerol to the liver (4) and 2) depletion of hepatic long-chain polyunsaturated FAs (LCPUFAs) (5). The latter factor may direct FAs away from oxidation and secretion and into triacylglycerol storage, because LCPUFAs, particularly those of the n-3 series, induce the expression of genes related to lipid oxidation and export and suppress that of lipogenic genes (3,6). Depletion of n-3 LCPUFAs is related to several factors: 1) lower adipose tissue levels of n-3 LCPUFAs and of their essential precursor 18:3n-3 (5), a parameter considered as a biomarker of dietary intake of these FAs (7); 2) a decreased liver (20:5 + 22:6)n-3/18:3n-3 ratio (5), suggesting desaturase inhibition (8); 3) higher adipose tissue content of the 18:1n-9 trans isomer (5), a potent desaturase inhibitor (9); and 4) liver oxidative stress (5,10) that may involve increased peroxidation of n-3 LCPUFA (3).

Deficiency of n-3 FA is characterized by loss of docosahexaenoic acid (22:6n-3; DHA) from the brain or retina in experimental animals (11) and in human infants fed vegetable oil–based formulas (12). Interestingly, DHA depletion is accompanied by a compensatory increase in n-6 FAs, mainly docosapentaenoic acid (22:5n-6; DPAn-6 or Osbond acid) (11–14), suggesting the existence of mechanisms conserving polyunsaturation of membrane phospholipids (15). DHA deficiency with DPAn-6 compensation observed in brain and retina (11,12,14) also occurs in erythrocytes (11,12,16) and liver (16), being a reversible phenomenon showing a faster recovery in liver compared with brain on the use of an n-3 adequate diet (16). In view of these considerations, the aim of this study was to test the hypothesis that depletion of hepatic DHA levels in obese patients with NAFLD is associated with a compensatory increase in those of DPAn-6. In addition, correlations between the changes in the content of DHA and DPAn-6 in liver phospholipids and erythrocyte membranes were established to validate the latter as a reliable biomarker of the hepatic DHA/DPAn-6 status.

Research Methods and Procedures

Patient and Laboratory Studies

Twelve patients with NAFLD (average BMI, 43.8 ± 2.4 kg/m^2; age range, 27 to 56 years), who underwent subtotal gastrectomy with a gastro-jejunal anastomosis in Roux-en-Y as therapy for obesity, were included in this study. Eight non-obese patients (BMI, 22.8 ± 0.6 kg/m^2; age range, 23 to 63 years) who underwent antireflux surgery were also studied as the control group. The protocol was explained in detail to the subjects, who gave their written informed consent to participate in the study before any procedure was undertaken. Nutritional and alcohol consumption histories with anthropometric measurements were obtained. Laboratory tests included serum liver enzymes (aspartate aminotransferase and alanine aminotransferase) and serum total cholesterol, triacylglycerols, and low- and high-density lipoprotein levels. Exclusion criteria included positive hepatitis B or C serology, positive antibodies (antinuclear, antimitochondrial, and antismooth muscle antibodies), smoking habits or nonsmokers <1-year cessation, and consumption of >40 grams of ethanol per week. Insulin resistance was calculated from the fasting insulin and glucose values by homeostasis model assessment (HOMA) of insulin resistance analysis [fasting insulin (μU/mL) × fasting glucose (mM/L)/22.5] (17).

The patients were subjected to a diet of 25 kcal/kg body weight (where 1 kcal = 4.184 kJ), with 30% of the energy given as lipids [10% saturated FAs, 10% monounsaturated FAs, and 10% polyunsaturated FAs (PUFAs)], 15% as proteins, and 55% as carbohydrates for at least 2 days before surgery, and liver biopsies of ~2 cm^3 for histological diagnoses and lipid composition determination were taken during surgery. The samples were fixed in 10% formalin and paraffin embedded, and sections were stained with hematoxylin/eosin and Van Gieson's stain. Sections of each liver sample were observed in a blinded manner and evaluated for histological alterations by means of previously defined codes (18). Steatosis was graded as absent or grade 1 (mild, 5% to 33% of hepatocytes affected), 2 (moderate, 33% to 66%), and 3 (severe, >66%) (18). Control patients exhibited a normal liver histology, whereas obese patients showed the presence of macrovesicular steatosis (mild/moderate/severe = 5/1/6). In the latter group, six patients exhibited steatosis alone, and six patients presented steatosis and lobular inflammation with hepatocyte ballooning, one of them with mild fibrosis (steatohepatitis). The Ethics Committee of the University of Chile Clinical Hospital approved the study protocol, according to Helsinki criteria.

Preparation Procedure and Gas-mass Analysis for Plasma F_2-isoprostane Determination

The preparation of plasma samples before gas-mass analysis of F_2-isoprostanes involved solid phase extraction on an octadecylsilane (C_{18}) and silica cartridge followed by thin layer chromatography (TLC), combined with aminopropyl (NH_2) cartridge solid phase extraction (19). The determinations were carried out by gas chromatography/negative ion chemical ionization tandem mass spectrometry analysis. Samples (1 μL) were injected into the gas chromatograph in undecane containing *N,O*-bis-(trimethylsilyl-)trifluoroacetamide. The carrier gas was helium, and methane was used as reagent gas at a flow of 1.2 mL/min. The collision energy used was 1.3 eV. The measured ions were m/z 299 and m/z 303 derived from the typical ions (m/z 569 and m/z 573) produced from 15-F_{2t}-IsoP (the most represented isomer) and the tetradeuterated derivative of prostaglandin $F_{2\alpha}$, respectively. The detection limit was 10 pg/mL (0.028 nM).

Extraction and Separation of Liver Phospholipids

Liver tissue dissociation was achieved by homogenization in ice-cold chloroform-methanol (2:1, vol/vol) containing 0.01% (wt/vol) butylated hydroxytoluene using an Ultraturrax homogenizer (Janke & Kunkel, Stufen, Germany). Phospholipids from liver were separated by TLC aluminum sheets (20 × 20-cm silica gel 60 F-254; Merck, Santiago, Chile), using a solvent system of hexane/diethyl ether/acetic acid (80:20:1, by vol) and phosphatidylcholine as standard (20). After development of the plate, the solvent was allowed to evaporate, and lipid bands were visualized by exposing the plates to a Camag UV (250 nm) lamp designed for use in the TLC laboratory. This solvent system separates phospholipids, cholesterol, triacylglycerols, and cholesterol esters in increasing order of relative mobility. Individual lipid zones were scraped from TLC plates and eluted from the silica gel with chloroform/methanol/water (10:10:1, by vol) (21).

Extraction and Separation of Phospholipids from Erythrocyte Membranes

Blood samples received in syringes containing 5% (wt/vol) EDTA as anticoagulant were centrifuged at 2500 rpm and 4 °C for 15 minutes to separate erythrocytes. Erythrocyte membranes were obtained according to Huertas et al. (22), and membrane lipids were extracted as described by Bligh and Dyer (23). Separation of phospholipid fractions was performed as described for liver phospholipids.

Preparation and Analysis of Fatty Acid Methyl Esters

Fatty acids from liver phospholipids and from erythrocyte membranes were methylated. The phospholipids were eluted from silica gel with two 15-mL portions of chloroform/methanol/water (10:10:1, by vol). The solvent was evaporated in a stream of nitrogen, and 10 mg of tricosaenoic acid (23:0, internal standard) was added before the esterification with 0.2 N sodium-methanol during 30 minutes at 40 °C and then with H_2SO_4 methanol as described for alkaline methylation. After the sample was cooled, the fatty acid methyl esters were extracted with 0.5 mL hexane.

Fatty acid methyl esters (FAMEs) of all samples were analyzed by gas-liquid chromatography. A Hewlett-Packard gas chromatograph (model 6890; Hewlett-Packard Co., Palo Alto, CA) equipped with a capillary column (50-m × 0.22-mm BPX70; 0.25U QC 0.08 SGE) was used to separate FAMEs. The temperature was programmed from 180 °C to 230 °C at 2 °C/min with a final hold, separating 12:0 to 22:6,n-3. The temperature of both detector and injector was 240 °C. Hydrogen was used as carrier gas, at a flow rate of 1.5 mL/min and split ratio of 1:80. The FAMEs were identified by comparison of their retention times with those of individual purified standards and quantified using a Hewlett-Packard integrator (HP 3396 Series III) (24).

Statistical Analysis

Results are expressed as means ± SD for the number of patients indicated. Statistical analysis of the differences between mean values was assessed by the nonparametric Mann-Whitney *U* test. The differences were considered statistically significant at $p < 0.05$. To analyze the association between different variables, the Spearman rank order correlation coefficient was used. All statistical analyses were computed using GraphPad Prism version 2.0 (GraphPad Software, San Diego, CA).

Results

The characteristics of the studied patients are shown in Table 1. Patients with NAFLD were significantly more obese than control subjects, as shown by their BMI being 92% higher than controls. Fasting blood glucose and insulin levels were significantly higher in patients with NAFLD compared with control values, with increased insulin resistance being established in the obese group as determined by the 4.5-fold ($p < 0.05$) enhancement in the HOMA index. Both plasma lipid levels (total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and triacylglycerols) and alanine aminotransferase activity in serum were within normal ranges in the studied groups; four patients with NAFLD exhibited serum aspartate aminotransferase levels >40 IU/L (range, 57 to 254 IU/L). Obese patients exhibited serum levels of F_2-isoprostanes 84% ($p < 0.05$) higher than those of the control group (Figure 1).

The hepatic composition of total LCPUFAs in obese patients with steatosis or steatohepatitis [steatosis, 31.2 ± 4.9 ($n = 6$) g/100 g of FAME; steatohepatitis, 31.0 ± 2.8 g/100 g of FAME ($n = 6$)] and that of total n-6 LCPUFA [steatosis, 20.5 ± 3.5 g/100 g of FAME ($n = 6$); steatohepatitis, 18.7 ± 4.8 g/100 g of FAME ($n = 6$)] and n-3 LCPUFA [steatosis, 8.79 ± 2.65 g/100 g of FAME ($n = 6$); steatohepatitis, 10.7 ± 3.4 g/100 g of FAME ($n = 6$)] were comparable, in agreement with earlier studies (5). Thus, all obese patients studied were included in a single group. Data presented in Table 2 show no significant differences in 14:0, 14:1n-7, 16:0, 16:1n-7, 18:1n-9, 18:3n-3, 20:0, and 22:0 in obese patients compared with controls, whereas 18:0 and 22:5n-6 (DPAn-6) were significantly higher by 71% and 350%, respectively. In addition, levels of 18:2n-6, 20:4n-6, 20:5n-3, 22:5n-3, and 22:6n-3 were significantly lower by 46%, 48%, 58%, 52%, and 70% in obese patients over controls, respectively (Table 2). These data represent no significant differences in total monounsaturated FAs and total n-6 LCPUFAs in the studied groups; however, total saturated FA content was 33% higher and that of total n-3 LCPUFAs was 63% lower in obese patients than controls. The above changes represent a significant net 40% diminution in the levels of total PUFAs and LCPUFAs of liver phospholipids from obese patients over control values, with a 2.4-fold increase in the n-6/n-3 LCPUFA ratio (Table 2).

Table 1. Clinical and biochemical variables for control subjects and obese patients with NAFLD

Parameters (normal range)	Controls ($n = 8$)	NAFLD patients ($n = 12$)
Age (years)	44 ± 11	41 ± 10
BMI (<25 kg/m^2)	22.8 ± 1.7	43.8 ± 8.3*
Fasting glucose (<100 mg/dL)	87 ± 14	104 ± 13*
Fasting insulin (4 to 25 μU/mL)	10 ± 5	37 ± 34*
HOMA index	2.1 ± 0.6	9.5 ± 10*
Total cholesterol (<200 mg/dL)	186 ± 42	218 ± 38
High-density lipoprotein-cholesterol (>40 mg/dL)	48 ± 42	44 ± 7
Low-density lipoprotein-cholesterol (<130 mg/dL)	98 ± 78	137 ± 34
Triacylglycerol (<150 mg/dL)	128 ± 115	167 ± 62
AST (<40 IU/liter)	25 ± 8	69 ± 96
ALT (<50 IU/liter)	24 ± 8	42 ± 34

Values represent means ± SD for the number of subjects indicated.
* $p < 0.05$ compared with controls, assessed by the Mann-Whitney U test.

The fatty acid composition of erythrocyte phospholipids (Table 3) was comparable in 14:0, 14:1n-7, 16:0, 16:1n-7, 18:0, and 20:5n-3 in control and obese patients, whereas 18:1n-9, 18:2n-6, 18:3n-3, 20:0, 22:0, and 22:5n-6 levels were higher in obese patients, and those of 20:4n-6, 22:5n-3, and 22:6n-3 were lower ($p < 0.05$), compared with controls. The above data represent no significant differences in total saturated FA and total n-6 LCPUFAs between controls and obese patients; however, total monounsaturated FA was 69% higher and total n-3 LCPUFA was 50% lower in obese patients over control values (Table 3). As in the case of liver phospholipids, erythrocyte phospholipids of obese patients exhibited total PUFA and LCPUFA levels 22% and 28% lower, respectively, and n-6 LCPUFA/n-3 LCPUFA ratios 105% higher, than control values (Table 3).

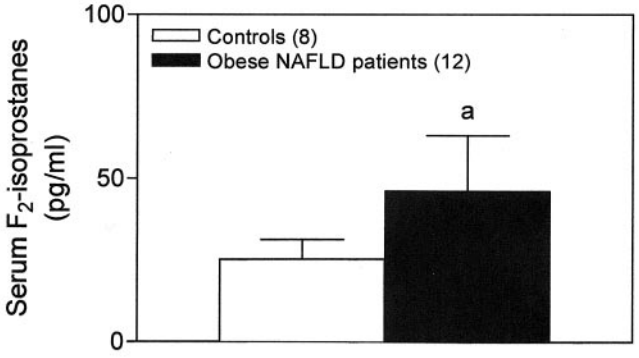

Figure 1: Serum F_2-isoprostanes levels in control subjects and obese patients with NAFLD. Values represent means ± SD for the number of patients indicated in parentheses. Values in obese patients with steatosis (48.5 ± 17.5 pg/mL; $n = 6$) and steatohepatitis (42.8 ± 16.5 pg/mL; $n = 6$) were comparable. [a]$p < 0.05$ compared with controls assessed by the Mann-Whitney U test.

The content of DHA and DPAn-6 in liver phospholipids (Table 2) was comparable to that in erythrocyte phospholipids (Table 3), in both control subjects and obese patients. Association analyses revealed significant correlations in DHA content between liver and erythrocyte phospholipids ($r = 0.77$; $p < 0.0001$), as well as in DPAn-6 levels ($r = 0.90$; $p < 0.0001$), from controls and obese patients with NAFLD. Furthermore, both liver phospholipids ($r = -0.79$, $p < 0.0001$) and erythrocyte phospholipids ($r = -0.58$; $p < 0.01$) from control subjects and obese patients showed significant inverse correlations between DHA and DPAn-6 contents, respectively, with respective DHA/DPAn-6 ratios of obese patients 90% ($p < 0.05$) lower than those of the control group (Figure 2).

Discussion

Obese patients in this study, showing marked insulin resistance as assessed by the HOMA index and higher oxidative stress status as evidenced by the elevated levels of serum F_2-isoprostanes, exhibit significant depletion of total LCPUFAs in liver phospholipids, in agreement with earlier observations (5). Oxidative stress in NAFLD (1–3,10) may be caused either by enhanced mitochondrial production of reactive oxygen species on triacylglycerol accumulation in liver steatosis (25) or to that coupled with cytochrome P4502E1 induction (2,10,26) and leukocyte infiltration of the liver in steatohepatitis (27). Liver LCPUFA depletion is

Table 2. Fatty acid composition of liver phospholipids in control subjects and obese patients with NAFLD

Fatty acid (g/100 g of FAME)	Controls ($n = 8$)	NAFLD patients ($n = 12$)
14:0	0.68 ± 0.42	1.31 ± 0.96
14:1n-7	0.32 ± 0.45	0.88 ± 1.07
16:0	13.1 ± 4.7	16.4 ± 2.4
16:1n-7	3.72 ± 0.22	4.68 ± 3.30
18:0	9.00 ± 4.2	15.4 ± 2.8*
18:1n-9	8.26 ± 2.05	10.9 ± 2.8
18:2n-6	1.70 ± 1.18	0.92 ± 0.48*
18:3n-3	1.05 ± 0.90	0.81 ± 0.38
20:0	1.96 ± 1.55	2.45 ± 1.41
20:4n-6	20.4 ± 0.6	10.6 ± 2,0*
20:5n-3	4.80 ± 2.50	2.04 ± 0.79*
22:0	2.94 ± 2.11	1.80 ± 1.0
22:5n-6 (DPAn-6)	2.02 ± 0.28	9.07 ± 5.08*
22:5n-3	6.53 ± 1.33	3.14 ± 2.21*
22:6n-3 (DHA)	15.1 ± 1.9	4.58 ± 2.21*
Total SAFA	31.0 ± 5.3	41.1 ± 3.21*
Total MUFA	19.3 ± 3.0	22.8 ± 5.2
Total PUFA	51.6 ± 5.9	31.1 ± 4.8*
Total LCPUFA	48.9 ± 4.6	29.4 ± 4.01*
Total n-6 LCPUFA	22.4 ± 0.6	19.6 ± 4.7
Total n-3 LCPUFA	26.4 ± 4.8	9.76 ± 3.66*
n-6 LCPUFA/n-3 LCPUFA ratio	0.84 ± 0.14	2.00 ± 1.45*

Values represent means ± SD for the number of patients indicated in parentheses.
* $p < 0.05$ compared with controls, assessed by the Mann-Whitney U test.

particularly evident for the n-3 LCPUFA DHA, which is paralleled by a substantial enhancement 1) in the n-6/n-3 LCPUFA ratio and 2) in the content of DPAn-6. These obesity-related changes in hepatic FA pattern are of major importance because they represent signals able to promote pathogenic responses (28). First, liver n-3 LCPUFA depletion may favor the development of steatosis by up-regulating lipogenic genes and down-regulating those for FA oxidation, caused by derangement in the activation of peroxisome proliferator-activated receptor α and/or increased availability of sterol response element binding protein 1c, which are expected to occur under conditions of low levels of 20:5n-3, 22:5n-3, and 22:6n-3 and/or their oxidized derivatives (6,8). Second, eicosanoid metabolic active products from n-6 LCPUFAs are formed in larger amounts than those from n-3 LCPUFAs (28), particularly under conditions of high n-6/n-3 LCPUFA ratios (15), with a consequent overproduction of prostaglandins and leukotrienes that enhance inflammation. Interestingly, DHA depletion and enhancement in the n-6/n-3 LCPUFA ratio is accompanied by significantly higher levels of DPAn-6 in the liver of obese patients compared with controls. It is well known that the liver does not synthesize significant amounts of DPAn-6, except under conditions of n-3 FA deficiency (29), as observed in experimental animals (11,14,16) and human infants (12,30). This inverse interrelationship between DHA and DPAn-6 levels could be explained in terms of induction of a microsomal n-6-specific Δ^4 desaturase catalyzing the hepatic biosynthesis of DPAn-6 from 22:4n-6 caused by the low levels of DHA, which normally represses the enzyme (13). However, this proposal has not received experimental support; it is generally accepted that DHA and DPAn-6 biosynthesis requires the formation of the respective 24-carbon PUFA (24:6n-3 and 24:5n-6) followed by partial peroxisomal oxidation, whose regulation remains to be established (31). Although enhancement in DPAn-6 in DHA depletion may represent a compensatory mechanism of FA homeostasis to maintain polyunsaturation of phospholipids for adequate membrane fluidity in n-3 FA deficiency (11,16,32), it does not seem to be completely oper-

Table 3. Fatty acid composition of erythrocyte phospholipids in control subjects and obese patients with NAFLD

Fatty acid (g/100 g of FAME)	Controls ($n = 8$)	NAFLD patients ($n = 12$)
14:0	0.88 ± 0.39	1.15 ± 1.28
14:1n-7	1.08 ± 0.28	2.00 ± 1.52
16:0	16.8 ± 2.5	13.9 ± 3.5
16:1n-7	2.27 ± 0.53	2.94 ± 1.04
18:0	19.5 ± 3.86	15.4 ± 3.8
18:1n-9	7.81 ± 1.24	10.1 ± 2.1*
18:2n-6	0.85 ± 0.34	2.29 ± 1.41*
18:3n-3	0.58 ± 0.28	1.49 ± 0.90*
20:0	1.24 ± 1.04	2.86 ± 1.66*
20:4n-6	17.2 ± 1.4	11.6 ± 3.5*
20:5n-3	2.61 ± 1.55	2.87 ± 1.56
22:0	1.32 ± 0.73	2.71 ± 1.70*
22:5n-6 (DPAn-6)	1.75 ± 0.59	7.75 ± 3.52*
22:5n-3	7.12 ± 2.26	2.16 ± 1.66*
22:6n-3 (DHA)	15.2 ± 2.3	7.37 ± 3.18*
Total SAFA	42.1 ± 4.93	40.4 ± 4.5
Total MUFA	14.3 ± 1.7	24.1 ± 5.4*
Total PUFA	45.3 ± 5.1	35.5 ± 3.5*
Total LCPUFA	43.9 ± 5.0	31.7 ± 5.5*
Total n-6 LCPUFA	19.0 ± 1.4	19.3 ± 5.67
Total n-3 LCPUFA	25.0 ± 3.9	12.4 ± 3.97*
n-6 LCPUFA/n-3 LCPUFA ratio	0.76 ± 0.11	1.56 ± 1.48*

Values represent means ± SD for the number of patients indicated in parentheses.
* $p < 0.05$ compared with controls, assessed by the Mann-Whitney U test.

ative in obesity, as shown by the lower levels of total PUFAs observed in the liver and erythrocytes of obese patients compared with control values. Thus, it remains to be determined whether additional factors regulating the intracellular metabolism of highly unsaturated n-3 and n-6 FAs play a role in the changes in PUFA composition found in obese patients. These may include 1) the possible involvement of separate chain-length-specific Δ^6 desaturases (31), 2) changes in the partial degradation–resynthesis cycles for 20-, 22-, and 24-carbon PUFAs (31), and/or 3) changes in the expression and activity of Δ^6 and Δ^5 desaturases (9) under conditions of insulin resistance and liver oxidative stress underlying obesity.

Similar changes in the composition of LCPUFAs found in liver phospholipids were observed in erythrocyte phospholipids from obese patients over control values. These include 1) diminution in 20:4n-6, 22:5n-3, 22:6n-3 (DHA), total LCPUFAs, and total n-3 LCPUFAs, 2) enhancement in 22:5n-6 (DPAn-6) and in the n-6/n-3 LCPUFA ratio, and 3) comparable levels of total n-6 LCPUFA. This is particularly

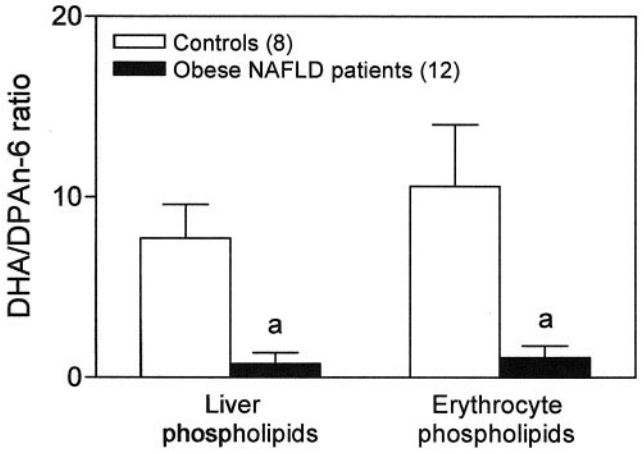

Figure 2: DHA/DPAn-6 ratios in liver and erythrocyte phospholipids in control subjects and obese patients with NAFLD. Values represent means ± SD for the number of patients indicated in parentheses. [a]$p < 0.05$ compared with controls assessed by the Mann-Whitney U test.

relevant for DHA and DPAn-6, whose levels in liver phospholipids exhibit significant correlations with those in erythrocyte phospholipids, whereas DHA and DPAn-6 show significant inverse associations in both liver and erythrocytes, with marked decreases in the DHA/DPAn-6 ratios. The above correlations are in agreement with previous reports showing significant associations in the percentage of 20:4n-6, 22:5 n-3, and 22:5n-6 from erythrocyte and skeletal muscle membranes in healthy men (33) and in DHA, n-3 PUFA, and n-6/n-3 PUFA ratios in young children (34). A similarity in the pattern of PUFA in brain and erythrocyte membranes of rhesus monkeys was reported under conditions of n-3 FA deficiency, which was completely corrected on dietary fish oil feeding (11). Collectively, data presented indicate that obese patients with NAFLD exhibit drastic alterations in the PUFA pattern of the liver, characterized by significant n-3 LCPUFA depletion, particularly DHA, which is partially compensated by DPAn-6 enhancement, with increase in the n-6/n-3 LCPUFA ratio. These changes are of major importance because they favor partitioning of hepatic FA metabolism from oxidation and secretion into lipogenesis, thereby contributing to steatosis. The underlying mechanisms may involve defective PUFA desaturation and higher LCPUFA peroxidation caused by oxidative stress, features that are related to insulin resistance. In addition, the correlations established between liver and erythrocyte phospholipid PUFA composition would validate the measures in erythrocytes as a reliable biomarker of derangements in liver lipid metabolism in obese patients. The usefulness of this proposal is further supported by the finding that erythrocyte FA composition is not modified on long storage when using low temperatures and antioxidants in the presence of N_2 (35).

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