

Abnormalities in the serum phospholipids fatty acid profile in patients with alcoholic liver cirrhosis - a pilot study

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The fatty acid composition of serum phospholipids were analyzed in 20 patients with alcoholic liver cirrhosis (11 with malnutrition and 9 with acceptable nutritional status); 25 healthy age and sex-matched adults were used as controls. Cirrhotic patients showed higher levels of palmitic acid and total saturated fatty acids than healthy subjects. Total *n*-6 and *n*-3 polyunsaturated fatty acids (PUFA), and levels of linoleic, dihomogamma linolenic, arachidonic, and docosahexaenoic acid were significantly lower ($p < 0.001$) in patients with alcoholic cirrhosis compared to healthy controls. Significant changes were also found between patients stratified according to nutritional status. In particular, the sum of *n*-3 PUFA was significantly lower ($p < 0.001$) and ratio of *n*-6/*n*-3 fatty acids was higher ($p < 0.01$) in malnourished patients when compared to the patients with acceptable nutritional status. Furthermore, important changes in the levels of saturated fatty acids, palmitoleic and oleic acid and long-chain PUFA were found in well-nourished patients with alcoholic cirrhosis as well. Our present data confirmed evidence that malnutrition is one of the factors that led to lower levels of polyunsaturated fatty acids in patients with alcoholic liver cirrhosis. PUFA supplementation in the latter needs further investigation.

Key Words: phospholipids fatty acid, alcohol, cirrhosis, malnutrition

Disturbances in the fatty acid (FA) metabolism have been reported in patients with cirrhosis.⁽¹⁻³⁾ They were often associated with protein energy malnutrition^(4,5) and the occurrence of encephalopathy.⁽⁶⁾ It has been found^(1,7-10) that chronic alcoholism can be associated with both essential fatty acid (EFA) and particularly long chain polyunsaturated fatty acid (LC-PUFA) deficiency in plasma, liver and adipose tissue. Cabre *et al.*⁽¹¹⁾ suggested that other factors, such as malnutrition and liver damage, probably override the effects of alcohol consumption on PUFA metabolism in patients with cirrhosis.

PUFA are essential not only because of their nutritional value, but also because they are a major component of the membrane structure, which could be modified by alcoholism.^(12,13) Furthermore, alcohol inhibits phospholipase activity.⁽¹⁴⁾ The importance of phospholipids (PL) for the structure and integrity of cellular membranes suggests that many functional disturbances in liver cirrhosis may be related to changes in phospholipids FA composition.^(12,13,15) It is known that the FA profile of serum phospholipids reflects the FA composition of cell membranes.⁽¹⁶⁾ LC-PUFA status results from intake, but also from the desaturation and elongation of EFA. This process requires an adequate nutritional status and normal liver function.⁽¹⁷⁾ Therefore, excessive alcohol consumption can be related to alterations in the liver PUFA metabolism.⁽⁹⁾

Regardless of etiology, cirrhosis was found to be associated with protein energy-malnutrition in more than 30% of the tested patients, particularly when a severe liver failure was present.^(18,19) Many factors are involved in the mechanisms of malnutrition, such as inadequate diet, impaired digestion and absorption, increased energy requirements, accelerated protein breakdown and inefficient protein synthesis.^(20,21) Irrespective of the pathophysiological mechanisms, malnutrition was found to have a negative prognostic value in patients with chronic liver disease.^(22,23)

Diet clearly plays an important role in liver pathology associated with chronic alcohol consumption.^(24,25) Combination of low protein and carbohydrate intake with high fat consumption modifies multiplicatively the risk of cirrhosis related to alcohol abuse.⁽²⁶⁾ In alcoholic patients, the quality of dietary fat may be important for the structure, physiological and biochemical recovery of the cirrhotic liver.^(10,13,15) The previous reports showed that Serbian population consumed a constant amount of alcohol throughout their lives, along with an energy-dense diet, which is high in saturated fat and sugars and low in complex carbohydrates and polyunsaturated fats.^(27,28)

The aim of this pilot study was to determine phospholipids FA composition in well-nourished and malnourished patients with alcoholic liver cirrhosis. These groups were compared mutual and to healthy subjects in order to assess whether disturbances in FA profiles in cirrhosis were a result of the cirrhotic process or of a patient's nutritional status.

Materials and Methods

Patients. We cross-sectionally evaluated 96 patients with liver cirrhosis from the Department of Gastroenterology, University Hospital "Dr Dragiša Mišović-Dedinje" (Belgrade). For this study we included cohort group of 20 patients (18 male, 2 female; mean age 62 ± 8 years old, range 46–72 years old) with alcoholic etiology of liver cirrhosis. Diagnosis of cirrhosis was based on clinical, biochemical and/or histological data. The severity of cirrhosis was assessed using Child-Pugh classification, which assigns an arbitrary score to plasma concentration of bilirubin, albumin and prothrombin time, and to the presence of ascites and hepatic encephalopathy.⁽²⁹⁾ All patients had a history of high alcohol intake (more than 150 g/day) for at least 5 years, tested negative for hepatitis B and C, and had elevated IgA and serum GGT levels. They were all in a stable clinical condition without acute complication of cirrhosis (hepatic encephalopathy, sepsis, gastro-intestinal bleeding). Patients were enrolled in the study between hospital days 4 and 7. No clinical or laboratory evidence

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of renal, cardiac, pulmonary or endocrine disease was found in the patients. None of them were taking lipid-lowering drugs for at least 6 months. All patients had low habitual consumption of soy and fish (one meal in two weeks), and no supplementation of oils rich in long chain fatty acids (fish, sesame or linseed oil). This was determined by a diet assessment made at the time of recruitment.

Our control group consisted of 29 well-nourished healthy volunteers (26 males, 3 female, mean age 61 ± 7 , range 45–70), matched by gender and age with the patients. Their routine laboratory values were in normal range. All control subjects were non-drinkers and were matched with the patient group based on their smoking habits and physical activity. The controls maintained a constant body weight (± 2 kg) for at least 6 months preceding our study.

All study participants signed an informed consent document. Study protocol was approved by the Institute's Ethical Committee and was conducted in line with the principles of the Declaration of Helsinki of the World Medical Association.

Nutritional status and dietary intake. Body weight was measured to the nearest 0.1 kg by a beam scale with the subject wearing light clothes and without shoes. Height was measured using a wall-mounted stadiometer. Body mass index (BMI) was imputed as the ratio between body weight (kg) and square height (m^2). Mid-upper arm circumferences were measured using a flexible steel tape to the nearest 0.1 mm. Measurements were taken midway between the tip of the acromion and olecranon process. Triceps skinfold (TSF) thickness was measured using a Harpenden skin fold caliper (John Bull British Indicators Ltd., Burgess Hill, England) to the pressure of 10 g/mm^3 of contact surface area to the nearest 0.2 mm. Mid-arm muscle circumference (MAMC) was calculated according to the equation $MAMC \text{ (cm)} = MAC \text{ (cm)} - \pi \times TSF \text{ (cm)}$. Mid-arm muscle area (MAMA) was calculated as $MAMA \text{ (cm}^2\text{)} = (MAC - \pi \times TSF)^2 / 4\pi$ and mid-arm fat area (MAFA) as $MAFA \text{ (cm}^2\text{)} = (TSF \times MAC)^2 - (\pi \times TSF^2) / 4$.^(30,31) Values of anthropometric parameters (TSF, MAMC, MAMA, MAFA) were compared with those of healthy subjects. Patients were considered malnourished when one or more of the anthropometric parameters were below the fifth percentile of an acceptable nutritional status.⁽³⁰⁾

We subdivided patients with alcoholic liver cirrhosis into two groups according to the presence of malnutrition. Based on their anthropometric measurements, 9 cirrhotic patients were classified as malnourished (MN) and 11 as having an adequate nutritional status (AN). These methods have proved reliable in the identification of malnutrition.^(23,31) To estimate energy and macronutrient intake, subjects were asked to complete a 3-day diary (2 week days 1 day of the weekend), which the dietitian carefully analyzed, taking into account calories deriving from alcohol consumption. Through this we were able to reproduce the usual eating pattern of our subjects.

Biochemical determination. Serum samples were prepared by 4°C centrifugation of venous blood collected after a 12–14 h fast. Total cholesterol and triglyceride levels were measured spectrophotometrically using a colorimetric enzymatic reaction kits (Eli Tech Diagnostic, Sées-France). Serum albumin concentration was determined by using a bromocresolgreen reagent (Eli Tech Diagnostic, Sées-France). The serum direct bilirubin concentration was determined with diazotized sulfanilic acid (BioSystems S.A., Barcelona, Spain). The assays were performed in triplicate on each sample and the total cholesterol and triglyceride levels were expressed as mM serum. Total serum phospholipids were determined by the Zilversmit and Davis method.⁽³²⁾ Serum lipids were extracted according to the method of Sperry and Brand⁽³³⁾ which uses chloroform-methanol mixture (2:1 v/v) with 10 mg/100 ml 2,6-di-*tert*-butyl-4-methylphenol (BHT) added as an antioxidant. The phospholipids fraction was isolated from the extracted serum lipids by one-dimensional thin-layer chromatography (TLC) in a neutral lipid solvent system hexane-diethyl

ether-acetic acid (87:12:1, v/v/v) using Silica Gel GF plates (C. Merck, Darmstadt, Germany).

Fatty acid determination. Methyl esters of phospholipids FA were prepared by methods that have already been reported.⁽³⁴⁾ Fatty acid methyl esters derivatives were then analyzed by gas chromatography using Varian GC (Model 3400, Varian Associates) equipped with DB-23 ($30 \text{ m} \times 0.53 \text{ mm i.d.}$, film thickness 0.5 μm , J&W Scientific Inc Bellefonte, Folsona, CA) fused silica capillary column. Analysis was performed in duplicate for each sample. Individual FA methyl esters were identified by comparing peak retention times with authentic standards (Sigma Chemical Company, St. Louis, MO) and/or the PUFA-2 standard mixtures (Supelco Inc., Bellefonte, PA). Varian 4290 integrator was used for the FA quantification, and results were expressed as the relative percentage of the total fatty acids.

Statistical analysis. All the results were expressed as means \pm SD. Normality was tested using the Shapiro-Wilk's test. The Student *t* test was used to compare the normally distributed variables, and Mann-Whitney *U* test for non-normally distributed variables. The differences were considered significant at $p < 0.05$. The SPSS 10.0 program for Windows (Chicago, IL) was used for statistical analysis.

Results

The key clinical, anthropometric and lipid parameters of alcoholic cirrhotic patients (all, AN and MN) and control subjects are presented in Table 1. The group of patients had significantly lower BMI ($p < 0.001$) when compared with the control group. There was no significant difference in anthropometric parameters between well-nourished patients with cirrhosis and healthy controls. Malnourished patients were younger than those with a satisfactory nutritional status (55 ± 6 vs 67 ± 3). As expected, BMI in malnourished cirrhotic patients was lower than in nourished patients and control subjects ($p < 0.001$). Seven patients were severely malnourished, their MAMC and/or TSF levels were below the 5th percentile, and 2 patients were moderately malnourished (MAMC and/or TSF < 10th percentile). In malnourished cirrhotic patients, a significant reduction in fat mass was observed ($p < 0.001$). In the last final week before hospitalization, patients consumed an average of 2276 ± 164 kcal/day, consisting of around 11% protein, 36% lipids (*n*-6 7.3% and *n*-3 0.6%), and 54% carbohydrates. In AN and MN groups daily energy intake of *n*-6 fatty acids was similar (7.06 ± 1.09 and 7.69 ± 1.02 , respectively). However, daily energy intake of *n*-3 fatty acids was significantly ($p < 0.01$) lower in MN patients when compared to AN group (0.52 ± 0.11 vs 0.66 ± 0.18). The ratio of polyunsaturated to saturated fats (PUFA/SFA) was 0.55 ± 0.07 in all patients and *n*-6/*n*-3 ratio was 15:1 in the MN group, but 10.7:1 in the AN group, similar as in the control group. The average alcohol intake was 229 ± 31 g/day (Table 1). Daily caloric intake was 31.8 ± 3.1 kcal/kgBW and 31.3 ± 3.9 kcal/kgBW, and protein intake was 0.91 ± 0.11 g/kgBW and 0.70 ± 0.10 g/kgBW ($p < 0.001$) in well-nourished and malnourished patients, respectively. No significant differences were found in serum total cholesterol between patients with alcoholic cirrhosis and the healthy controls, but patients with malnutrition had significantly lower ($p < 0.001$) concentration of serum cholesterol than well-nourished patients. Serum triglycerides levels were significantly higher, and PL level was lower ($p < 0.001$) in patients with alcoholic cirrhosis than in the control subjects. Higher levels of serum triglycerides and lower PL concentration were observed in the MN than in the AN group.

Serum phospholipids FA compositions of alcoholic cirrhotic patients and of healthy controls are shown in Table 2. The amount of saturated fatty acid (SFA) was significantly higher in the patients than in the control subjects. This increase was due to higher levels of palmitic acid ($p < 0.001$) in spite of lower levels ($p < 0.01$) of stearic acid. The proportions of palmitoleic acid,

Table 1. Clinical, anthropometric, lipid parameters and dietary intake of patients with alcoholic liver cirrhosis and healthy controls

	Healthy controls	Patients with cirrhosis (AN + MN)	Patients with adequate nutrition status (AN)	Malnutrition patients (MN)
<i>n</i> (M/F)	29 (26/3)	20 (18/2)	11 (9/2)	9(9/0)
Age (years old)	61 ± 7	62 ± 8	67 ± 3**	55 ± 6* ###
Child Pugh A/B/C	-	11/9/0	9/2/0	3/6/0
Body mass index (BMI)	24.00 ± 0.93	22.66 ± 1.16***	23.47 ± 0.70	21.67 ± 0.76*** ###
MAC (cm)	31.11 ± 0.80	28.92 ± 2.62***	31.14 ± 0.84	26.20 ± 0.67*** ###
TSF (cm)	20.87 ± 0.25	15.46 ± 0.61***	20.44 ± 3.00	9.38 ± 0.88*** ###
MACA (cm)	24.56 ± 1.05	24.07 ± 1.32	24.72 ± 1.44	23.28 ± 0.53*** #
MAMA (cm ²)	48.12 ± 4.05	46.26 ± 5.14	48.79 ± 5.59	43.16 ± 1.98*** #
MAFA (cm ²)	29.02 ± 3.29	20.87 ± 8.99***	28.45 ± 3.46	11.62 ± 1.27*** ###
Serum Lipids				
Triglycerides (mmol/l)	1.50 ± 0.12	1.78 ± 0.26***	1.67 ± 0.26**	1.91 ± 0.18*** #
Total cholesterol (mmol/l)	4.66 ± 0.24	4.79 ± 0.64	5.27 ± 0.43***	4.21 ± 0.24*** ###
Total phospholipids (mmol/l)	3.01 ± 0.46	2.36 ± 0.35***	2.53 ± 0.34**	2.19 ± 0.28*** #
Dietary intake				
Energy (kcal/d)	2253 ± 107	2276 ± 164	2374 ± 99	2160 ± 156##
Protein (% en)	16.59 ± 2.11	10.71 ± 1.42***	11.81 ± 0.71***	9.40 ± 0.55*** ###
Carbohydrates (% en)	52.63 ± 1.71	53.58 ± 3.46	55.48 ± 3.61**	52.05 ± 2.53#
Total fat (% en)	30.78 ± 1.27	35.97 ± 2.79***	36.47 ± 1.88***	35.26 ± 3.58***
Saturated fat (% en)	10.17 ± 0.58	14.32 ± 1.03***	14.57 ± 1.29***	14.12 ± 2.14***
Monounsaturated fat (% en)	12.68 ± 0.96	13.71 ± 2.04**	14.18 ± 2.14**	12.93 ± 2.39
Polyunsaturated fat (% en)	7.93 ± 0.61	7.94 ± 0.84	7.72 ± 1.12	8.21 ± 1.03
<i>n</i> -6 (% en)	7.22 ± 0.59	7.32 ± 0.92	7.06 ± 1.09	7.69 ± 1.02
<i>n</i> -3 (% en)	0.71 ± 0.18	0.62 ± 0.11	0.66 ± 0.18	0.52 ± 0.11**
PUFA/SFA	0.78 ± 0.11	0.55 ± 0.07***	0.53 ± 0.07***	0.59 ± 0.05*** #
<i>n</i> -6/ <i>n</i> -3	10.17 ± 0.80	12.18 ± 2.55***	10.67 ± 2.32	14.79 ± 2.78*** ##
Energy kcal/BW	29.2 ± 3.4	31.6 ± 3.4	31.8 ± 3.1	31.3 ± 3.9
Protein kg/BW	1.29 ± 0.15	0.81 ± 0.15***	0.91 ± 0.11***	0.70 ± 0.10*** ###
Alcohol intake (g/d)	0	229 ± 31	221 ± 25	239 ± 35

MAC, Mid-upper arm circumference; MAMC, Mid-arm muscle circumference; MAMA, Mid-arm muscle area; MAFA, mid-arm fat area; TSF, Triceps skinfold; BW, body weight; Child Pugh A/B/C, arbitrary score, classification of the severity liver disease. Values are means ± SD. **p*<0.05, ***p*<0.01, ****p*<0.001 vs healthy controls; #*p*<0.05, ##*p*<0.01, ###*p*<0.001 vs alcoholic cirrhosis patients well nourished.

oleic acid, and total monounsaturated fatty acid (MUFA) were significantly higher (*p*<0.001) in patients than in healthy controls. Alcoholic cirrhotic patients also showed lower levels of linoleic (LA), dihomo-gamma linoleic (DGLA) and arachidonic acid (AA) and higher levels of docosahexaenoic acid (22:4) (*p*<0.001). Alfa-linolenic (ALA; 18:3*n*-3), which is a precursor of the *n*-3 fatty acids, tended to be lower in alcoholic cirrhotic patients. Its metabolites eicosapentaenoic (EPA), docosapentaenoic and docosahexaenoic acid (DHA) were significantly lower (*p*<0.001) in alcoholic patients than in the control group (Table 2). Consistent with this, *n*-3, *n*-6 and total PUFA levels were lower (*p*<0.001) in patients with alcoholic cirrhosis. There was not a significant difference in the ratio of *n*-6/*n*-3 FA between the patients and controls, but the PUFA/SFA ratio was lower (*p*<0.001) in cirrhotic patients than in the healthy subjects.

The differences in FA profiles between AN and MN patients are also shown in Table 2. SFA levels in serum phospholipids were unaffected in alcoholic cirrhotic patients in relation to their malnutrition. However, malnourished patients with alcoholic liver cirrhosis had higher values of MUFA than patients with an acceptable nutritional status (*p*<0.01). LA *n*-6 and AA *n*-6 were markedly decreased in malnutrition (*p*<0.001, *p*<0.05). All *n*-3 PUFA were significantly lower in MN than in the AN group (*p*<0.001). The *n*-6/*n*-3 ratio was significantly higher in malnourished patients than in the patients with an acceptable nutritional status (*p*<0.001), who even had a lower ratio than the control group. In addition, malnutrition lowered the PUFA/SFA ratio, from 0.83 ± 0.14 to 0.54 ± 0.08 (*p*<0.001).

Discussion

This study focused on the effect of the nutritional status on the composition of serum phospholipids in alcohol liver cirrhosis. It has been previously observed that in patients with cirrhosis the degree of liver function impairment and survival rates correlate with their nutritional status.^(18,22,23,35) For this reason, we categorized the nutritional status of our patients according to their anthropometric parameters. Narayanan *et al.*⁽³⁶⁾ demonstrated that body fat mass is more affected in patients with non-alcoholic cirrhosis and muscle mass in patients with alcoholic cirrhosis. However, in our study 9 malnourished patients had a significant reduction in body fat mass. It is probably not induced by poor dietary intake. Namely, well-compensated cirrhotic patients need about 30 kcal/kgBW,⁽¹⁹⁾ and in our study, the caloric intake was 32 ± 3 kcal/kgBW and 31 ± 4 kcal/kgBW in the well-nourished and malnourished patients. Depending on the clinical condition of the cirrhotic patients, the recommended daily protein intake is 1.0 to 1.5 g/kgBW.⁽³⁷⁾ Malnutrition cirrhotic patients in our study had lower protein intake (0.70 ± 0.10 g/kgBW) compared to well nourished patients (0.91 ± 0.11 g/kgBW). Protein intake is important because a protein-deficient diet decrease PUFA biosynthesis.⁽⁴³⁾ It has been shown on animal models⁽³⁸⁾ that low-protein intake lowered plasma and liver DHA status. In addition to different *n*-6/*n*-3 intake, it could contribute to marked differences in this ratio in serum phospholipids between the well-nourished and malnourished patients in our study.

Lower levels of total serum cholesterol and PL in malnourished patients than in healthy subjects found in our study are in agree-

Table 2. Fatty acid composition of serum phospholipids in patients with alcoholic cirrhosis and healthy controls

	Healthy controls	Patients with cirrhosis (AN + MN)	Patients with adequate nutrition status (AN)	Malnutrition patients (MN)
C16:0 palmitic acid	26.53 ± 2.44	30.76 ± 4.75***	31.12 ± 5.92**	30.32 ± 3.05***
C18:0 stearic acid	16.14 ± 2.41	14.07 ± 2.72**	14.80 ± 3.19	13.18 ± 1.79**
SFA saturated FA	41.97 ± 2.82	45.85 ± 7.03*	46.33 ± 7.26*	43.97 ± 2.11
C16:1 <i>n</i> -7 palmitoleic acid	0.29 ± 0.16	1.55 ± 0.54***	1.13 ± 0.37***	1.93 ± 0.35*** ##
C18:1 <i>n</i> -9 oleic acid	11.71 ± 1.44	18.83 ± 4.54***	16.08 ± 4.16***	22.19 ± 2.06*** ##
MUFA monounsaturated FA	12.59 ± 1.69	20.39 ± 5.19***	17.32 ± 4.48***	24.01 ± 2.33*** ##
C18:2 <i>n</i> -6 linoleic acid	27.02 ± 3.08	17.81 ± 3.76***	20.40 ± 2.51**	14.65 ± 2.29*** ##
C20:3 <i>n</i> -6 dihomo- γ -linolenic acid	2.43 ± 0.72	1.58 ± 0.46***	1.54 ± 0.33**	1.63 ± 0.59*
C20:4 <i>n</i> -6 arachidonic acid	11.56 ± 2.32	6.61 ± 1.75***	7.28 ± 1.90***	5.62 ± 1.12*** #
C22:4 <i>n</i> -6 docosatetraenoic acid	0.39 ± 0.19	0.50 ± 0.19***	0.47 ± 0.18	0.52 ± 0.22
Σ <i>n</i> -6	40.98 ± 2.86	25.55 ± 4.50***	28.30 ± 3.69***	22.50 ± 3.23*** ##
C18:3 <i>n</i> -3 α -linolenic acid	0.14 ± 0.05	0.11 ± 0.04	0.14 ± 0.03	0.09 ± 0.028** #
C20:5 <i>n</i> -3 eicosapentaenoic acid	0.33 ± 0.24	0.19 ± 0.11*	0.28 ± 0.10	0.10 ± 0.03** ##
C22:5 <i>n</i> -3 docosapentaenoic acid	0.57 ± 0.10	0.46 ± 0.16*	0.60 ± 0.10	0.33 ± 0.09*** ##
C22:6 <i>n</i> -3 docosahexaenoic acid	3.62 ± 1.12	2.09 ± 0.91***	2.74 ± 0.68*	1.29 ± 0.28*** ##
Σ <i>n</i> -3	4.69 ± 1.35	2.92 ± 1.28***	3.85 ± 0.98	1.79 ± 0.31*** ##
<i>n</i> -6/ <i>n</i> -3	9.98 ± 2.20	10.20 ± 4.21	7.78 ± 1.24**	12.62 ± 0.47** ##
PUFA polyunsaturated FA	45.34 ± 3.42	28.69 ± 4.46***	31.72 ± 3.53***	24.98 ± 1.88*** ##
PUFA/SFA	1.09 ± 0.15	0.70 ± 0.19***	0.83 ± 0.14***	0.54 ± 0.08*** ##

Values are means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs healthy controls; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs alcoholic cirrhosis patients well nourished.

ment with previous studies.^(5,39) It is known that light alcohol consumption is associated with decreased triglyceride levels whereas alcohol abuse increases triglyceride levels.⁽⁴⁰⁾ In addition, plasma triglyceride concentration represents a functional indicator of the *n*-3 PUFA status because *n*-3 PUFA exerts a consistent hypotriglyceridemic effect, which is dose dependent and persistent.⁽⁴¹⁾

Low levels of LA, AA and DHA were the most responsible for the observed reduction in the total PUFA status and PUFA/SFA ratio in patients with alcoholic cirrhosis. Possible reasons include a poor dietary intake of LA and EPA, changes of Δ -6, Δ -5 and Δ -9 desaturase activity due to chronic alcohol consumption and hepatocellular insufficiency⁽⁴²⁻⁴⁴⁾ and/or an increased degradation of PUFA due to lipid peroxidation.⁽⁴⁵⁾ Previously published data⁽⁴⁶⁻⁴⁸⁾ suggest that a high level of palmitic acid in cirrhotic patients (found also in our study) is related to the cirrhotic process and not to alcohol consumption. High level of palmitic acid in our cirrhotic patients does not seem to be affected by malnutrition. Additionally, Alvaro *et al.*⁽⁴⁹⁾ found that an impaired FA elongation in cirrhosis might explain this alteration. The hypothesis of increased activity of Δ -9 desaturase and decreased activity of elongase could explain the decrease in stearic acid and the increase in palmitoleic acid in cirrhosis patients.^(42,50) This process is even more exerted in the malnourished patients. Namely, low level of LA increased the activity of Δ -9 desaturase⁽⁵¹⁾ increasing levels of palmitoleic and 18:1 *n*-9, with the FA status usually characterised by low LA *n*-6^(50,52) as observed in malnutrition.

The amount of *n*-6 and *n*-3 PUFA in the diet strongly influences serum PUFA levels.^(53,54) Generally, the habitual diet of Serbian adults is characterized by a high intake of energy derived from dietary fats, combined with a lower protein and complex carbohydrate intake.^(27,28) Dietary *n*-6/*n*-3 PUFA ratio should be maintained between 2.8 and 3.2 in chronic liver disease,^(55,56) but this ratio in the patients in our study was 12.2 (10.7 in well-nourished and 14.8 in malnourished patients). Consequently, it is important to emphasize that patients have dietary habits to consume (sunflower-oil based) *n*-6 PUFA cooking oil as a part of their

usual diet with low fish intake.

The tested subjects had a long-term daily consumption of a constant amount of alcohol (mainly in the form of spirits). Studies showed that serum FA levels were affected by alcohol consumption.^(14,16) For instance, as the quantity of alcohol per week increased, the levels of palmitoleic and oleic acid also increased, but the levels of linolenic, stearic and PUFA/SFA decreased.^(57,58,59) Warnet *et al.*⁽⁵⁸⁾ reported that palmitoleic acid is an independent marker of alcohol consumption and could be useful in epidemiological and clinical studies as a variable of consumption. Several studies attempted to address the fact that in patients with advanced cirrhosis, malnutrition appeared to be a major additional mechanism which contributed to PUFA-deficiency.^(5,6,60) A deficiency of LA and LNA may occur in malnourished patients and may result in the modification of the PUFA profile.^(8,9,61) We found that well-nourished patients with alcoholic cirrhosis had lower levels of LC-PUFA in both the *n*-6 and the *n*-3 FA series than healthy subjects, and lower LA. However, normal level of LA serves as a marker of a good nutritional status. González *et al.*⁽⁵⁾ found decreased proportion of LA in patients with severe malnutrition. In contrast, LA and LNA did not seem to be affected in alcoholic subjects with liver disease in the Pita *et al.*⁽⁴⁸⁾ study. Furthermore, levels of AA (derived from LA) decreased in serum phospholipids in our patients, particularly in the MN group. Plasma AA deficiency is univariately associated with higher mortality rates in patients with advanced liver cirrhosis,⁽¹¹⁾ and may be an important factor in the pathogenesis of altered coagulation, immunological and renal functions in cirrhosis.⁽⁶²⁾

Some studies^(1,43,44) found a decrease in DHA and total *n*-3 PUFA in serum and erythrocytes in alcoholic cirrhosis patients, but not in patients with other etiologies of liver cirrhosis. An explanation may be a more active oxidative degradation process⁽⁴⁵⁾ which is possibly linked to alcohol consumption. In addition, Watanabe *et al.*⁽⁶³⁾ showed decreased plasma levels of DHA in alcoholic and non-alcoholic liver cirrhosis depending on the severity of liver failure. Their research suggests that a deficit of this fatty acid, which is concentrated in nerve tissues, might be

related to impaired neural function observed in hepatic encephalopathy of cirrhotic patients. In our patients, DHA status was mainly responsible for a decrease in *n*-3 series PUFA; the lowest values were found in accordance with the state of nutrition and generally low dietary intake of DHA and EPA. Patients with malnutrition often have compromised taste and dislike seafood⁽⁶⁴⁾ which partly explained lowered dietary *n*-3 PUFA intake and status in our patients. However, there was no difference in dietary *n*-6 fatty acid intake in our malnutrition patients compared to well-nourished patients. This contributed to very high *n*-6/*n*-3 ratio in serum phospholipids. In addition to low PUFA intake, factors that may induce PUFA deficit in malnutrition include poor lipid digestion, absorption and transport, as well augmented β -oxidation and PUFA peroxidation.⁽⁵²⁾ It is important to note that lower levels of LA found in our malnutrition patients could be to an impaired fat absorption related to decrease levels of bile salt and to lymphangiectasia,⁽⁵⁾ frequently observed in cirrhosis. The derangement of the serum phospholipids fatty acid profile in patients with an acceptable nutritional status depends mainly on hepatocellular insufficiency^(2,11,55) which led to low *n*-6/*n*-3 ratio in this cirrhotic patients. The clinical significance of low PUFA status is still unknown, although abnormal FA profile may contribute to clinical problems such as itching, pruritus, abnormal

perspiration, susceptibility to infection, delayed wound healing, anemia, thrombocytopenia, diminished platelet aggregation, dysfunction in eicosanoid synthesis and diminished immune system status as seen in patients with cirrhosis.^(43,65,66)

Our pilot study clearly showed that alcoholic cirrhotic patients had an abnormal FA status. Moreover, patients with an acceptable nutritional status have a poor LA and LC-PUFA status, while malnutrition associated with cirrhosis leads to lower levels of *n*-6 and *n*-3 PUFA in patients with alcoholic liver cirrhosis. These findings imply that alcoholics who have a marginal intake of LA and *n*-3 PUFA may be at a greater risk for developing liver cirrhosis. PUFA supplementation in these patients could be of interest.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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