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Abnormal polyunsaturated fatty acid patterns of serum lipids in alcoholism and cirrhosis: Arachidonic acid deficiency in cirrhosis

(essential fatty acids/polyunsaturated fatty acid deficiency/liver damage/alcohol abuse)

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ABSTRACT **Patterns of polyunsaturated fatty acids of serum phospholipids were measured for groups of alcoholics without cirrhosis, alcoholics with cirrhosis, cirrhotics without alcoholism, and a control population. Alcoholics without cirrhosis showed increased polyunsaturated fatty acids derived from linoleic and linolenic acids, but in cirrhotics these products were decreased. Alcoholism accentuated the abnormal polyunsaturated fatty acid pattern of cirrhosis. In alcohol abuse without cirrhosis, the level of 20:3ω9 (20 acyl carbon atoms:3 double bonds, ω, 9 carbon atoms beyond last double bond) was significantly increased, despite adequate levels of linoleic and arachidonic acids. Liver involvement appears necessary for development of deficiencies of polyunsaturated fatty acids in serum phospholipids, of which arachidonic acid deficiency is of the largest magnitude.**

Aberrations in fatty acid composition of serum and tissue lipids, indicating marginal or overt essential fatty acid (EFA) deficiency, occur in many disease states (1–6). A preliminary study of chronic alcoholism indicated that EFA deficiency may occur (7). This was suspected because changes in lipid metabolism occur in chronic alcoholism. Accumulation of triacylglycerols and cholesteryl esters in the liver and disturbances in lipoprotein metabolism have been reported (8). Many functional disturbances in alcoholism may relate to changes in phospholipid (PL) composition of cellular membranes (9, 10). Resolution of the mechanisms underlying these changes is complicated by dependence of observed changes on amount and duration of ethanol abuse and on nutritional status of the subject (8, 11).

Two cooperative studies were undertaken to examine fatty acid profiles of serum PL in relation to liver function. Chronic alcoholism, with and without liver disease, was compared to appropriate control populations and to cirrhosis not associated with alcoholism. The studies were made on two geographically and culturally distinct populations.

MATERIALS AND METHODS

Study 1 included patients attending clinics or hospitalized in Montreal General, St. Anne's, and Douglas or Queen Mary Veteran Hospitals in Montreal, Quebec, Canada. Subjects of both sexes ranged from 16 to 80 yr of age. Males and females were considered together because no significant differences were found between the polyunsaturated fatty acid (PUFA) patterns of serum lipids of male and female controls (12). The study included 9 alcoholics with cirrhosis, 90 alcoholics without cirrhosis, 16 cirrhotic patients without alcoholism, and 29 controls from the same area and culture. The cirrhotics without alcoholism had a previous history of alcohol abuse but had abstained from alcohol for some time prior to the study, many for >2 yr. Patients were diagnosed as alcoholics by a physician, using the criteria of Spitzer *et al.* (13). Information was obtained from histories and interviews with members of their families. Four standard tests of liver function were made (see below). Alcoholics suspected of cirrhosis because of high enzyme levels were biopsied. All diagnoses of cirrhosis were made from histological examination of liver needle biopsy samples by a pathologist. The alcoholics were compared as a whole and were later subdivided according to drinking habits by duration of alcohol intake, level of alcohol consumption, and duration of abstinence.

In the second study, 20 male patients with alcoholic cirrhosis from the Minneapolis Veterans Administration Hospital, having a mean age of 56 ± 2 yr, were evaluated in comparison with 33 age-matched volunteers from the Minneapolis area. Diagnosis of alcoholic cirrhosis was confirmed by liver biopsy. Eighteen of the 20 cirrhotics had 2+ or greater ascites at the time of the study. All patients were housed in the Minneapolis Veterans Administration Special Diagnostic and Therapeutic Unit as part of a study evaluating nutritional status in alcoholic liver disease. The following liver tests and indicators of nutritional status were performed on these 20 alcoholic cirrhotics; results are expressed as mean ± SEM and normal values for the institution are in parentheses: albumin, 3.1 ± 0.1 g/dl (3.5–5.3); serum glutamic oxalacetic transaminase, 61 ± 7 international units (IU) (6–26); bilirubin, 2.9 ± 0.5 mg/dl (0.2–1.2); alkaline phosphatase, 144 ± 15 IU (24–96); prealbumin, 11.9 ± 1.2 mg/dl (10–40); retinal binding protein, 2.6 ± 3.6 mg/dl (3–6); creatinine/height, 71 ± 7 (100%).

Single fasting samples from each patient in the study were analyzed. To test whether one sample is representative of one's EFA status, fasting blood was drawn from 7 volunteer normal healthy laboratory personnel at 8:00 a.m. Blood was drawn 2, 4, 6, 8, and 12 hr later, and a second fasting sample was taken the following morning. The subjects ate normally during the day. Fatty acid pattern of serum PL was determined on each specimen for comparison to a control population of 33.

In all studies, fasting blood was drawn, clotted at room temperature, and the serum was removed and sent frozen to the Hormel Institute. Extractions, lipid separations by thin-layer chromatography and gas chromatography of fatty acid methyl esters (FAME) were described previously (14). In study 1, only PL was analyzed, but for study 2 all lipid classes were examined. For study 1, gas chromatography analysis was carried out on a packed column, and for study 2, it was done on a capillary column (14).

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Abbreviations: PUFA, polyunsaturated fatty acids; PL, phospholipids; EFA, essential fatty acids; FAME, fatty acid methyl esters; IU, international units; NS, not significant.

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Means of relative percentages of each fatty acid and each parameter used in assessment of EFA status were calculated for each population and compared. Graphic PUFA profiles were constructed from normalcy ratios (experimental values/control values). Significances of differences were measured using Student's *t* test. Because PUFA occur principally in the PL of membranes, which are essentially two-dimensional surfaces, the effective concentration of PUFA in the surfaces accessible to enzyme systems is more correctly expressed as relative percentage in the total PL than as concentration in the bulk aqueous system (mg per g of tissue). Therefore, all data discussed are relative percents.

Serum levels of total bile acids were determined according to the method of Murphy *et al.* (15), and alkaline phosphatase, glutamyltransferase, and serum glutamic oxalacetic transaminase activities were determined on an SMAC-12 analyzer in the clinical laboratories.

RESULTS AND CONCLUSIONS

Diurnal Variation of PUFA Profile. In the PUFA profiles of 7 normal subjects, significant differences occurred only among minor acids not involved in assessment of EFA status. Fatty acids germane to evaluation of EFA status fell within 1 SD of the mean of a larger group of controls (n = 33). For example, means ± SD of total ω6 fatty acids at each time point fell within ±1 SD of the mean of the control population (38.3 ± 3.3%). Only two individual values for the entire study (n = 49) fell slightly outside 1 SD. All individual values for fasting 0 and 24 hr samples were within ±1 SD of the control mean, and means at each time point were not significantly different from each other. The group of 49 analyses from 7 time periods had 37.4 ± 2.0% total ω6 acids. For the 14 fasting samples, the value was 37.6 ± 1.9 and for the 35 nonfasting samples, the value was 37.4 ± 2.1. For each person, means ± SD of the 5 nonfasting samples were nearly identical to the two fasting values. Thus, the ω6 acids of PL, the best composite measure of EFA status, varied slightly over a 24-hr period but remained within the mean ± SD of the control group despite variation in time of day and state of nutrition. Because all specimens in both studies were from fasting blood samples, we conclude that the single specimens analyzed from each patient were representative of their individual PUFA status. Moreover, values compared were means of 9 or more individuals per group, and we considered meaningful only those differences for which $P <$ 0.05. Aberrations in profile in alcoholism and in cirrhosis far exceeded the daily variations found in free-living individuals and were statistically significant.

Study 1: Alcoholism. To indicate magnitude of pertinent fatty acids and to permit estimates of percentage values from the profiles, values for the FAME from serum PL of the control group pertinent to evaluation of EFA status were as follows, in percent of total FAME (mean ± SD): 18:1ω9, 14.9 ± 3.2; 18:2ω6, 19.9 ± 4.3; 18:3ω6, 0.04 ± 0.05; 18:3ω3, 0.31 ± 0.38; 20:2ω6, 0.24 ± 0.27; 20:3ω9, 0.27 ± 0.27; 20:3ω6, 2.7 ± 1.1; 20:4ω6, 9.4 ± 3.2; 20:5ω3, 0.81 ± 0.81; 22:4ω6, 0.49 ± 0.49; 22:5ω6, 0.42 ± 0.49; 22:5ω3, 0.71 ± 0.65; 22:6ω3, 2.3 ± 2.2; total ω6, 33.1 ± 5.9; ω6 products, 13.2 ± 4.9; total ω3, 4.2 ± 3.2; ω3 products, 3.9 ± 3.2; monoenoic acids, 16.1 ± 3.2; saturated acids, 45.6 ± 6.5.[‖] PUFA profiles of serum PL in 90 alcoholics without cirrhosis compared to controls are shown in Fig. 1*A*. For alcoholics, the level of linoleic acid (18:2ω6) was 93% of the control value [not significant (NS)], indicating adequate intake and absorption of linoleate. Metabolic products of 18:2ω6 were above normal, some significantly so. Total ω6 fatty acids in PL were 99% of control level. Linolenic acid (18:3ω3) was 217% of control and all its products were above control levels. Total ω3 acids were significantly increased ($P <$ 0.05), and monoenoic and saturated acids were 103% and 95% of control values, respectively (NS). The principal PUFA derived from oleic acid, 20:3ω9, was increased to 232% of control value ($P < 0.001$), although there was no deficiency of linoleic acid or its major metabolic product, arachidonic acid (20:4ω6). Overall, the alcoholic population received adequate EFA and utilized them at normal or above normal levels.

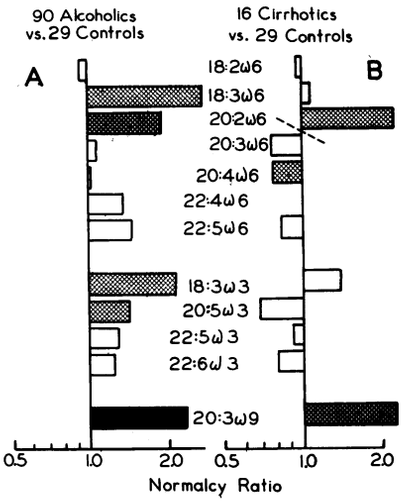

FIG. 1. Study 1: Profiles of PUFA in serum PL for 90 alcoholics compared to 29 controls (*A*) and for 16 cirrhotics compared to 29 controls (*B*). Normalcy ratio is the ratio of the observed value to the control value. Significance is indicated by shading: open bars, not significant; light cross-hatch, $P < 0.05$; dark cross-hatch, $P < 0.01$; black, $P < 0.001$. Pointed bars extend beyond scale.

Cirrhosis. Because alcohol abuse often involves cirrhosis, 16 patients with confirmed cirrhosis but without alcoholism were compared to the control population to discern the contribution of cirrhosis alone to abnormal PUFA profile (Fig. 1*B*). Cirrhotics exhibited normal levels of 18:2ω6 and of its product 18:3ω6. The 20:2ω6 was significantly increased ($P < 0.05$) but 20:4ω6 was significantly deficient (77%; $P <$ 0.05). The total ω6 acids were significantly below control levels (89%; $P < 0.05$). Linolenic acid was not significantly affected. The level of 20:3ω9 was increased (227%; $P <$ 0.01), indicating that Δ5 desaturation was not impaired.

When alcoholic cirrhotics were compared to controls (9 vs. 29) (Fig. 2*A*), impairment in the metabolism of both 18:2ω6 and 18:3ω3 was observed, which was not seen in the comparison of alcoholics vs. controls (Fig. 1*A*). The levels of these dietary PUFA precursors were normal (98% and 147%, respectively), whereas levels of most products were below normal; only 20:4ω6 was depressed significantly (65%; $P <$ 0.01). The 20:3ω9 was increased ($P < 0.01$), indicating no inhibition of Δ5 desaturation by which 20:4ω6, 20:5ω3, and 20:3ω9 are produced, and suggesting that the defect in ω6 and ω3 metabolism could be structure specific, probably at the elongation step from C_{18} to C_{20}.

Comparison of alcoholic cirrhotics to alcoholics (Fig. 2*B*), to assess again the effect of cirrhosis, showed many of the differences found between alcoholic cirrhosis and controls (Fig. 2*A*) but with a higher degree of significance. Values for 20:3ω6 (68%) and 20:4ω6 (63%), and for the ω3 products 20:5ω3 (40%), 22:5ω3 (42%), and 22:6ω3 (49%) were significantly suppressed. Comparison of the alcoholic cirrhotics to cirrhotics, which assesses the effect of alcoholism (Fig. 2*C*),

[‖]The ω nomenclature for PUFA consists of the number of acyl carbon atoms:number of double bonds, ω, and number of carbons beyond last double bond including the terminal (ω) carbon atom. This nomenclature is used because metabolic changes do not alter terminal (ω) structure.

showed that the levels of the major ω6 and ω3 fatty acids were not significantly different except for 22:5ω3.

To test whether the drinking habits of alcoholics were reflected in the fatty acid profile, the group of alcoholics was subdivided based on duration of alcohol abuse (0–1 yr, 1–5 yr, >5 yr, and episodic), amount of alcohol consumed (0–150 g/wk or 150–200 g/wk), and duration of abstinence (1–10 days or 10 days to 6 mo). These subgroups were compared to 29 controls and with each other. No significant correlation was found between fatty acid pattern and drinking habits in the alcoholics (data not shown), suggesting that alcoholism without liver damage does not significantly alter the PUFA pattern of serum PL.

Values for 4 measures of liver function in normal subjects (N), controls (C), alcoholics (A), and alcoholic cirrhotics (AC), respectively, were as follows—glutamyltransferase (IU): N, 0–30; C, 21.3 ± 16.2; A, 46.0 ± 35.7 ($P < 0.001$); AC, 120 ± 26 ($P < 0.001$); alkaline phosphatase (IU): N, 30–110; C, 56.0 ± 17.6; A, 72.6 ± 25.7 ($P < 0.01$); AC, 182 ± 33 ($P < 0.001$); serum glutamic oxalacetic transaminase (IU): N, 8–40; C, 19.5 ± 10.9; A, 27.9 ± 25.7; AC, 79.7 ± 9.1 ($P < 0.001$); bile acids (μmol/liter: N, 0–15; C, 7.0 ± 5.8; A, 13.6 ± 7.3 ($P < 0.001$); AC, 55.9 ± 8.0 ($P < 0.001$). Significances given are for comparisons with the control group. For alcoholism without cirrhosis, serum glutamic oxalacetic transaminase levels were within normal limits, glutamyltransferase were slightly increased from normal and above control, and bile acids and alkaline phosphatase were significantly above control values but within normal limits. For alcoholics with cirrhosis, all measures were above normal and all were significantly elevated compared to the controls. These data agree with other reports (16–18).

Study 2: Alcoholism with Cirrhosis. To confirm and extend findings of study 1, a larger group ($n = 20$) of patients with alcoholism and cirrhosis was examined. Capillary chromatography provided better separation of the FAME and more reproducible measurement of individual PUFA. The content of fatty acids germane to the evaluation of EFA status in serum PL of the control population, expressed as mean relative percent ± SD, was as follows: 18:1ω9, 8.8 ± 1.7; 18:2ω6, 22.9 ± 3.4; 18:3ω6, 0.13 ± 0.06; 18:3ω3, 0.21 ± 0.17; 20:2ω6, 0.33 ± 0.11; 20:3ω9, 0.15 ± 0.06; 20:3ω6, 3.1 ± 0.6; 20:4ω6, 11.0 ± 2.9; 20:5ω3, 0.65 ± 0.46; 22:4ω6, 0.42 ± 0.11; 22:5ω6, 0.41 ± 0.17; 22:5ω3, 0.77 ± 0.17; 22:6ω3, 2.2 ± 0.6; total ω6, 38.3 ± 3.4; ω6 products, 15.4 ± 2.9; total ω3, 3.9 ± 1.1; ω3 products, 3.7 ± 1.1; monoenoic acids, 13.8 ± 1.7; saturated acids; 40.7 ± 2.9. Fig. 3 shows the PUFA profile of PL for study 2. Dietary EFA was adequate because the levels of 18:2ω6 and 18:3ω3 were within control range. The Δ6 desaturation of 18:2ω6 was functional because 18:3ω6 was at control level. Metabolic products beyond this step were deficient, notably 20:3ω6 (84%; $P < 0.05$) and 20:4ω6 (74%; $P < 0.001$), which are the products of greatest relative abundance and best known function. The products of 18:3ω3 (20:5ω3, 53%, $P < 0.01$; 22:5ω3, 55%, $P < 0.001$; and 22:6ω3, 60%, $P < 0.001$) were significantly below normal, indicating a parallel blockage in this pathway. Oleic acid was elevated (154%, $P < 0.001$) as was its product 20:3ω9 (180%, $P < 0.01$), indicating that Δ5 desaturation was not impaired. Impairment of metabolism may occur at elongation from C_{18} to C_{20}. Profiles for serum PL of alcoholic cirrhotics vs. controls for both studies (Figs. 2*A* and 3) indicate significant relative deficiencies of arachidonate, and in the larger more discriminating study 2, 20:3ω6, 20:5ω3, 22:5ω3, and 22:6ω3 were also significantly deficient in the serum PL.

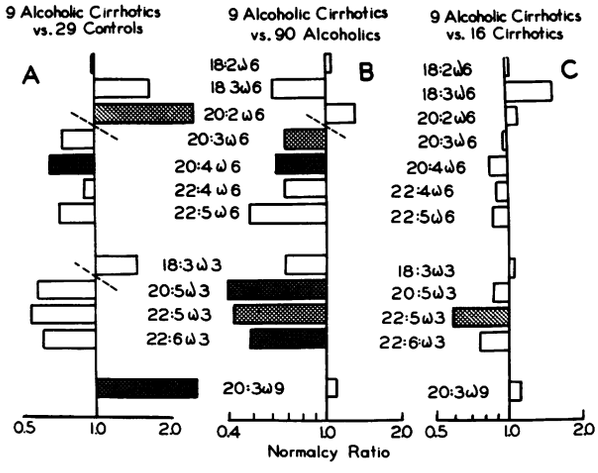

FIG. 2. Study 1: Profiles of PUFA of serum PL for 9 alcoholic cirrhotics compared to 29 controls (*A*), for 9 alcoholic cirrhotics compared to 90 alcoholics (*B*), and 9 alcoholic cirrhotics compared to 16 cirrhotics (*C*). For details, see legend to Fig. 1.

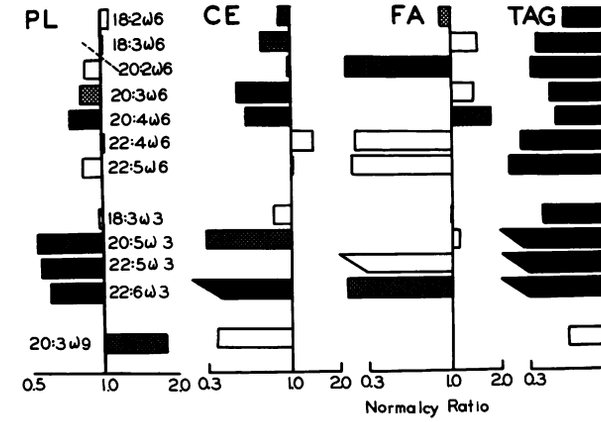

FIG. 3. Study 2: Profiles of PUFA of serum PL for 20 males with alcoholic cirrhosis compared with 33 age-matched controls measured in serum PL, cholesteryl esters (CE), free fatty acids (FA), and triacylglycerols (TAG). For details, see legend to Fig. 1.

PUFA profiles of all four lipid classes are shown in Fig. 3. The major changes in free fatty acids, which may be substrates for prostanoid and autocoid synthesis, were significant decreases in 18:2ω6, 20:2ω6, and 22:6ω3, and an increase in 20:4ω6. Triacylglycerols showed significant decreases in all ω6 and ω3 fatty acids and high proportions of oleic (114%, $P < 0.001$) and stearic acids (152%, $P < 0.001$). Cholesteryl esters showed significant decreases in major metabolic products, 20:3ω6, 20:4ω6, 20:5ω3, and 22:6ω3. Thus, the four major lipid classes in serum indicated suppression of PUFA in circulating lipids and impaired transport of PUFA.

DISCUSSION

In our preliminary study of chronic alcoholism, decreased PUFA were observed in serum PL (7). In the present study, larger groups of controls from the same cultural and geographic areas as the patients were studied, and alcoholism was carefully differentiated from cirrhosis by liver function tests and liver biopsies. The results indicate that alcoholism elevates PUFA somewhat, but that cirrhosis is accompanied by significant deficiencies in PUFA.

Nutritional deficiencies of PUFA underlie some abnormalities in lipid metabolism. EFA deficiency develops when fat-free high-glucose preparations are given intravenously or orally (1, 2), suppressing release of linoleic acid from adipose triacylglycerols. Chronic malnutrition causes a decrease in 18:2ω6 and its products because of low intake of EFA and the protein necessary for synthesis of enzymes and serum

lipoproteins (3). Faulty absorption of EFA causes the marginal EFA deficiency of cystic fibrosis (4). Normal levels of linoleic acid and subnormal levels of arachidonic acid and other ω6 acids occur in Sjögren–Larsson syndrome (5), suggesting faulty metabolism of linoleic acid to longer chain PUFA. The essentiality of linolenic acid was shown in a child with neurological symptoms and low ω3 acids, both of which were reversed when an emulsion rich in linolenic acid was administered (6). In study 1, elevation of 20:3ω9 was the only evidence of EFA deficiency in the alcoholics. They had normal or elevated amounts of PUFA in their serum PL, reflecting the status of tissue PL (2), and their liver function tests were normal. Decreased 18:2ω6 has been observed after a drinking bout, with slow recovery when normal diet was administered in subjects alcoholic >5 yr (19). Most subjects in our study were alcoholic <5 yr, and no relationship of PUFA status to duration of abuse was found, perhaps because liver impairment had not yet become measurable.

Cirrhosis alone significantly decreased 20:4ω6, the major metabolic product of 18:2ω6 and a major prostanoid precursor (Fig. 1*B*). Consumption of alcohol in conjunction with liver disease (study 1) exacerbated the general paucity of PUFA (compare Figs. 1*B* and 2*A*), although not significantly so (Fig. 2*C*). In a larger group of alcoholics with cirrhosis (study 2), PUFA products were suppressed more significantly, although levels of the precursors were normal (Fig. 3).

The 20:3ω9 increased in alcoholism, with (Fig. 2*A*) or without (Fig. 1*A*) cirrhosis. Others have reported no increase in 20:3ω9, even with depressed levels of 18:2ω6 and increased 18:1ω9 (19). In our study, the 18:1ω9 was significantly increased in cirrhosis or alcoholic cirrhosis but not in alcoholism alone. The alcoholics had normal levels of 18:2ω6 and 20:4ω6 in serum PL. Alcoholism increased desaturation and elongation of 18:1ω9 to 20:3ω9, despite normal levels of its precursor 18:1ω9 and despite elevated levels of competing ω3 and ω6 acids.

In our studies of alcoholism, the products of Δ5 desaturation were elevated [20:3ω9, 232%, $P < 0.001$; 20:5ω3, 144%, $P < 0.05$; 20:4ω6, 102%, (NS)]. This is seemingly contrary to the reports of Nervi *et al.* (20) and Wang and Reitz (21), who measured enzyme activities in rat liver. Alcoholism may affect differently enzyme activities and proportions of PUFA in structural lipids. Alcoholism alone significantly increased 18:3ω6, a product of Δ6 desaturation (304%, $P < 0.05$), but had little effect on the sums of products of the other desaturating or elongating enzymes (elongation to C_{20}, 112%, NS; Δ5 desaturation, 109%, NS; elongation to C_{22}, 132%, NS; Δ4 desaturation, 128%, NS; Δ9 desaturation, 103%, NS).

Cirrhosis and alcoholism suppressed the Δ5 desaturation products, 20:4ω6 (65%, $P < 0.01$) and 20:5ω3 (58%, NS) but 20:3ω9 was enhanced (254%, $P < 0.01$) in study 1. The product of Δ6 desaturation, 18:3ω6, was increased (NS). Products of Δ4 desaturation were significantly less than control values (64%, $P < 0.001$) in study 2, but products of Δ9 desaturation were increased in study 1 (133%, $P < 0.001$) and study 2 (156%, $P < 0.001$). Products of elongation were decreased in study 2 (C_{20}, 84%, $P < 0.05$; C_{22}, 71%, $P < 0.01$). These data, illustrating perturbed PUFA patterns in liver disease, differ from those reported by Alling *et al.* (16). However, Reye's syndrome, another disease state with liver malfunction and hepatic accumulation of lipids, showed decreased PUFA in serum PL (22).

In our study, excessive alcohol consumption alone did not induce essential fatty acid deficiency. Alcohol seemed to stimulate Δ5 desaturase to elevate 20:5ω3 and 20:3ω9 especially. The stress of alcohol may demand more PUFA, and because 20:3ω9 is derived from the endogenous oleic acid, it may be most readily produced in a degenerating state of nutrition. However, when alcoholism is accompanied by cirrhosis, metabolism of all families of PUFA is abnormal, suggesting that the liver, site of much PUFA metabolism, must be damaged to initiate deficiencies in fatty acid patterns of serum PL.

Cirrhosis, with or without alcoholism, is accompanied by deficiencies of arachidonic acid and other C_{20} and C_{22} PUFA, suggesting that faulty patterns of PUFA in hepatic membranes, and reflected in serum PL, may suppress metabolic capabilities of the cirrhotic liver. Because arachidonic acid is the major long-chain PUFA in tissue and serum PL, because it is significantly suppressed in cirrhosis, and because arachidonic acid and its precursors are naturally occurring components of food, enhanced provision of arachidonic acid by nutritional means may be feasible when it cannot be generated in adequate amounts by metabolic means.

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