Effects of Different Quantities of Fat on Serum and Liver Lipids, Phospholipid Class Distribution and Fatty Acid Composition in Alcohol-Treated Rats

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Summary The present study investigated the quantitative effect of dietary fats and ingestion of alcohol on serum and liver lipids, fatty acid bound to phospholipids and their class distribution of male Wistar rats. The rats in C (control) and A (alcohol) groups were fed a standard laboratory diet, HFC (high fat-control) and HFA (high fat-alcohol) groups were fed a high fat diet (standard diet supplemented with 20 g%w/w, sunflower oil : lard mixture 1 : 1) for 6 wk. Alcohol-treated rats consumed alcohol at the rate of 9 g/kgbw/d (15–20% energy). Liver phospholipid (PL) content was decreased, and phospholipid/cholesterol liver molar ratio increased in the alcohol treated rats. The proportion of serum sphingophospholipid (Sph) was significantly lower and proportion of phosphatidylcholin (PC) significantly higher in serum PL in alcohol-treated rats. Phospholipid class distribution was unaffected by alcohol feeding in liver. Significantly lower levels of 16:1*n*-7 and higher levels of 20:5*n*-3 and 22:4*n*-6 in the serum PL were observed in the alcohol-treated rats. The groups on the HF diet increased levels of 20:4*n*-6, 22:4*n*-6 and total *n*-6, polyunsaturated fatty acid (PUFA) and decreased levels of 18:1*n*-9 and total monounsaturated fatty acids (MUFA) in both liver and serum PL, but *n*-3 fatty acid increased in serum PL and decreased in liver PL compared to groups on the standard diet. Alcohol fat interaction was evident in MUFA and PUFA/SEA in serum PL and *n*-6, MUFA, PUFA and polyunsaturated/saturated fatty acid ratios (PUFA/SEA) in liver PL. This study showed that the high fat intake in alcohol-treated rats increased levels of 20:4*n*-6, 22:4*n*-6 and 20:4/18:2 ratio, and decreased level of 18:1*n*-9 in liver and serum phospholipids.

Key Words alcohol, dietary fats, phospholipids, fatty acids, rats

It is well known that lipid composition of most tissues is critically dependent upon that of the diet. The amount of dietary fat is important in pathogenesis of alcoholic liver disease (1, 2). Diets with high levels of fat greatly enhance liver steatosis as well as liver membrane damage and fibrosis (3, 4).

The phospholipid class and its fatty acid composition and cholesterol content in biomembranes are basic determinants of the physical properties of membranes, and alterations in these lipid species are of special interest, as functional and pathological consequences may be correlated (5). Many functional disturbances in alcoholism may be related to changes in phospholipid composition of cellular membranes (3, 6). It is believed that animals can compensate the increased fluidity of their membranes by altering the incorporation of polyunsaturated fatty acids as a mechanism of adaptation to alcohol (7, 8). Most researchers have suggested that chronic alcohol exposure is accompanied by a decrease in the percentage of arachidonic acid, together with an increased percentage of saturated and monounsaturated

fatty acids, in the different plasma lipid fractions, blood and parenchymal cells in both human (9, 10) and animal models (11, 12).

The objective of the present study was to compare the effects of the different quantities of fat and ingestion of alcohol over a 6 wk period, on the serum and liver lipids, phospholipid class distribution and fatty acid composition of rats.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (VMA, Belgrade) two months old, with initial weight of approx. 200 g were used in this study. The rats were divided into four groups with 9 animals in each, according to diet: control-standard (C), alcohol-standard (A), high fat-control (HFC) and high fat-alcohol (HFA) groups. C and A groups were fed a standard normoprotein pelted cereal-based on a nutritionally and energetically adequate laboratory diet (Veterinary Institute, Subotica) containing by weight (w/w) 17.2% protein, 60.9% carbohydrate, 3.7% fat with a polyunsaturated/saturated (P:S) fatty acid ratio of 1.3, 5.6% fibre and adequate amount of vitamins and minerals (ash 7.6%) for 6 wk. One gram

Table 1. Fatty acid composition of experimental diets (wt% of total fatty acids).

Fatty acids	C	HFC
16:0	19.62	16.84
16:1n-7	0.62	0.80
18:0	7.31	8.77
18:1n-9	36.53	40.00
18:2n-6	32.32	30.88
18:3n-3	1.63	1.20
20:4n-6	0.41	0.34
SEA	26.93	25.60
MUFA	37.15	40.79
PUFA	34.35	32.41
PUFA/SEA	1.3	1.3

SEA, saturated fatty acids.

MUFA, monounsaturated fatty acids.

PUFA, polyunsaturated fatty acids.

represented an estimated metabolizable energy of 14.9 kJ with 10% being derived from fat. HFC and HFA groups were fed a standard diet supplemented with 20 g% (w/w) sunflower oil (Vital, Vrbas) and lard (Slavia, Belgrade) (1:1) mixture, prepared in our laboratory, containing a P:S ratio (1.3) similar to the control-standard diet. The high fat (HF) diet was composed (w/w) of protein, 13.7%, carbohydrate, 48.7%, fat, 23.7%, fibre, 4.5% and an adequate amount of vitamins and minerals (ash 6.1%). One gram represented an estimated metabolizable energy of 19.7 kJ with 46% being derived from fat. The fatty acid composition of experimental diets is given in Table 1.

The rats in the groups A and HFA drank a 32% alcohol solution in the 25% sucrose (weight per volume) in Richter drinking tubes ad libitum while alcohol in the control groups was isocalorically substituted with sucrose. The daily food and fluid intake of the rats in the control groups was monitored so as to be equal to the intake consumed by the alcohol-fed pair on the previous day. The rats were housed in separate cages in order to accurately determine quantities of the food and alcohol consumed by each animal in temperature controlled room ($19 \pm 1^\circ\text{C}$) with a 12 h light-dark cycle. Two month old animals were matched for initial body weight and were weighed at weekly intervals thereafter. All rats, after 6 wk of treatment, were sacrificed during i.p. nembutal anaesthesia. The samples of blood were taken by heart puncture using heparinized tubes. Serum was separated by centrifugation and lipids extracted. The liver was rapidly excised, weighed and the lipids were extracted immediately.

Lipid analysis. Concentration of total phospholipids (PL) in the serum and liver was determined by the method of Zilversmit and Davis (13). Serum and liver cholesterol were analysed by the modified Liebermann-Bürchard method (14) and triglyceride levels were determinate with colorimetric methods by Fletcher (15). Serum lipids were extracted by the method of Alling (16). During the extraction procedure lipids were

protected against oxidation by addition of 10 mg/100 mL butylated hydroxytoluene to the solvents. Serum phospholipids classes were separated from the serum-extracted lipids, by thin-layer chromatography (TLC), one aliquot (contain 10 μg lipid phosphors) was spotted on thin layer glass plates (0.25 mm, silica gel H and florisil) using a one-dimensional solvent system: chloroform-methanol-20% ammonia solution (70:25:5, v/v). The following phospholipid classes: phosphatidylcholine (PC), phosphatidylethanolamin (PE), lysophosphatidylcholine (LPC) and sphingomyelin (Sph) were visualised by iodine vapour and after complete sublimation of the iodine from the plate, areas of individual phospholipid were scraped and analysed for their phosphorus content (17).

The total lipids from standard and HF laboratory diet and the lipids from rat liver tissues were extracted by the method of Harth et al. (18). Liver phospholipids classes were separated from the liver extracted lipids by TLC, one aliquot (containing 60 μg lipid phosphors) was spotted on thin layer glass plates using a two-dimensional solvent system: 1. Chloroform-methanol-20% ammonia solution (65:25:5, v/v) and 2. Chloroform-acetone-methanol-acetic acid-water (75:17.5:12.5:10:4.4, v/v) into the following fractions: PC, PE, Sph, phosphatidylinositol (PI), diphosphatidylglycerol (DPG) and phosphatidylserine (PS).

Fatty acid analysis. The phospholipid fraction was isolated from the extracted lipids by one-dimensional (TLC) neutral lipid solvent system of hexane-diethyl ether-acetic acid (87:2:1, v/v) using Silica Gel GF plates (C. Merck, Darmstadt, Germany). The phospholipid fraction was scraped into glass tubes and phospholipid fatty acids methyl esters were prepared by transmethylation with 2 M NaOH methanol (heated at 85°C for 1 h) and 1 M sulfuric acid-methanol (heated at 85°C for 2 h). Fatty acid methyl esters derivatives formed from isolated plasma phospholipid fraction were separated by gas chromatography using a Varian GC (model 3400 Varian Associates) equipped with a flame ionisation detector and DB-23 (30 m $\times$ 0.53 mm i.d., film thickness 0.5 μm , J&W Scientific Inc., Folsom, Ca, USA) fused silica capillary column. The flame ionisation detector was set at 250°C , the injection port at 220°C , and the oven temperature programmed from 130 to 190°C at the heating rate of $3^\circ\text{C}/\text{min}$.

Comparing sample peak retention times with authentic standards (Sigma Chemical Company) and/or the polyunsaturated fatty acids (PUFA)-2 standard mixture (Supelco Inc, Bellefonte) identified individual fatty acid methyl esters. Peak areas were integrated using Varian 4290 integrator, and the results were expressed as the relative percentage of total identified fatty acids.

Statistical analysis. The data are expressed as mean $\pm$ SD. The effects of alcohol treatment and dietary fat supplementation were evaluated using a repeated measure two-way analysis of variance (ANOVA) model. When significant differences were found, means were partitioned by Fisher's protected least significant differ-

ence. Comparisons between control and alcohol groups were made using students'-*t*-test. Main effects of fat and alcohol and interactions between them of *p* value ≤ 0.05 were considered statistically significant.

RESULTS

Food intake, alcohol intake, body weight gain, relative liver weight

There were no significant differences in food intake in alcohol-treated rats compared with pair-fed controls (Table 2). Rats maintained on the alcohol-standard diet regularly consumed 9.36 ± 1.90 g. alcohol per kg body weight per day (g/kgbw/d) (20% of the daily energy intake) and rats maintained on the alcohol-high fat diet consumed 8.10 ± 1.78 g/kgbw/d (15% of the daily energy intake). There were no significant differences in body weight gain among the groups. Relative liver weight in rats of group HFC and HEA that were fed the high fat diet were significantly decreased compared to rats on the standard diet. No significant differences in liver weight were found by alcohol feeding.

Lipid contents in liver and serum

Serum and liver lipids and cholesterol/phospholipid

molar ratio in liver are shown in Table 3. The liver phospholipid (PL) content was significantly lower ($p < 0.01$) in alcohol-treated rats and alcohol $\times$ fat interaction was evident in serum phospholipids ($p < 0.01$). Serum phospholipid decrease in alcohol-treated rats on the standard diet compared to the control group. Alcohol induced a decrease ($p < 0.05$) in total cholesterol (CH) in the serum but did not affect the liver cholesterol contents. Consequently, cholesterol/phospholipid (CH/PL) liver molar ratio was significantly higher ($p < 0.05$) in the liver of alcohol-treated rats. The high fat diet increased serum ($p < 0.05$) and liver ($p < 0.001$) total cholesterol content and the CH/PL molar liver ratio ($p < 0.01$). There were no significant differences in liver triglyceride levels according to fat and alcohol intake but the serum triglyceride levels decreased in alcohol and fat treated rats compared with the control group.

Phospholipid class distribution

Table 4 and 5 show the phospholipid class distribution in serum and liver as a molar percentage of total lipids phosphorous. The serum phospholipid class distribution was unchanged according to fat content in diet. The proportion of serum Sph was significantly lower

Table 2. Food intake, body weight gain and relative liver weight.

Group	Food intake (g/d)	Alcohol intake (g/kg BW) (en%)	Initial body weight (g)	Final body weight (g)	Body weight gain (g/d)	Relative liver weight (% BW)
C	28.50 ± 2.29	—	234 ± 13	472 ± 45	5.66 ± 0.83	2.48 ± 0.13
A	28.43 ± 2.96	9.36 ± 1.90 (20)	256 ± 13	498 ± 32	5.76 ± 0.71	2.49 ± 0.08
HFC	28.42 ± 1.30	—	241 ± 9	461 ± 46	5.33 ± 1.06	2.38 ± 0.11
HEA	25.83 ± 2.01	8.10 ± 1.78 (15)	245 ± 11	472 ± 26	5.52 ± 0.58	2.33 ± 0.14^c
ANOVA						
Fat	NS		NS	NS	NS	≤ 0.001
Alcohol	NS		NS	NS	NS	NS
Fat $\times$ Alcohol	NS		NS	NS	NS	NS

Values are means $\pm$ SD for 9 rats. C, control; A, alcohol; HFC, high fat control; HEA, high fat alcohol groups.

^c Significantly different from A ($p \leq 0.05$).

Table 3. Serum and liver lipids and cholesterol/phospholipid molar ratio in liver.

	Phospholipids		CH/PL	Total cholesterol		Triglyceride	
	serum (mmol/L)	liver (mg/g)	liver molar ratio	serum (mmol/L)	liver (mg/g)	serum (mmol/L)	liver (mg/g)
C	2.13 ± 0.22	46.91 ± 4.61	0.09 ± 0.01	2.04 ± 0.14	2.11 ± 0.14	0.82 ± 0.06	24.16 ± 2.61
A	1.83 ± 0.12^a	38.82 ± 3.18^a	0.11 ± 0.02^a	1.74 ± 0.14^a	2.11 ± 0.15^a	0.82 ± 0.10	25.83 ± 2.13
HFC	2.11 ± 0.21	45.77 ± 3.81	0.12 ± 0.02^a	2.33 ± 0.80^a	2.61 ± 0.32^a	0.93 ± 0.08^a	24.62 ± 1.67
HEA	1.98 ± 0.20	40.28 ± 5.06^c	0.14 ± 0.02^c	2.09 ± 0.16^{bc}	2.93 ± 0.45	0.83 ± 0.05^b	25.19 ± 2.65
ANOVA							
Fat	NS	NS	≤ 0.001	$0 \leq 0.01$	≤ 0.001	NS	NS
Alcohol	NS	≤ 0.01	≤ 0.05	≤ 0.05	NS	NS	NS
Fat $\times$ Alcohol	≤ 0.01	NS	NS	≤ 0.05	NS	NS	NS

Values are means $\pm$ SD for 9 rats.

^a Significantly different from C ($p \leq 0.05$).

^b Significantly different from HFC ($p \leq 0.05$).

^c Significantly different from A ($p \leq 0.05$).

Table 4. Phospholipid class distribution in serum as a molar percentage of total lipid phosphorus.

Phospholipids	C	A	HFC	HEA	ANOVA		
					Fat	Alcohol	Fat×Alcohol
PC	48.70±1.88	49.91±1.97	48.58±1.13	50.83±0.95 ^b	NS	≤0.025	NS
Sph	25.97±1.67	24.28±0.56	25.56±1.28	24.75±0.76	NS	≤0.025	NS
LPC	14.84±1.25	14.58±1.25	14.82±0.94	13.25±0.65	NS	NS	NS
PE	10.49±1.13	10.44±1.03	11.02±0.92	11.15±1.30	NS	NS	NS
Sph/PC	0.52±0.05	0.48±0.03	0.53±0.04	0.49±0.02	NS	≤0.05	NS
PE/PC	0.21±0.03	0.21±0.02	0.23±0.03	0.21±0.03	NS	NS	NS

Values are means±SD for 9 rats.
PC, phosphatidylcholine; Sph, sphingomyelin; LPC, lysophosphatidylcholine; PE, phosphatidylalcoholamine.
^b Significantly different from HFC ($p\leq0.05$).

Table 5. Phospholipid class distribution in liver as a molar percentage of total lipid phosphorus.

Phospholipids	C	A	HFC	HEA	ANOVA		
					Fat	Alcohol	Fat×Alcohol
PC	54.87±1.35	54.02±0.98	54.42±2.01	55.18±1.34	NS	NS	NS
PE	25.90±1.79	28.02±1.33 ^a	24.36±1.12	24.76±1.95 ^c	≤0.01	NS	NS
PI	12.35±1.71	11.91±0.85	15.09±2.22 ^a	13.34±1.66	≤0.01	NS	NS
PS	3.59±0.83	3.07±0.62	3.18±0.39	2.93±0.49	NS	NS	NS
Sph	1.85±0.35	1.70±0.29	1.91±0.44	2.03±0.37	NS	NS	NS
DPG	1.66±0.25	1.43±0.44	1.80±0.23	1.78±0.31	NS	NS	NS

Values are means±SD for 9 rats.
PC, phosphatidylcholine; PE, phosphatidylalcoholamine; PI, phosphatidylinositol; PS, phosphatidylserine; Sph, sphingomyelin; DPG, diphosphatidylglycerol.
^a Significantly different from C ($p\leq0.05$).
^c Significantly different from A ($p\leq0.05$).

Table 6. Fatty acid composition of serum phospholipids.

Fatty acids	C	A	HFC	HEA	ANOVA		
					Fat	Alcohol	Fat×Alcohol
16:0	24.34±3.80	23.77±4.32	21.73±1.28	23.38±3.52	NS	NS	NS
16:1 n -7	0.49±0.14	0.33±0.09 ^a	0.79±0.22 ^a	0.64±0.07 ^c	≤0.001	≤0.025	≤0.025
18:0	24.11±2.60	26.00±1.60	26.17±3.73	22.59±2.46 ^c	NS	NS	≤0.05
18:1 n -9	12.67±3.11	9.09±1.63 ^a	8.43±0.99 ^a	8.57±1.47	≤0.025	NS	≤0.05
18:2 n -6	13.16±1.65	13.63±1.00	12.73±1.82	11.69±0.58	NS	NS	NS
18:3 n -3	0.87±0.33	0.51±0.13	1.28±0.36	1.50±0.22 ^c	≤0.001	NS	≤0.025
20:3 n -6	0.44±0.11	0.55±0.11	0.64±0.16	0.54±0.14	NS	NS	NS
20:4 n -6	11.78±1.87	11.90±1.96	17.68±1.35 ^a	15.55±1.62 ^c	≤0.001	NS	NS
20:5 n -3	0.42±0.10	0.63±0.27	0.41±0.10	0.86±0.13 ^{bc}	≤0.001	≤0.001	≤0.001
22:4 n -6	0.59±0.10	0.93±0.26 ^a	0.73±0.17 ^a	1.91±0.34 ^{bc}	≤0.001	≤0.001	≤0.001
22:5 n -3	0.76±0.14	0.96±0.26	0.93±0.12	0.64±0.15 ^{bc}	NS	NS	≤0.01
22:6 n -3	4.87±1.07	5.08±1.58	6.74±1.00 ^a	6.20±1.25	≤0.025	NS	NS
20:4/18:2 n -6	0.90±0.15	0.87±0.08	1.43±0.31 ^a	1.33±0.11 ^c	≤0.001	NS	NS
SEA	48.45±3.98	49.76±5.38	47.90±3.93	45.97±2.67	NS	NS	NS
UFA	45.93±4.29	43.31±5.00	50.36±3.36	48.04±3.51	≤0.025	NS	NS
MUFA	13.16±3.09	9.42±1.62 ^a	9.22±1.10 ^a	9.20±1.54	≤0.05	NS	≤0.05
Σ n -6	25.86±2.84	27.03±3.17 ^a	31.68±1.77 ^a	29.69±2.08	≤0.01	NS	NS
Σ n -3	6.92±1.19	7.18±1.61	9.36±1.30 ^a	9.20±1.48 ^c	≤0.01	NS	NS
n -6/ n -3	3.79±0.36	3.76±0.64	3.44±0.50	3.29±0.47	≤0.025	NS	NS
PUEFA	32.77±3.84	33.92±4.41	41.03±2.35 ^a	38.89±2.95	≤0.001	NS	NS
PUEFA/SEA	0.68±0.11	0.70±0.15	0.85±0.09 ^a	0.85±0.10	≤0.01	NS	≤0.025

Values are means±SD for 9 rats (mol%).
SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUEFA, polyunsaturated fatty acids.
^a Significantly different from C ($p\leq0.05$).
^b Significantly different from HFC ($p\leq0.05$).
^c Significantly different from A ($p\leq0.05$).

Table 7. Fatty acid composition of total liver phospholipids.

Fatty acids	C	A	HFC	HEA	ANOVA		
					Fat	Alcohol	Fat×Alcohol
16:0	18.47±0.99	17.38±0.87	14.89±0.79 ^a	15.63±0.85 ^c	≤0.001	NS	≤0.05
16:1 n -7	2.42±0.55	2.02±0.40	0.83±0.16 ^a	0.92±0.21 ^c	≤0.001	NS	NS
18:0	19.44±1.52	20.39±1.50	24.04±1.03 ^a	24.02±0.71 ^c	≤0.001	NS	NS
18:1 n -9	11.64±1.01	9.96±0.94 ^a	8.58±0.72 ^a	9.03±0.77	≤0.001	NS	≤0.01
18:2 n -6	13.98±1.04	15.55±0.94	14.02±1.17	14.33±1.30	NS	NS	NS
18:3 n -3	0.23±0.09	0.28±0.09	0.29±0.07	0.18±0.04	NS	NS	NS
20:3 n -6	1.10±0.24	1.14±0.11	0.69±0.32 ^a	1.01±0.26	≤0.025	NS	NS
20:4 n -6	20.47±1.82	21.45±1.37	27.14±0.75 ^a	25.90±2.35 ^c	≤0.001	NS	NS
20:5 n -3	1.30±0.33	1.13±0.46	0.24±0.06 ^a	0.22±0.02 ^c	≤0.001	NS	NS
22:4 n -6	0.13±0.01	0.16±0.00	0.24±0.02	0.27±0.12 ^c	≤0.01	NS	NS
22:5 n -3	1.18±0.14	1.12±0.21	0.74±0.15 ^a	0.68±0.10 ^c	≤0.01	NS	NS
22:6 n -3	9.28±0.36	8.78±0.99	7.83±1.22 ^a	7.04±0.61 ^c	≤0.01	NS	NS
20:4/18:2 n -6	1.47±0.21	1.38±0.14	1.94±0.15 ^a	1.83±0.29 ^c	≤0.01	NS	NS
SEA	37.92±1.28	37.77±1.13	39.10±0.96	39.66±0.95 ^c	≤0.01	NS	NS
UFA	61.61±1.31	61.47±0.92	60.41±0.91	59.57±1.07 ^c	≤0.01	NS	NS
MUEA	14.06±1.55	11.99±1.25 ^a	9.42±0.72 ^a	9.95±0.71 ^c	≤0.001	NS	≤0.025
Σ n -6	35.61±1.77	38.18±1.46 ^a	42.05±1.56 ^a	41.51±1.70 ^c	≤0.001	NS	≤0.05
Σ n -3	11.95±0.74	11.53±1.66	8.94±1.33 ^a	8.11±0.99 ^c	≤0.001	NS	NS
n -6/ n -3	3.00±0.26	3.40±0.51	4.82±0.83 ^a	5.20±0.81 ^c	≤0.001	NS	NS
PUFA	47.56±1.56	49.70±0.84 ^a	50.99±1.08 ^a	49.62±1.47	≤0.01	NS	≤0.01
PUFA/SEA	1.25±0.08	1.32±0.06	1.31±0.05	1.25±0.08	NS	NS	≤0.025

Values are means±SD for 9 rats (mol%).

SEA, saturated fatty acids; UFA, unsaturated fatty acid; MUEA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

^a Significantly different from C (p ≤0.05).

^b Significantly different from HFC (p ≤0.05).

^c Significantly different from A (p ≤0.05).

(p <0.025) but proportion of PC was significantly higher (p <0.025) in alcohol-treated rats. The high fat diet decreased PE percentage (p <0.01) and increased PI percentage in the liver (p <0.01). Phospholipid class distribution was unaffected by alcohol feeding in liver. There are no effects of alcohol×fat interaction on phospholipid class distribution in serum or the liver.

Fatty acid composition

The relative fatty acid compositions of serum and liver phospholipids are shown in Table 6 and 7. Unsaturated fatty acids (UFA) were increased (p <0.025) with the high fat diet in serum phospholipids and decreased (p <0.01) in total liver phospholipids, whereas the saturated fatty acid (SEA) levels remained unaffected in serum phospholipids and increased (p <0.01) in liver phospholipids. No difference was found in the linoleic acid (18:2 n -6) in serum and liver phospholipids, but major metabolite arachidonic acid (20:4 n -6) as well as docosatetraenoic acid (22:4 n -6) was increased with the high fat diet in both serum and liver phospholipids. Total n -6 fatty acid content was elevated (p <0.01) in serum and liver (p <0.001) phospholipids by the high fat diet. The amounts of total n -3 fatty acids and especially docosahexaenoic (22:6 n -3) acid, the main fatty acid of the n -3 family, increased (p <0.025) with the high fat diet in serum phospholipids. By contrast n -3

fatty acids were significantly lowered (p <0.001) in liver phospholipids, particularly eicosapentaenoic acid (20:5 n -3), docosapentaenoic acid (22:5 n -3) and docosahexaenoic acid (22:6). Total PUFA (n -6+ n -3) were elevated with high-fat diet in serum (p <0.001) and liver (p <0.001) phospholipids. The high fat diet decreased oleic acid (18:1 n -9) in serum and liver phospholipids. High fat diet also increased palmito-oleic acid (16:1 n -7) in serum but decreased it in liver phospholipids. Total monounsaturated fatty acids (MUEA) were decreased in both serum (p <0.05) and liver phospholipids (p <0.001).

Alcohol consumption was associated with a decrease of 16:1 (p <0.025), and an increase of (p <0.001) in 20:5 n -3 and 22:4 n -6 in serum phospholipids. The fatty acid in the liver phospholipids remained unchanged in alcohol treated rats. Alcohol×fat interaction was evident in the levels of 18:0, 16:1 n -7, 18:1 n -9, 18:3 n -3, 20:5 n -3, 22:4 n -6, 22:5 n -3, total MUEA and PUFA/SEA in serum phospholipids. Alcohol×fat interaction affected the proportional composition of 16:0, 18:1 n -9, total MUEA, n -6, total PUFA and PUFA/SEA levels in liver phospholipids.

DISCUSSION

Most diets in which standard pelleted food is used in

combination with alcohol in drinking water are low in fat content and high in carbohydrate content. The food pelted cereal used in our study (contained 20% of the energy content (en%) as protein, 10% as fat, 70% as carbohydrates) was based on the modification formula recommended by the AIN'77-standard diet (19). The alcohol/total food energy intake percentage was 15 to 20% (8.10 ± 1.78 and 9.36 ± 1.90 g/kgBW/d) for rats maintained on high fat and standard diet, respectively. Our results are in agreement with findings of Van de Wiel et al. (20) that 5–13 g/kg/d alcohol intake resulted in a considerable energy intake 12–20% from alcohol. Moderate drinking in humans is defined as long term consumption of a maximum of 20 en% as alcohol (21). Therefore treated animals should have similar dosage procedure with an alcohol dose at least 15 en% to lead to observable damage in the liver (20). Some observation suggests that alcohol consumption influenced food consumption and weight gain in animals (22). The dietary condition in the present study observed that combination of 25% sucrose with 32% alcohol (weight per volume) in the drinking water did not cause a change of weight gain, when animals consumed diet ad libitum. No differences were found in growth rate per day between the groups. Relative liver weights decreased with the high fat diet in our study. Decreased liver weight with increasing levels of dietary fats Boazer et al. (23) due to less liver glycogen and water storage with a higher fat lower carbohydrate diet was observed.

The change in fluidity of membrane from ethanol treated animals has been attributed to a change in lipid composition of the membrane (6, 24). The importance of phospholipids for structure and integrity of cellular membranes suggests that many functional disturbances in alcoholism may be related to changes in phospholipid content and composition (3). Some studies showed that chronic alcohol intake resulted in the change of phospholipid content (11, 25) but several studies have reported minor changes in phospholipid distribution (6, 26, 27). Resolution of the mechanisms underlying this change is complicated by changes of amount and duration of alcohol abuse. The major finding in the present study is the significant decrease in phospholipid content in liver with a tendency of lowering serum phospholipids levels in alcohol-treated rats when compared with sucrose-fed controls. The decrease of the total phospholipid levels in the liver after alcohol treatment in our study was independent of the amount of dietary fat but such treatment did not cause changes in the percentage of liver phospholipid class distribution. The present results indicate changes in serum phospholipid class distribution: a decrease in Sph and an increase in PC phospholipid fraction after 6 wk of alcohol consumption.

The fat intake has been studied as a modulator of liver disease in animal models of alcoholism. Previous studies (1, 28) have shown that hepatic lipid accumulation after alcohol ingestion in experimental animals is dependent on the dietary fat content. In the contrast to previous studies, Simpson et al. (29) found that hepatic

triglyceride accumulation occurs in alcohol-treated rats even when consumed with low fat diet (4.4 en%). Our results show that a standard fat diet (10 en%) and a high-fat (46 en%) with alcohol did not affect the liver triglyceride content. These results are in agreement with the findings of Okita et al. (30) who reported that alcohol-treated rats did not show a significant difference in the accumulation of liver triglycerides as compared to control groups. In many previous reports, chronic alcohol administration raised total serum cholesterol (31, 32), our study presents that a high fat diet increased the serum and liver cholesterol levels and that alcohol decreases serum cholesterol with no effect on the cholesterol content in the liver. Goheen et al. (33) observed a reduction in the liver content of triglycerides in alcohol-treated rats induced by dietary arachidonic acid. Under the present experimental conditions it is reasonable to consider that a decrease of 20:4n-6 may induce the pathologic change in the liver associated with alcoholism. In our study, arachidonic acid increased by high-fat diet but 20:4n-6 remained unchanged in alcohol-treated rats and liver triglyceride content unaffected according to fat and alcohol intake.

Yamanda and Lieber (34) reported significant decrease in cholesterol and CH/PL molar ratio in liver plasma membranes while observing no change in the phospholipid content in liver plasma membranes after chronic alcohol administration with high fat content (35%) in the diet. Lee and Hosein (27) observed no statistical difference in the total cholesterol, phospholipid and CH/PL molar ratio of liver plasma membranes with a low fat diet (5%). Several studies (8, 34) in animal models for alcoholism examining the effects of chronic ethanol consumption have found that decreased levels of membrane cholesterol will lead to an increase in the fluidity of liver plasma membranes. In our study the CH/PL liver molar ratio was significantly higher in the liver of alcohol-treated rats with a different amount of dietary fat, because total phospholipid content decreased with no difference in cholesterol content in the liver according to alcohol intake. In contrast to previous studies, Gutiérrez-Ruiz et al. (35) showed that alcohol intake increased fluidity with an increase in the CH/PL liver molar ratio of plasma membranes. They found a decrease in phospholipid content after acute alcohol intake and increased cholesterol content in acute and chronic treatments in the liver plasma membrane. The high fat diet in our study increased liver cholesterol content, consequently increasing CH/PL liver molar ratio with no difference in phospholipid content according to different amounts of dietary fat.

It was expected that the percentage of fatty acids found in the rat liver phospholipids on HF-diet would be similar to those found in control animals on standard diets, since the P : S ratio (1.3) of the two diets was similar. To the contrary, in our experiment the levels of fatty acids found in liver total phospholipids in HFC-group were significantly different from the fatty acid profile in the control group. The HF diet increased PUFA and decreased MUFA in both liver and serum PL, but n-3

fatty acid increased in serum PL and decreased in liver PL compared to groups on the standard diet. Mlekush et al. (36) observed that the level of dietary fat seemed to influence the phospholipid and fatty acid profile of liver to a greater extent than the fatty acid content of the diet. The difference between the standard diet and the high-fat diet in our study was primarily in the amount of fat (3.7% and 23.7%w/w) and not in the fatty acid composition (see Table 1).

Alcohol is known to alter the levels of tissue and serum fatty acid (for review see 37). Previous analysis of the PUFA content of the liver and blood from animals fed alcohol chronically have produced widely conflicting results but most studies with a alcoholic liver disease model showed that chronic alcohol exposure is capable of altering the fatty acid composition in liver phospholipids. Such a wide variation in the results may be attributed to differences in the type, duration and dose of alcohol, on the techniques and animal species used, diet, the lipid composition, tissues, and subcellular fraction analysed. The most consistent findings have been increases in oleic and linoleic acid and decreases in arachidonic acids together with decreases in other PUFA of *n*-6 and *n*-3 series (12, 37, 38). In our study, the percentages of linoleic acids were unaffected by either alcohol or a high fat diet, probably indicating an adequate intake and absorption of linoleate. Linoleic acid represented 2.2% and 10.8% on dietary energetic intake of the groups on the standard diet and the high fat diet, respectively, and was considerably above the minimum requirement of 1.3% for rats (39). Our study also showed that the high fat intake in alcohol-treated rats increased 20:4*n*-6, 22:4*n*-6 and 20:4/18:2 ratio, and decreased 18:1*n*-9 in liver and serum phospholipids. Our results point that the fatty acid synthesis rates are dependent on the fat content of the diet.

The liver is one of the primary target organs for the metabolism of ingested alcohol, and the consequences of the metabolic response are often reflected in the serum components. Serum phospholipids are primarily formed and secreted by the liver. The lower PUFA in serum phospholipid in alcohol-treated rats (34% and 39%) as compared to that in the liver (50% and 50%) noted in the present study reflects a selective transfer and difference in phospholipid transport and metabolism into serum in alcohol-treated rats. The primary conclusion from the present study is that chronic alcohol intake decreased the liver phospholipid content. The data indicated that the alternations of the fatty acid composition in total liver and serum phospholipids in alcohol-treated rats (15–20% of total daily energy intake) did not show a tendency of polyunsaturated deficiency.

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