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EFFECT OF DIETARY FAT UPON ETHANOL METABOLISM IN RATS

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Abstract—The effect of dietary fat upon ethanol metabolism was studied in rats. Wistar strain male rats were divided into four groups according to diet, namely alcohol-high fat, alcohol-low fat, control-high fat, and control-low fat. After 4 weeks of feeding, blood ethanol levels following an intraperitoneal injection of 0.2 g ethanol/100 g of body weight were measured. The disappearance rate of blood ethanol was faster and the metabolic rate of ethanol was significantly greater in the alcohol-high fat group compared to the alcohol-low fat or non-alcoholic groups. Microsomal enzymes, such as the microsomal ethanol-oxidizing system, aniline hydroxylase, and glucose-6-phosphatase, were significantly higher in the alcohol-high fat group than in the alcohol-low fat or non-alcoholic groups. The ethanol uptake rate of the isolated perfused liver was increased significantly in the alcoholic groups. In the alcoholic rats, the high fat group showed significantly higher uptake than the low fat group. Although the ethanol uptake rate after 4-methylpyrazole treatment was not significantly different among the four groups, its fraction of the total ethanol uptake was increased significantly in the alcohol-high fat group. These results suggest that high fat diets accelerate ethanol metabolism through the microsomal ethanol-oxidizing system.

Recently, a direct hepatotoxic action of ethanol has been emphasized [1]. However, dietary factors also play a role in the development of alcoholic liver injury. Attention to dietary factors in the pathogenesis of alcoholic liver injury has focused on protein malnutrition [2]. The role of dietary fat has received less attention, except for the pathogenesis of fatty liver.

We recently became aware that acceleration of ethanol metabolism seen with long-term ingestion of alcohol becomes less clear when rats are fed a low fat diet. There are numerous reports concerning the relation between alcoholic fatty liver and dietary fat [3–5]. However, studies concerning ethanol metabolism and dietary fat are few [6], and the relationship between both factors remains obscure. In the present report, the effect of dietary fat upon ethanol metabolism was studied in rats.

MATERIALS AND METHODS

Animals. Ninety Wistar strain male rats, weighing about 200 g each, were divided into two groups according to diets, one receiving a liquid diet containing 36% of total calories from ethanol (alcoholic group) and the other a diet replaced equicalorically by sucrose (control group). Each group was further subdivided into two subgroups of high fat and low fat. Calories from fat were 36% (high) and 15% (low) of total calories. Consequently, the experimental groups were twenty-five rats fed alcohol-high fat (AL-HF), twenty-five rats fed alcohol-low fat (AL-LF), twenty rats fed control-high fat (C-HF), and twenty rats fed control-low fat (C-LF). As the protein source of the diets, an amino acid mixture, as shown in Table 1, was used, and corn oil was used as the fat source. The liquid diet contained 1 calorie/1 ml. The composition of the diet of each group is shown in Fig. 1. Each group of rats was group pair-fed with the alcohol-high fat group for 4 weeks. Ten rats of each group were used for the perfusion experiment with isolated liver. Blood ethanol clearance rates were measured in the remaining rats of each group.

Blood ethanol clearance. To determine the blood ethanol clearance rate, 0.2 g of ethanol/100 g of body weight, in 25% saline solution, was injected intraperitoneally into the overnight fasted rats, and blood was taken from the retro-orbital sinus at 30, 60, 120, 180, 240 and 300 min after the ethanol injection. Blood ethanol levels were estimated by gas chromatography according to the method of Roach and Creaven [7] using *t*-butanol as the internal standard.

Biochemical study of the liver. After the blood ethanol clearance determination, the rats of each

Table 1. Composition of the amino acid mixture

Essential	%	Non-essential	%
L-Lysine HCl	6.53	L-Glutamic acid	10.53
L-Arginine HCl	3.95	DL-Serine	2.63
DL-Tryptophan	1.05	Glycine	3.69
DL-Phenylalanine	4.74	DL-Tyrosine	14.74
DL-Leucine	8.43	L-Cystine	1.05
DL-Isoleucine	5.27	L-Proline	4.74
DL-Valine	7.37	L-Asparagine monohydrate	7.32
DL-Histidine HCl	2.84	DL-Alanine	6.32
DL-Methionine	3.53		
DL-Threonine	5.27		

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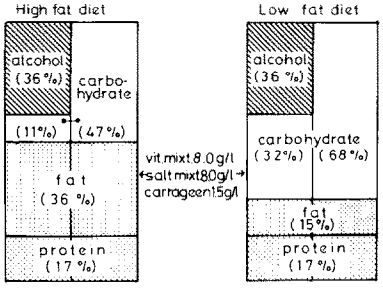

Fig. 1. Dietary compositions of the liquid diets. As the protein source, an amino acid mixture, as shown in Table 1, was used. Fat and carbohydrate of the diets were corn oil and sucrose.

group continued the same diet for 1 more week and were killed for the biochemical analysis of the liver after overnight fasting. The livers were homogenized in a 150 mM ice-cold KCl solution by a Teflon–glass homogenizer. The microsomal fraction was separated by ultracentrifugation according to the description of Hogeboom and Schneider [8]. Protein content of the liver and microsomal fraction was determined by the method of Lowry *et al.* [9]. Microsomal ethanol-oxidizing system (MEOS) activity, glucose-6-phosphatase (G-6-Pase) activity, aniline hydroxylase activity and cytochrome P-450 content in the microsomal fraction were estimated by the methods of Lieber and DeCarli [10], Swanson [11], Imai *et al.* [12] and Omura and Sato [13] respectively. Alcohol dehydrogenase (ADH) activity [14] in the supernatant fraction and triglyceride content [15] of the liver were also determined.

Perfusion of the isolated liver. Ten rats of each group were anesthetized by an intraperitoneal injection of nembutal. Teflon tubes which were connected to an extracorporeal perfusion unit (Ambec Co. Ltd., U.S.A.) were inserted into the portal vein and the inferior vena cava, and liver perfusion was started *in situ*. After ligation of the vena cava at the level of the diaphragm, the liver was isolated while continuing the perfusion. Liver perfusion was carried out in the closed circulating system with 250 ml of Hanks' balanced salt solution (pH 7.4) containing

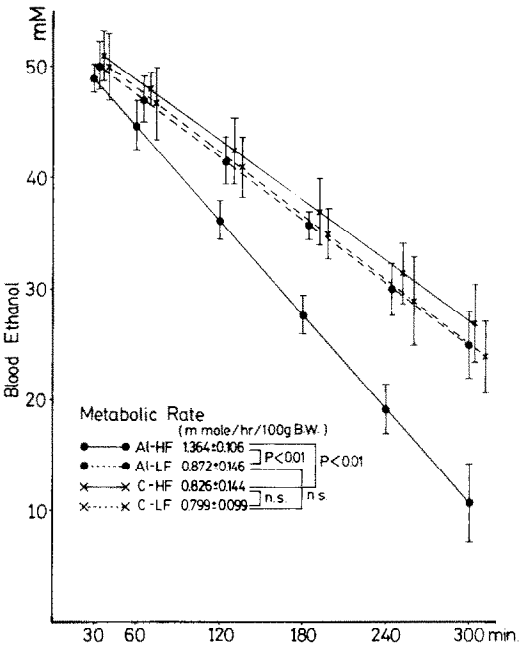

Fig. 3. Blood ethanol clearance rate after an i.p. injection of 0.2 g ethanol/100 g of body weight. The metabolic rate of ethanol was significantly greater in the alcohol-high fat group.

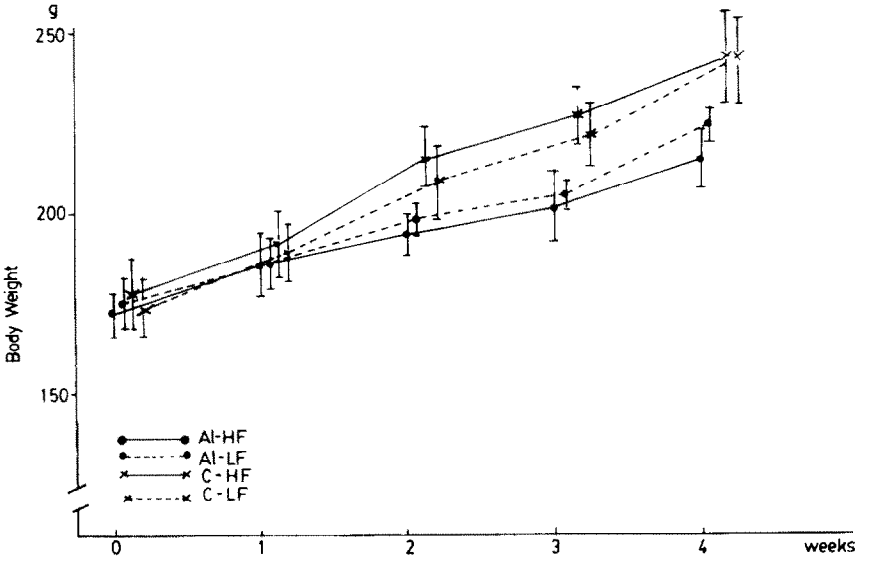

Fig. 2. Serial changes of body weight. Body weights at week 4 were not significantly different among the four groups. Abbreviations: AL-HF, alcohol-high fat group; AL-LF, alcohol-low fat group; C-HF, control-high fat group; and C-LF, control-low fat group. The same abbreviations will be used in the following figures.

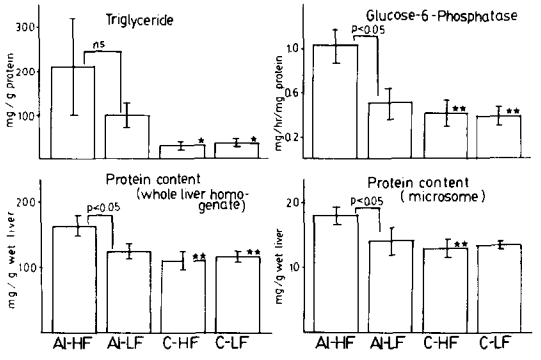

Fig. 4. Biochemical analysis of the liver (I). Key: (*) $P < 0.05$, and (**) $P < 0.001$ compared with the corresponding alcoholic groups.

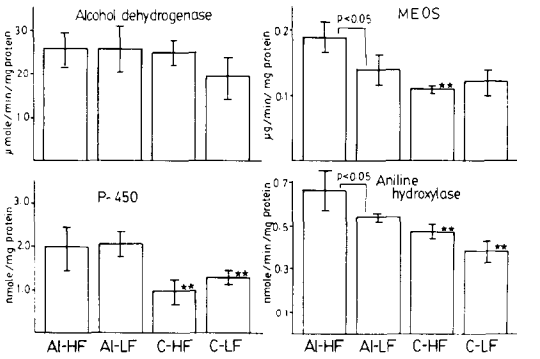

Fig. 5. Biochemical analysis of the liver (II). Key: (**) $P < 0.001$ compared with the corresponding alcoholic groups.

20% of human red blood cells and 100 mg of ampicillin at a rate of 24 ml/min, at 37°. The perfusate was oxygenated by carbogen gas at a rate of 0.3 ml/min.

Uptake rate of ethanol by the isolated liver. After 20 min of preperfusion, 25 mmoles of ethanol was added to 1000 ml of perfusate, and the ethanol concentration of the perfusate was measured by gas chromatography [7] at a constant interval. The uptake rate of ethanol was calculated from the serial changes of ethanol concentration of the perfusate. To measure the ethanol oxidation rate in the non-ADH pathway, the livers of some rats in each group were reperfused with Hanks' balanced solution for 10 min, and then 2 mmoles of 4-methylpyrazole was added to 1000 ml of the perfusate. After 20 min of the preperfusion, the ethanol uptake was determined again.

The condition of the isolated liver was monitored by bile flow. When constant bile flow (over 0.2 ml/hr) was not obtained, the observed results were excluded.

Statistics. All experimental values are expressed as mean ± standard deviation. Statistical analysis was done by Student's *t*-test.

RESULTS

Average daily dietary intake was 61 ml (61 calories). Serial changes of body weight in each group are shown in Fig. 2. All groups showed constant weight gain, although weight gain of alcoholic groups was smaller than that of non-alcoholic groups.

Blood ethanol clearance of each group is shown in Fig. 3. The disappearance rate of blood ethanol was clearly faster in the AL-HF group than in the other three groups. The metabolic rate of ethanol calculated from the blood ethanol level was significantly greater in the AL-HF group than in the other three groups. A significant difference in metabolic rate was not observed between the two low fat groups, and the two non-alcoholic groups.

Liver weight, calculated per 100 g of body weight, was significantly greater in the AL-HF group (3.7 ± 0.3 g) than in the other three groups (2.7 ± 0.1 g in AL-LF, 2.4 ± 0.1 g in C-HF, and 2.5 ± 0.1 g in C-LF). Among the three other groups, significant differences in liver weight were not observed.

Biochemical changes in the liver in each group are shown in Figs. 4 and 5. Hepatic triglycerides and cytochrome P-450 content were significantly higher in the two alcoholic groups than in the corresponding non-alcoholic groups. However, differences between high fat and low fat groups were not significant. Hepatic protein content, microsomal G-6-Pase and aniline hydroxylase activity were significantly higher in the alcoholic groups than in the corresponding non-alcoholic groups. The difference between high fat and low fat groups was also significant in the alcoholic groups, but not in the non-alcoholic groups. Microsomal protein content and MEOS activity were

Table 2. Metabolic rates of ethanol in the isolated perfused liver (nmoles/hr/100 g body wt)

Group	No. of rats	Total	4-methylpyrazole treatment			
			No. of rats	(A) before	(B) after	% of B/A
AL-high fat	8	1.09 ± 0.22*†	4	1.19 ± 0.24	0.54 ± 0.21	45.1 ± 7.9*
AL-low fat	10	0.89 ± 0.14†	3	0.97 ± 0.16	0.35 ± 0.12	36.2 ± 10.9
Con-high fat	7	0.74 ± 0.16	3	0.90 ± 0.10	0.29 ± 0.05	23.4 ± 6.1
Con-low fat	7	0.63 ± 0.08	3	0.79 ± 0.51	0.22 ± 0.11	28.8 ± 9.4

* $P < 0.05$ vs corresponding low fat group.
† $P < 0.05$ vs corresponding non-alcoholic group.

significantly higher in the AL-HF group than in the other three groups, and the difference between the two low fat groups was not significant. ADH activities were not significantly different among the four groups.

Metabolic rates of ethanol in the perfused liver in each group are shown in Table 2. In the alcoholic groups, the mean metabolic rates were 1.09 ± 0.22 mmoles per hr per 100 g of body weight in the high fat group and 0.89 ± 0.14 in the low fat group. These were significantly higher than those of the corresponding non-alcoholic groups. Differences between high fat and low fat groups were significant in the alcoholic rats, but not in the non-alcoholic rats. The ethanol metabolic rate after 4-methylpyrazole treatment tended to be high in the AL-HF group; however, differences among the four groups were not significant. The ratios of metabolic rates of ethanol through the pyrazole-insensitive pathway (non-ADH pathway) to total metabolic rate were calculated. The ratio in the AL-HF group was significantly higher than that in C-HF but not than that in AL-LF.

DISCUSSION

The precise mechanism of ethanol metabolism following chronic alcohol consumption has not been fully elucidated yet. The effect of the basal diet on ethanol metabolism is also an important problem to be clarified. In the present experiment, it was clearly shown that high fat diets accelerated the increase of ethanol metabolism due to chronic ingestion of ethanol. Recently, an alcohol-containing liquid diet described by DeCarli and Lieber [16] has been widely used for experiments in rats. Dietary composition in the high fat group in the present study was similar to that of DeCarli and Lieber [16]. Increased ethanol metabolism and induction of the microsomal enzymes including MEOS and the AL-HF group were compatible with our previous report [17] and with other reports [18, 19] in chronic alcoholic rats.

When skim milk is used as the protein source of the alcohol-containing liquid diets, fat content of the diet cannot exceed 15% of total calories, because of the high lactose content in skim milk. We have become aware that the increase in ethanol metabolism by chronic ethanol administration in rats becomes less clear when the liquid diet is prepared by skim milk. However, studies concerning the relationship between dietary fat and ethanol metabolism are very rare. In the present experiment, we tried to clarify the role of dietary fat in ethanol metabolism. Ishii *et al.* [6] reported that a reduction of dietary fat content did not influence the effect of ethanol on the hepatic endoplasmic reticulum, including MEOS activity, in a 6-week experiment with rats. However, they did not compare results between high fat (35%) and low fat (10%) groups. In the data reported by Ishii *et al.* [6], MEOS activity was higher in the 35%-fat alcoholic group than in the 10%-fat alcoholic group (7.00 ± 0.28 vs 5.4 ± 0.38 nmoles/mg protein), which may be compatible with the difference we found between AL-HF and AL-LF groups. In the present experiment, the AL-LF group showed higher cytochrome P-450 content, G-6-Pase activity and aniline hydroxylase activity in the microsome, and higher ethanol oxidation rate in the isolated perfused liver than the C-LF group, indicating that ethanol had an effect on the hepatic endoplasmic reticulum, even with a low fat diet. These results are compatible with those of Ishii *et al.* [6]. In the present experiment, the AL-HF group showed a significantly higher ethanol metabolic rate and higher microsomal enzyme activity compared to the AL-LF group, indicating that high fat diet accelerated the effect of ethanol on the hepatic endoplasmic reticulum. MEOS activity in the AL-LF group was not significantly different from that in the C-LF group in the present experiment. The discrepancy between MEOS activity in the present study and the results of Ishii *et al.* [6] might reflect the difference of the experimental model or the duration of ethanol feeding. In the experiment of Ishii *et al.* [6], the low-fat alcoholic group was fed *ad lib.* with a pair-fed control, while in the present experiment the group was pair-fed with a high-fat alcoholic group. Therefore, the impact of ethanol *ad lib.* may operate more strongly in the experiment of Ishii *et al.* [6]. In that experiment [6], a mixture of corn and olive oil was used as a source of fat, which may account for some differences. However, in our recent study [20], qualitative changes of dietary fat did not affect the blood ethanol clearance rate in chronic alcoholic rats, indicating that quantitative changes may be a more important factor in the acceleration of ethanol metabolism.

It has been shown that enhancement of ethanol metabolism by chronic ethanol administration is due mainly to an increase of ethanol oxidation in the non-ADH pathway, probably by an increase of MEOS activity [17–19]. The present study also indicates that acceleration of ethanol metabolism by a high fat diet was attributable to the increased oxidation rate of ethanol in the non-ADH pathway, because both MEOS activity and pyrazole-insensitive oxidation rate of ethanol were increased in the AL-HF group.

It has been reported that variation in the content and composition of the dietary fat markedly affects the drug-metabolizing enzyme activities in the microsome [21, 22]. Wade and Norred [21] pointed out that maximal drug-metabolizing activity occurred with a 10% corn oil diet. In the present experiment, the low fat diet contained 15% corn oil, and high and low fat diets in the non-alcoholic rat did not show a difference in ethanol metabolic rate and microsomal enzyme activities. These results indicate that the dietary fat content used in the present experiment did not influence the induction of microsomal enzymes in the liver. Marshall and McLean [23] reported that polyunsaturated fatty acids have little inducing power on their own, but permit massive increases in the quantity of cytochrome P-450 induced by phenobarbitone. They called this a permissive effect. The same effect migh occur in high fat diets in the livers of rats given ethanol chronically.

Recently, Teschke *et al.* [24] reported that a low carbohydrate content of the diet by itself increased MEOS activity and accelerated the inductive effect of ethanol on MEOS. In the present study, the high fat diet had a low carbohydrate content. Therefore, low carbohydrate content may partially explain the

acceleration of ethanol metabolism by the high fat diet. However, the main factor was the high fat content, since an increase of MEOS activity was not observed in the non-alcoholic rats fed a high fat diet.

Although the actual mechanism of the toxic effect of ethanol on the liver is still obscure, the effect of acetaldehyde overproduced in the liver by increased ethanol oxidation has been proposed as one of the causes of the hepatotoxicity of ethanol [25]. It seems to us that acceleration of ethanol oxidation in the non-ADH pathway by a high fat diet might also be harmful to the liver by producing a large amount of acetaldehyde. Therefore, high fat diet should be considered as an enhancing factor of alcoholic liver injury, apart from the pathogenesis of fatty liver.

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