Gender Differences in the Response of Hepatic Fatty Acids and Cytosolic Fatty Acid-Binding Capacity to Alcohol Consumption in Rats (43293)

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Abstract. **To investigate possible gender differences in the response of hepatic fatty acids and cytosolic fatty acid-binding capacity to ethanol consumption, both female and male rats (41 days of age) were pair fed liquid diets (with a littermate of the same sex) for 28 days. The diets contained 36% of energy either as ethanol or as additional carbohydrate. After ethanol feeding, the hepatic concentration of fatty acids increased 155% in females ($P < 0.01$), whereas there was only a trend for an increase (22%) in males. This was associated with a much smaller increase of cytosolic fatty acid-binding capacity in females (58%) than in males (161%). Whereas the ethanol-induced increase in fatty acid-binding capacity provided an ample excess of binding sites for the fatty acids in the males, the increase in females was barely sufficient for the binding of the large increase of fatty acids produced by ethanol in the females. The cytosolic protein responsible for this binding, the liver fatty acid-binding protein of the cytosol (L-$FABP_c$), also promotes esterification of the fatty acids. In keeping with the postulated role of this protein, the ethanol-induced increases in hepatic triacylglycerols, phospholipids, and cholesterol esters were smaller in females than in males. The gender difference in cholesterol esters was associated with parallel changes in acyl-CoA transferase activity. A possible implication of the relatively small and most likely inadequate increase in liver fatty acid-binding capacity and fatty acid esterification during alcohol consumption in the females is that under these circumstances the risk for development of a potentially deleterious accumulation of fatty acids in the liver is increased, thereby contributing to the enhanced vulnerability of females to alcohol-induced hepatotoxicity.**

[P.S.E.B.M. 1991, Vol 198]

Ethanol inhibits hepatic oxidation of fatty acids (1) and could promote accumulation of potentially toxic nonesterified fatty acids. Normally, however, a hepatic cytosolic fatty acid-binding protein (L-$FABP_c$) attenuates such an accumulation by binding fatty acids and promoting their esterification (2). Consistent with this possibility, we found that chronic ethanol administration to male rats produced only a small increase in the hepatic concentration of fatty acids (3), despite strong inhibition of fatty acid oxidation. This was associated with marked increases in both the cytosolic capacity to bind fatty acids and the content of L-$FABP_c$. However, previous results in female rats fed similar ethanol-containing diets showed no effects of alcohol feeding on what was then called Z protein (4), which is now considered to be identical with the L-$FABP_c$ (5). We wondered, therefore, whether there is a gender difference in the response of fatty acid concentration and cytosolic binding capacity to alcohol consumption.

Materials and Methods

Materials. [1-^{14}C]Palmitic acid (58 mCi/mmol), [9,10-^{3}H]oleic acid (5 Ci/mmol), and [1-^{14}C]palmitoyl-CoA (50–60 mCi/mmol) were purchased from the Amersham Corporation (Arlington Heights, IL), had a radiochemical purity greater than 98%, and were used

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Received February 14, 1991. [P.S.E.B.M. 1991, Vol 198]
Accepted May 9, 1991.

0037-9727/91/1981-0584$3.00/0

fresh. With the exception of Lipidex 1000 and glyceryl diheptadecanoate, which were obtained from the Packard Instruments Co. (Downers Grove, IL) and Nu Chek Prep (Elysian, MN), respectively, all other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO) and were at least 98% pure. All the organic solvents were of a chromatographic grade of purity. The liquid diets were purchased from Dyets (Bethlehem, PA). They contain 1% fiber, 18% of energy as protein, 35% as fat, 11% as carbohydrate, and 36% either as ethanol or additional carbohydrate. The fatty acid composition of the diets consisted primarily of oleic (53%), linoleic (28%), and palmitic (13%) acids. Further details on the composition of these diets has been reported previously (6).

Animal Procedures. Animal experimentation was carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (7). Seventy weanling rats (21 days old), 38 males and 32 females, of a Sprague-Dawley strain (CRL-CR[SD]-BR) were purchased from Charles River Breeding Laboratories (Wilmington, MA) and fed *ad libitum* Rat Chow 5012 diet (Ralston Purina Co., St. Louis, MO). At 41 days of age, they were housed in individual cages and pair fed the liquid diets described above for 4 weeks. The day prior to sacrifice, they received one third of their usual daily intake at 9:00 AM and two thirds at 5:00 PM. The following morning they were given, by gastric tube, 6 ml of the corresponding diets per 100 g of body weight, 90 min before the sacrifice. In the case of the alcohol-containing diet, this corresponds to an acute ethanol dose of 3 g/kg body wt.

Under pentobarbital anesthesia (40 mg/kg, ip), the abdomen was opened and a liver lobe was freeze-clamped between aluminum prongs cooled in liquid nitrogen and stored at −80°C for fatty acid determination. The remainder of the liver was excised and thoroughly cleared of blood by reverse perfusion through hepatic vein branches with 40–50 ml of a cold isotonic buffer solution, consisting of 0.25 *M* sucrose, 1.0 m*M* EDTA, and 5.0 m*M* potassium phosphate buffer (pH 7.4). A portion was weighed and stored frozen at −80°C for further lipid analyses. The remaining liver was weighed and homogenized in 5 vol of 0.25 *M* sucrose for preparation of cytosol and microsomes.

The homogenate was centrifuged at 4°C for 15 minutes at 15,000*g*. The postmitochondrial supernatant was aspirated in order to minimize contamination by floating fat and recentrifuged at 100,000*g* for 1 hr at 4°C. The supernatant (cytosol) was stored frozen at −20°C. The microsomal pellet was resuspended in 2.5 vol of the same solution, containing, in addition, 1.0 m*M* dithiothreitol, and recentrifuged at 100,000*g* for 30 min. The washed microsomal pellet was resuspended in 1–1.5 ml of 0.25 *M* sucrose per gram of liver and stored at −80°C until assayed. To assess the losses of cytosol during fractionation, lactic dehydrogenase was used as a marker; its activity was measured (8) in the homogenates and the cytosol, which was diluted 1/200–400 (without the use of detergents) and expressed as μmoles of NADH produced per minute. Cytosolic proteins were calculated by dividing the lactic dehydrogenase activity in the homogenate (per gram of liver) by the specific activity of the cytosol (per milligram of cytosolic protein). The recovery of microsomes was assessed using the activity of glucose 6-phosphatase (9), as described previously (10).

Analytical Procedures. Liver lipids were extracted according to Folch *et al.* (11). Phospholipids were measured by the method of Bartlett (12). After separation by thin layer chromatography (13), triacylglycerols were measured by the method of Snyder and Stephens (14) and the free and esterified fractions of cholesterol were measured by the methods of Searcy and Berquist (15) as modified by Zak and Epstein (16).

Fatty acids were extracted by a modified Folch *et al.* (11) procedure and measured by gas liquid chromatography, as reported previously (3). Approximately 0.5 g of frozen liver was homogenized in 7 vol of chloroform containing 25 μg each of pentadecanoic acid (for assessing the recovery of fatty acids) and heptadecanoyl esters of glycerol (mono-, di-, and triacylglycerols), cholesterol, phosphatidylcholine, and lysophosphatidylcholine (for the assessment of *ex vivo* lipolysis). Then, a half-volume of methanol was added to form a single 2/1 (v/v) chloroform/methanol phase. The homogenate was centrifuged for 20 min at 250*g* and supernatant aliquots were dried under a N_2 stream and solubilized in chloroform, and the lipids were separated by thin layer chromatography using hexane/ethyl ether/acetic acid (83/16/1, v/v/v) as a solvent system. The plates were prewashed with chloroform/methanol (4/1, v/v) overnight, then heat activated at 100°C for 1 hr and cooled prior to use. The lipid classes were identified by spraying with 0.25% rhodamine B in ethanol (17) by co-migration with appropriate standards. The fatty acid band was scraped off, extracted in chloroform/methanol (2:1, v/v), and dried under N_2. The residue was methylated at 100°C for 2 min in a 3-ml mixture of equal volumes of isooctane, methanol, and 14% (w/v) boron trifluoride in methanol. After the addition of 6 ml of hexane and 3 ml of distilled water, the methyl fatty acid esters were extracted in the hexane phase (18, 19), which was dried and stored at −20°C under N_2. The methylated fatty acids were measured, within 48 hr, in a Hewlett Packard 5840-A gas liquid chromatography apparatus, using a 6′ × 1/8″ glass column packed with 10% SP-2330 cyanosilicone on 100/120 chromosorb W-AW. The temperatures of the injector, column, and flame ionization detector were 240°, 186°, and 260°C, respectively. The carrier gas

used was N_2, the flow of which was maintained at a rate of 25 ml/min. Peaks were identified and quantified by comparison with standards of methyl fatty acid esters, and the amounts were corrected by the recovery of the pentadecanoic internal standard. Appearance of detectable amounts of heptadecanoic acid was considered evidence for *ex vivo* lipolysis and an indication for rejection of the sample.

To assess liver cytosolic fatty acid-binding capacity, Lipidex 1000 was used for both delipidation and assay of fatty acid binding, as described by Glatz and Veerkamp (20). For delipidation, 1 ml of cytosol (7–10 mg of protein) was mixed with Lipidex (1.5 g in 15–20 ml) equilibrated with 10 m*M* potassium phosphate buffer (pH 7.4), and incubated at 37°C for 1 hr. Proteins were eluted through a Buchner funnel and concentrated by ultrafiltration using a Diaflo YM-5 membrane (Amicon Corp., Danuers, MA). To assess the completion of the delipidation, a tracer dose of [^{3}H]oleate was incorporated into the cytosol. The procedure was repeated if delipidation was not complete after the first incubation. The aqueous solutions of labeled fatty acids were prepared fresh by saponification in ethanol, followed by evaporation of the ethanol (20). For assay of fatty acid binding, delipidated samples containing 20 μg of protein were incubated in polyethylene vials with various concentrations of [^{14}C]palmitate (0.05–2.0 μM) in 0.45 ml of 10 m*M* potassium phosphate buffer (pH 7.4) at 37°C for 10 min. After incubation for 10 min at 0°C, the unbound fatty acids were bound to Lipidex by addition of 0.05 ml of an ice-cold suspension in the same buffer (1/1, v/v) and sedimented by centrifugation. Fatty acid binding was calculated from the radioactivity of the supernatants and the specific activity of palmitate and expressed as picomoles of fatty acid bound per microgram of cytosolic protein. The maximal binding capacity (B_{max}) was determined by computer-assisted Scatchard's plot analysis. Since fatty acid binding to a single cytosolic protein with the molecular weight similar to that of L-FABP$_c$ was observed on gel filtration (3), B_{max} provides an estimate of the amount of L-FABP$_c$, as confirmed by immunochemical assay (3).

Protein concentrations were determined by the method of Lowry *et al.* (21), after precipitation of the samples in 5% trichloroacetic acid, using bovine serum albumin as the standard. Acyl-CoA acyltransferase activity was determined by method of Einarsson *et al.* (22).

Statistics. All the results are expressed by their means ± SE. The significance of the differences was calculated by two-way analysis of the variance, using gender and alcohol consumption as the two factors, followed by *post hoc* Newman-Keuls tests for the assessment of individual differences (23). The significance of the percentage changes induced by ethanol in each gender was compared by Student's *t* test (23).

Results

Effect of Gender and Ethanol Consumption on Body and Liver Weight. Female rats were smaller and grew less than the males of the same age (Table I). The liver to body weight ratios tended to be slightly higher in the females than in the males. The amount of ethanol consumed per kilogram of body weight was similar in both genders. Ethanol-fed rats tended to gain less weight than the pair-fed controls, despite isocaloric feeding. Compared to their respective pair-fed littermates, the weight of the liver per 100 grams of body weight increased by 31 ± 3% in the ethanol-fed males and by 26 ± 3% in the ethanol-fed females ($P < 0.05$, by Student's *t* test).

Effects of Gender and Ethanol Consumption on Hepatic Fatty Acids. In the rats pair fed the control diets, there were no gender differences in the concentration and composition of fatty acids (Table II). However, ethanol feeding in females resulted in a 155% increase in the hepatic concentration of fatty acids, whereas no significant changes occurred in the males. Both genders responded to ethanol feeding with a similar change in fatty acid composition, namely an increase in oleic and linoleic acids and a decrease in palmitic acid.

Effects of Gender and Ethanol Consumption on Fatty Acid Binding. In the pair-fed control rats, the cytosolic binding capacity was 38% higher in the fe-

Table I. Effects of Gender and Ethanol Consumption on Body and Liver Weight[a]

	Males		Females	
	Alcohol-fed rats	Pair-fed controls	Alcohol-fed rats	Pair-fed controls
Initial body wt (g)[b]	162 ± 3	162 ± 4	143 ± 5	143 ± 6
Ethanol consumption (g/kg/day)[c]	12.1 ± 0.6	—	11.6 ± 0.4	—
Body wt gain (g/day)[d]	2.6 ± 0.3	3.1 ± 0.2	1.4 ± 0.1	1.6 ± 0.1
Liver wt (g/100 g body wt)[e]	4.2 ± 0.1	3.2 ± 0.1	4.4 ± 0.1	3.5 ± 0.1

[a] Mean ± SE of 38 male and 32 female rats of the same age (41 days) pair fed (with littermates of the same gender) liquid diets containing 36% of energy, either as ethanol or as additional carbohydrate, for 28 days.
[b] Significant difference by gender ($P < 0.001$), but not by ethanol feeding (two-way analysis of variance).
[c] Nonsignificant difference.
[d] Significant effect of both gender ($P < 0.001$) and ethanol ($P < 0.02$), with no significant interaction.
[e] Significant effect of both gender ($P < 0.01$) and ethanol ($P < 0.001$), with no significant interaction.

Table II. Effects of Gender and Ethanol Consumption on Nonesterified Fatty Acids[a]

	Males		Females	
	Alcohol-fed rats	Pair-fed controls	Alcohol-fed rats	Pair-fed controls
Total fatty acids (nmols/g of liver)[b]	235.6 ± 24.3	193.3 ± 23.4	526.0 ± 143.6	206.0 ± 41.9
C16:0 (%)[c]	28.6 ± 1.8	52.9 ± 4.4	28.0 ± 2.1	47.1 ± 4.8
C16:1 (%)	4.1 ± 0.7	5.1 ± 1.2	0.4 ± 0.3	ND[d]
C18:0 (%)	12.4 ± 1.1	13.2 ± 0.7	11.5 ± 1.5	15.5 ± 2.3
C18:1 (%)[c]	42.0 ± 2.6	25.1 ± 2.8	49.0 ± 2.5	23.3 ± 4.9
C18:2 (%)[c]	12.4 ± 1.6	7.8 ± 1.5	13.1 ± 2.0	8.5 ± 1.4
C20:4 (%)	3.5 ± 1.5	2.5 ± 1.8	1.2 ± 0.1	0.4 ± 0.1

[a] Mean ± SE of 32 male and 20 female rats of the same age (41 days) pair fed (with littermates of the same gender) liquid diets containing 36% of energy, either as ethanol or as additional carbohydrate, for 28 days.
[b] Significant effect of gender ($P < 0.01$), ethanol ($P < 0.001$), and interaction ($P < 0.01$) by two-way analysis of variance.
[c] Significant effect of ethanol ($P < 0.05$), but not of gender.
[d] ND, nondetectable.

males than in the males, although this difference did not reach a level of statistical significance (Table III). However, after chronic ethanol administration, the Scatchard's plots of the palmitate binding by delipidized cytosolic proteins revealed a marked increase in the number of binding sites (B_{max}) in the males, whereas there was an increase of smaller magnitude in the females. The recovery of cytosol was similar in both genders, as assessed by the recovery of lactic dehydrogenase activity (97 ± 10% in males vs 95 ± 2% in females; not significant), and unaffected by chronic ethanol administration (90 ± 6% in males vs 92 ± 2% in females; not significant. When compared to the concentration of fatty acids (Table II), the maximal binding capacity of the cytosolic proteins greatly exceeded the concentration of fatty acids in all experimental groups, except the alcohol-fed females, in which the mean cytosolic binding capacity was similar to the concentration of fatty acids. The increase in binding capacity produced by ethanol consumption in the male rats was associated with a decreased affinity, as judged by the increased dissociation constant (K_d).

Effects of Gender and Ethanol Consumption on Hepatic Glycerolipids. There was also a gender difference in the hepatic concentration of glycerolipids and in their response to ethanol feeding (Table IV). In the pair-fed controls, the concentration of triacylglycerols was higher and that of phospholipids tended to be lower in the females than in the males. Compared with their respective controls, ethanol feeding resulted in a 3- to 4-fold increase of the hepatic triacylglycerol concentration in the males versus a 2-fold increase in the females. Also, there was a trend for an ethanol-induced increase in phospholipid concentration in the males, but not in the females, although this difference did not reach a level of statistical significance. In general, the gender differences in the response of the hepatic esterified fatty acids to alcohol consumption were opposite to those of the nonesterified fatty acids.

Effects of Gender and Ethanol Consumption on Hepatic Cholesterol Esterification. In the controls, the concentration of free cholesterol was higher and that of cholesterol esters was lower in the females than in the males (Table V). Again, a striking gender difference occurred in the response of cholesterol esters to ethanol consumption; whereas there was a 3- to 4-fold increase in cholesterol esters in the alcohol-fed males, there was no significant change in the alcohol-fed females, when compared with their respective pair-fed controls. Hepatic free cholesterol concentrations remained unaffected by ethanol feeding in either sex. The gender difference in the capacity of ethanol to produce

Table III. Effects of Gender and Ethanol Consumption on Fatty Acid Binding by Liver Cytosol[a]

	Males		Females	
	Alcohol-fed rats	Pair-fed controls	Alcohol-fed rats	Pair-fed controls
B_{max} (nmol/g of liver)[b]	630 ± 113	241 ± 28	525 ± 62	332 ± 38
K_d (nM)[c]	1.29 ± 0.20	0.32 ± 0.04	0.64 ± 0.15	0.66 ± 0.10

[a] Mean ± SE of 12 male and 12 female rats of the same age (41 days) pair fed (with littermates of the same gender) liquid diets containing 36% of energy, either as ethanol or as additional carbohydrate, for 28 days.
[b] Significant effect of ethanol ($P < 0.01$) on the maximal binding capacity (B_{max}) by two-way analysis of variance. *Post hoc* Newman-Keuls tests indicate significant effect of ethanol consumption in both males ($P < 0.01$) and females ($P < 0.05$).
[c] Significant effect of ethanol ($P < 0.01$) on the dissociation constant (K_d) by two-way analysis of variance. *Post hoc* Newman-Keuls tests indicate that this effect was significant ($P < 0.01$) only in the males.

Table IV. Effects of Gender and Ethanol Consumption on Glycerolipids[a]

	Males		Females	
	Alcohol-fed rats	Pair-fed controls	Alcohol-fed rats	Pair-fed controls
Triacylglycerols (mg/g of liver)[b]	53.0 ± 5.1	14.9 ± 1.6	52.5 ± 5.4	24.8 ± 4.0
Phospholipids (mg/g of liver)[c]	32.1 ± 4.4	27.2 ± 1.7	25.2 ± 1.0	24.4 ± 1.0

[a] Mean ± SE of 32 male and 32 female rats of the same age (41 days) pair fed (with littermates of the same gender) liquid diets containing 36% of energy, either as ethanol or as additional carbohydrate, for 28 days.
[b] Significant effect of ethanol ($P < 0.001$), but not gender (two-way analysis of variance).
[c] Significant effect of gender ($P < 0.05$), but not ethanol (two-way analysis of variance).

Table V. Effects of Gender and Ethanol Consumption on Cholesterol Esterification[a]

	Males		Females	
	Alcohol-fed rats	Pair-fed controls	Alcohol-fed rats	Pair-fed controls
Free cholesterol (mg/g liver)[b]	1.4 ± 0.2	1.6 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Cholesterol esters (ng/g liver)[c]	2.78 ± 0.27	0.81 ± 0.13	0.86 ± 0.14	0.52 ± 0.09
Acyl-CoA transferase (pmol/min/mg protein)[d]	62.8 ± 13.7	39.6 ± 4.4	30.7 ± 2.5	29.6 ± 2.4

[a] Mean ± SE of 20 male and 20 female rats of the same age (41 days) pair fed (with littermates of the same gender) liquid diets containing 36% of energy, either as ethanol or as additional carbohydrate, for 28 days.
[b] Significant effect of gender ($P < 0.01$), but not of ethanol.
[c] Significant effects of gender ($P < 0.01$), ethanol ($P < 0.001$), and interaction ($P < 0.001$). *Post hoc* Newman-Keuls test showed a significant effect of ethanol in males ($P < 0.01$), but not in the females.
[d] Significant effects of gender ($P < 0.01$) and ethanol ($P < 0.05$) without interaction.

hepatic accumulation of cholesterol esters was associated with increased microsomal acyl-CoA transferase activity in the males (62.8 ± 13.7 pmol/min/mg microsomal protein vs 39.6 ± 4.4, in male controls; $P < 0.01$), but not in the females (30.7 ± 2.5 vs 29.6 ± 2.4, in controls; not significant). The recovery of microsomes, as judged by the recovery of glucose 6-phosphatase, was similar in both genders (30.3 ± 0.9% in males vs 28.4 ± 1.7% in females; not significant) and was not significantly affected by ethanol treatment (31.8 ± 2.0% in males vs 30.7 ± 0.7 in females; not significant).

Discussion

Our results show striking gender differences in the effect of chronic ethanol administration on the hepatic concentration of fatty acids, their major esters (triacylglycerols, phospholipids, and cholesterol esters), and the capacity of cytosolic proteins to bind fatty acids.

One of the most conspicuous effects of alcohol consumption is the accumulation of triacylglycerols, phospholipids, and cholesterol esters in the liver (24), but much less attention has been paid to the hepatic concentrations of their precursor fatty acids. This is due, at least in part, to their low concentration and the possibility that the latter could be dramatically affected by *ex vivo* hydrolysis of the large amount of glycerolipids and cholesterol esters accumulated. However, this possibility was not substantiated in this as well as in previous studies (3, 18); the addition of either a mixture of esters of heptadecanoic acid (a fatty acid normally absent in the rat liver) or labeled triolein (3) revealed no detectable *ex vivo* lipolysis under the conditions used.

Previous studies in a similar model of chronic alcohol administration (25) showed that the fatty acids which accumulate in the liver originate from dietary fatty acids, rather than from endogenous sources. In the present study, the fatty acid composition was similar in both genders, and the changes induced by ethanol were in the direction of the fatty acid content of the liquid diet. Thus, it is unlikely that the gender differences in fatty acids could be ascribed to differences in *de novo* synthesis. One of the most consistent effects of ethanol is its interference with mitochondrial fatty acid oxidation (26). If the increased accumulation of fatty acids in the female rats was due primarily to a gender difference in oxidation, one would expect a greater accumulation of fatty acid esters, but actually the opposite occurred: triacylglycerols, phospholipids, and cholesterol esters increased less in the female than in the male rats fed alcohol-containing diets. Although in the present series of male rats, the ethanol-induced increase in phospholipids did not reach a level of statistical significance, larger series of male rats similarly treated with ethanol-containing diets have previously shown the increase in phospholipids to be significant (24). Moreover, the secretion of triglycerides into the plasma is more enhanced by ethanol in male than in

female rats (27). Thus, this gender difference in the response to ethanol is most likely due to a difference in fatty acid esterification.

Fatty acids bind to L-FABP$_c$, a low molecular weight protein that is abundant in liver cytosol (2). In the present study, the maximal fatty acid-binding capacity of the delipidated cytosol was measured by the Lipidex 1000 procedure of Glatz and Veerkamp (20). We found the cytosolic binding capacity to be strikingly increased in the alcohol-fed males, while there was a smaller increase in the alcohol-fed female rats. It has been reported recently (28) that significant binding of oleate and of purified rat heart FABP (with an amino acid sequence and length distinct from L-FABP$_c$) to the walls of glass, polypropylene, or polystyrene vials can occur. This could result in a substantial overestimation of K_d and an underestimation of B_{max}. However, the procedures used in that study (28) differ from those in the present investigation: the fatty acid and the binding protein used were different, the incubation times were significantly shorter, and the volume of the present assay mixture was nearly double (decreasing the surface to volume ratio). We also used polyethylene tubes, as recommended in the original procedure by Glatz and Veerkamp (20). Using these tubes, recovery of [^{14}C] palmitate from the assay tube mixture ranged from 91 to 95%. Moreover, when we compared the binding capacity of the delipidized cytosol (B_{max}) using the procedure as originally described, it correlated and actually provided a close estimate of the immunologically determined concentration of L-FABP$_c$ in both alcohol-fed and pair-fed control male rats (3). We do not know why the affinity for palmitate binding of the cytosol from alcohol-fed males decreased (and K_d increased) as the apparent number of binding sites (B_{max}) increased in alcohol-fed male rats. We showed previously by gel filtration that virtually all the fatty acid bound eluted in a single protein peak with a molecular weight similar to that of L-FABP (3), whereas the binding to cytosolic albumin was negligible even after the increase of this protein in the cytosol of alcohol-fed rats (29). Since FABP binds a variety of anions other than fatty acids, it is possible that these have been incompletely removed by delipidation with Lipidex, resulting in an apparent decrease in affinity for palmitic acid.

Several studies have shown that untreated female rats have higher levels of both L-FABP$_c$ and its mRNA than males (30, 31). A similar gender difference was observed in the present study (Table III). However, the capacity of several drugs to increase fatty acid binding in female rats has been found to be about half of that in males (32), suggesting that females may respond differently than males to agents that increase the hepatic content of L-FABP$_c$. The present finding of a considerable increase of the cytosolic capacity to bind fatty acids after chronic ethanol administration in male rats, but not in female rats, suggests that a similar gender difference pertains to the ability of ethanol to increase this protein. This may also explain why previous studies using this model of alcohol administration in female rats failed to detect significant effects on Z protein (4), which at present is considered to be identical to the L-FABP$_c$. The hormonal mediation of these changes in normal animals has not been fully established (33). Therefore, it is difficult to speculate on the possible role of the ethanol-induced hormonal changes (34).

There is evidence that L-FABP$_c$ stimulates fatty acid esterifying activities of the microsomes (35, 36). The relationship between the changes in cytosolic fatty acid-binding and the accumulation of the major fatty acid esters, found in the present study, is consistent with this possibility. The apparent changes in esterification did not affect uniformly the major ester classes, suggesting that primary gender differences in some of the esterifying enzyme activities may affect the degree of accumulation after ethanol. In the present study, a gender difference in the ethanol-induced changes of acyl-CoA acyltransferase activity, noted previously by others (37), was associated with a prominent gender difference in accumulation of cholesterol esters.

Females are known to be especially vulnerable to the development of alcohol-induced liver injury (34, 38). We found recently that one factor that could contribute to this greater vulnerability was increased bioavailability of ethanol in the females (39), but other mechanisms could also be involved. Fatty acids, as well as their CoA esters, are potentially deleterious compounds (40, 41) and their concentrations are maintained at very low levels by powerful homeostatic mechanisms, such as oxidation or esterification to glycerolipids and cholesterol esters. The observed gender difference in terms of the ethanol-induced accumulation of fatty acids and their esters is consistent with one of the postulated roles of L-FABP$_c$, namely to protect against detrimental accumulation of fatty acids (2). Indeed, contrasting with the excess of fatty acid binding sites available in males, the increase in these sites after ethanol administration in females was barely enough to bind the excess of fatty acids available, thereby resulting in increased vulnerability to fatty acid toxicity. If a similar gender difference occurs in human subjects, this could contribute to the increased vulnerability of women for the development of alcoholic liver injury.

This study was supported by the Department of Veterans Affairs and DHHS Grants AA-03508 and AA-07275.

The expert technical assistance of L. Schoichet and N. C. Bailey are gratefully acknowledged.

1. Lieber CS, Pignon J-P. Ethanol and lipids. In: Fruchart JC, Shepherd J, Eds. Human Plasma Lipoproteins: Chemistry, Phys-

iology and Pathology. Berlin, New York: Walter De Gruyter and Co., p245–280, 1989.
2. Ockner RK. Historic overview of studies on fatty acid-binding proteins. Mol Cell Biochem **98**:3–9, 1990.
3. Pignon JP, Bailey NC, Baraona E, Lieber CS. Fatty acid-binding protein: A major contributor to the ethanol-induced increase in liver cytosolic proteins in the rat. Hepatology **7**:865–871, 1987.
4. Reyes H, Levi AJ, Gatmaitan Z, Arias IM. Studies on Y and Z, two hepatic cytoplasmic organic anion-binding proteins: Effects of drugs, chemicals, hormones, and cholestasis. J Clin Invest **50**:2242–2252, 1971.
5. Glatz JFC, van der Vusse GJ. Cellular fatty acid-binding proteins: Current concepts and future directions. Mol Cell Biochem **98**:237–251, 1990.
6. Lieber CS, DeCarli LM. Liquid diet technique of ethanol administration: 1989 update. Alcohol Alcohol **24**:197–211, 1989.
7. Guide for the Care and Use of Laboratory Animals. Bethesda, MD: Office of Science and Health Reports, DRR/NIH, 1985; DHEW publication no. (NIH) 86-23.
8. Bergmeyer H-U, Bernt E, Hess B. Lactate dehydrogenase. In: Bergmeyer H-U, Ed. Methods of Enzymatic Analysis. New York: Academic Press, pp736–741, 1963.
9. Harper AE. Glucose-6-phosphatase. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis. New York: Academic Press, pp788–792, 1963.
10. Ishii H, Joly J-G, Lieber CS. Increase of microsomal glucose-6-phosphatase activity after chronic ethanol administration. Metabolism **22**:799–806, 1973.
11. Folch J, Lees M, Sloane-Stanley GH. A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem **226**:497–509, 1957.
12. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem **234**:466–468, 1959.
13. Amenta JD. A rapid chemical method for quantitation of lipids separated by thin-layer chromatography. J Lipid Res **5**:270–272, 1964.
14. Snyder F, Stephens N. A simplified spectrophotometric determination of ester groups in lipids. Biochim Biophys Acta **34**:244–245, 1959.
15. Searcy RL, Berquist LM. A new color reaction for the quantitation of serum cholesterol. Clin Chim Acta **5**:192–199, 1960.
16. Zak B, Epstein E. New cholesterol reagent. Clin Chem **7**:268–270, 1961.
17. Jones D, Bowyer DE, Gresham GA, Howard AN. An improved spray reagent for detecting lipids on thin-layer chromatograms. J Chromatogr **23**:172–174, 1966.
18. Mavrelis PG, Ammon HV, Gleysteen JJ, Komorowski RA, Charaf UK. Hepatic free fatty acids in alcoholic liver disease and morbid obesity. Hepatology **3**:226–231, 1983.
19. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res **5**:600–608, 1964.
20. Glatz JFC, Veerkamp JH. A radiochemical procedure for the assay of fatty acid binding by proteins. Anal Biochem **132**:89–95, 1983.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
22. Einarsson K, Benthin L, Ewerth S, Hellers G, Stahlberg D, Angelin B. Studies on acyl-coenzyme A: Cholesterol acyltransferase activity in human liver microsomes. J Lipid Res **30**:739–746, 1989.
23. Snedecor GW, Cochran WG. Statistical Methods, Seventh Edition. Ames, IA: The Iowa State University Press, 1980.
24. Lieber CS, Jones DP, DeCarli LM. Effects of prolonged ethanol intake: Production of fatty liver despite adequate diets. J Clin Invest **44**:1009–1021, 1965.
25. Lieber CS, Spritz N, DeCarli LM. Role of dietary, adipose and endogenously synthesized fatty acids in the pathogenesis of the alcoholic fatty liver. J Clin Invest **45**:51–62, 1966
26. Lieber CS, Schmid R. The effect of ethanol on fatty acid metabolism: Stimulation of hepatic fatty acid synthesis *in vitro*. J Clin Invest **40**:394–399, 1961.
27. Hernell O, Johnson O. Effects of ethanol on plasma triglycerides in male and female rats. Lipids **8**:503–508, 1973.
28. Vork MM, Glatz JFC, Surtel DAM, van der Vusse GJ. Assay of the binding of fatty acids by proteins: Evaluation of the Lipidex 1000 procedure. Mol Cell Biochem **98**:111–117, 1990.
29. Baraona E, Leo MA, Borowsky SA, Lieber CS. Pathogenesis of the alcohol-induced accumulation of protein in the liver. J Clin Invest **60**:546–554, 1977.
30. Ockner RK, Burnett DA, Lysenko N, Manning JA. Sex differences in long chain fatty acid utilization and fatty acid-binding protein concentration in rat liver. J Clin Invest **64**:172–181, 1979.
31. Bass NM, Manning JA, Ockner RK, Gordon JI, Seetharam S, Alpers DH. Regulation of the biosynthesis of two distinct fatty acid-binding proteins in rat liver and intestine. Influences of sex difference and of clofibrate. J Biol Chem **260**:1432–1436, 1985.
32. Kawashima Y, Nakagawa S, Kosuka H. Effects of some hypolipemic drugs and phthalic acid esters on fatty acid-binding protein in rat liver. J Pharmacobiodyn **5**:771–779, 1982.
33. Bass NM. Fatty acid-binding protein expression in the liver: Its regulation and relationship to the zonation of fatty acid metabolism. Mol Cell Biochem **98**:167–176, 1990.
34. Van Thiel DH, Tarter RE, Rosenblum E, Gavaler JS. Ethanol, its metabolism and gonadal effects: Does sex make a difference? Adv Alcohol Subst Abuse **7**:131–169, 1989.
35. Mishkin S, Stein L, Fleishner G, Gatmaitan Z, Arias IM. Z protein in hepatic uptake and esterification of long-chain fatty acids. Am J Physiol **228**:1634–1640, 1975.
36. Burnett DA, Lysenko N, Manning JA, Ockner RK. Utilization of long chain fatty acids by rat liver: Studies of the role of fatty acid binding protein. Gastroenterology **77**:241–249, 1979.
37. Hashimoto S, Wisnieskie BJ, Wong H. Gender-related effects of chronic ethanol ingestion on rat hepatic acyl-CoA: cholesterol acyltransferase. Biochim Biophys Acta **879**:66–72, 1986.
38. Morgan MY, Sherlock S. Sex-related differences among 100 products with alcoholic liver disease. Br Med J **282**:1140–1143, 1977.
39. Frezza M, DiPadova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. N Engl J Med **322**:95–99, 1990.
40. Katz AM, Messineo FC. Lipid membrane interactions and the pathogenesis of ischemic damage in the myocardium. Circulation Res **48**:1–16, 1981.
41. Shaw W. Possible role of lysolecithins and nonesterified fatty acids in the pathogenesis of Reye's syndrome, sudden infant death syndrome, acute pancreatitis, and diabetic ketoacidosis. Clin Chem **31**:1109–1115, 1985.