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SEX DIFFERENCES IN THE RESPONSE OF SEVERAL LIPOGENIC ENZYMES TO CHRONIC ETHANOL INGESTION *

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Abstract—The activities of glucose 6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase, NADP-malic dehydrogenase and ATP-citrate lyase have been measured in male and female Long–Evans rats fed both high-fat (34.2% of calories) and low-fat (4.6% of calories) liquid diets. Ethanol was incorporated into the diets by replacing 36% of the carbohydrate calories. Glucose 6-phosphate dehydrogenase activity was decreased by ethanol only in the males. A 40 per cent decrease was observed regardless of which diet was fed. Because ethanol affected this enzyme only in males, the phosphogluconate pathway may be more important in females during chronic ethanol metabolism. Ethanol had small and variable effects on isocitrate dehydrogenase, and the high-fat diet seemed to modify the differential response of this enzyme to ethanol between males and females. The NADP-malate dehydrogenase was decreased 57 per cent in males and 53 per cent in females after ethanol ingestion with the low-fat diet. With the high-fat diet, these decreases were more pronounced (74 and 68 per cent, respectively). Changes in the activity of ATP-citrate lyase have been said to parallel those of malate dehydrogenase; however, this was not observed in the female animals. Instead of decreasing, the activity of this enzyme increased in females after feeding ethanol with the low-fat diet. A kinetic analysis of the ATP-citrate lyase showed that the apparent V_{max} had beenn altered, while the K_m for citrate remained unchanged. These data suggest that ethanol may have altered the content of the enzyme in both males and females. The high-fat diet enhanced the effect of ethanol on the activity of this enzyme in males and tended to reverse the effect of ethanol in females. All of the above data suggest that lipogenesis may not be decreased in females as a result of ethanol and that sex hormones may play a role in the effects of ethanol on lipid metabolism in the rat.

In a few studies in which the effects of ethanol feeding on lipid metabolism as a function of sex were investigated, differences were observed. For example, the plasma triglyceride concentration was increased 6 hr after a single large dose of ethanol in both male and female rats but to a greater extent in the males [1]. Ethanol enhanced the Triton-induced hypertriglyceridemia to a greater extent in male rats than in females [2]. Further, depending upon the sex of the animal, chronic ethanol ingestion affected the oxidation of certain mitochondrial substrates differently [3, 4]. More recently, our laboratory reported findings that emphasized the importance of sex as well as dietary levels of fat on hepatic mitochondrial structure and function after chronic ethanol ingestion and metabolism [5].

The accumulation of lipids in the liver following chronic ingestion of ethanol has been well documented [6], and essentially four mechanisms have been postulated to account for the alcoholic fatty liver [7, 8]. These include increased peripheral fat mobilization, decreased hapatic lipoprotein release, decreased fatty acid oxidation and enhanced hepatic lipogenesis. The preponderance of studies which have resulted in these various mechanisms being postulated was conducted with animals of only one sex. However, comparing normal lipid metabolism of normal males and females has revealed several sex related differences. For instance, the rate of uptake of free fatty acids by perfused livers from female rats was greater than from males [9], and the hepatic triglyceride concentration of female rats exceeded that of males [10]. Also, the output of triglycerides by livers from female rats is greater than that from livers of male rats *in vitro* [11, 12] and *in vivo* [13]. These differences in normal lipid metabolism as a function of sex were related to hormonal regulation [14–17]. Further, it has been demonstrated that females show greater susceptibility than males to the induction of fatty liver by ethionine [12], tetracycline [10], and cyclohexamide [18]. However, with respect to the induction of a fatty liver by chronic ethanol ingestion, the majority of data evaluating the metabolic events leading to ethanol-induced accumulation of hepatic lipid has been collected from male experimental animals.

In order to evaluate the effects of chronic ethanol ingestion as it may be related to sex differences, four enzymes were selected which represent metabolic loci historically considered to be markers of lipogenic activity. First, glucose 6-phosphate dehydrogenase catalyzes oxidation at carbon 1 of glucose 6-phosphate to produce 6-phosphogluconate, the first substrate in the hexose monophosphate shunt. This pathway is responsible for much of the reducing power (NADPH) needed for *de novo* synthesis of fatty acids. Second, the NADP-malic enzyme also has been well documented as a source of reducing equivalents for lipid biosynthesis [19–21], and under certain conditions can generate approximately one half the reducing power necessary for lipid biosynthesis [22]. Third, the ATP-citrate

* A preliminary report of these data has been published in the 1978 Proceedings of the Western Pharmacology Society.

lyase has been widely accepted as the main enzyme responsible for the production of acetyl-CoA, the precursor of fatty acids in the cytoplasm of the cell. Fourth, the NADP-linked isocitrate dehydrogenase converts isocitrate to α-ketoglutarate in the cytoplasm and, thus, to citrate. To account for the possibility of acetyl-CoA generation from α-ketoglutarate, isocitrate dehydrogenase was assayed in this study. Further, this enzyme has been demonstrated to be a potential source of reducing equivalents for lipid biosynthesis as indicated by its response to different carbohydrate diets as well as being significantly elevated in the obese hyperglycemic syndrome [23].

In the experiments described here, the activities of these marker enzymes were assayed in both male and female rats to determine how certain metabolic events leading to alcoholic fatty liver might differ as a function of sex and possibly dietary fat.

MATERIALS AND METHODS

Animals and treatment. Male and female Long–Evans rats (140–160 g) were obtained from Simonsen Labs, Gilroy, CA. Upon arrival, the animals were housed in separate cages and maintained on Purina Laboratory Chow and water *ad lib.* for 3–4 days. The animals were then weight-paired and fed a regimen of a specifically formulated liquid diet containing 36% of the total calories as ethanol. The low-fat diet contained 4.6% of the total calories as lipid, and the high-fat diet contained 34.2% of the calories as lipid. The composition and preparation of the liquid diets have been described in detail elsewhere [5]. Pair-fed controls received the same diet except that glucose was isocalorically substituted for ethanol. All animals were allowed to consume the diet *ad lib.* Richter tubes were used for measuring daily consumption of the liquid diets, and a 1:1 light to dark ratio (07:30–19:30 hr) lighting schedule was used. Animals were maintained on the diet for a period of 35–45 days prior to killing. The livers were excised immediately and placed in ice-cold 0.25 M sucrose containing 3.4 mM Tris–HCl at pH 7.4 The liver was diced rapidly with stainless steel scissors and then homogenized with a Potter–Elvehjem homogenizer attached to a variable speed electric drill. The enzyme fraction used in all assays was the 105,000 *g* supernatant fluid obtained by centrifuging the post-mitochondrial supernatant fluid for 70 min at 3° in a Beckman model L5-65 preparative ultracentrifuge.

Assays of enzymes. All enzymes were assayed spectrophotometrically by standard techniques using a Beckman model 25 spectrophotometer: glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP oxidoreductase; EC 1.1.1.49) [24]; malate dehydrogenase (L-malate:NADP oxidoreductase; EC 1.1.1.40) [25]; citrate oxaloacetate-lyase (EC 4.1.3.8) [26]; and isocitrate dehydrogenase (L-isocitrate:NADP oxidoreductase; EC 1.1.1.41) [27]. Protein was determined by the method of Lowry *et al.* [28].

Substrates. Glucose 6-phosphate (monosodium salt), L-malic acid (monosodium salt), DL-isocitric acid (type 1: trisodium salt), citric acid (trisodium salt), β-NADP (sodium salt), coenzyme A (lithium salt), and ATP (sodium salt) all were purchased from Sigma Chemical Co., St. Louis, MO, and used without further purification.

Enzymes. Malic dehydrogenase from porcine heart (cytoplasmic) was purchased from Sigma Chemical Co., St. Louis, MO, and used without further purification.

Statistical analysis. Paired *t*-test analysis was used to evaluate the significance of the observed differences.

RESULTS

A previous report from this laboratory [5] described the appearance of livers from male and female animals chronically fed ethanol in either the high- or low-fat diet. Ethanol ingestion did not alter liver weights from either sex when the low-fat diet was used. The male liver weights were about 40 g/kg, and the female liver weights were about 38 g/kg. When the fat content of the diet was increased, ethanol ingestion caused the male liver weights to increase from 39.2 to 61.4 g/kg and the female liver weights to increase from 32.0 to 49.0 g/kg. Total liver triacylglycerol concentrations were not determined, but the liver weights should be a very good indication of liver triglyceride as suggested by the data of Lieber and DeCarli [29]. Thus, ethanol only induced liver triacylglycerol accumulation when the dietary fat content was high.

In previous studies with males fed the low-fat diet, blood ethanol concentrations were observed to plateau between 250 and 300 mg/dl after 8 or 9 days and remain there until at least day 12. Blood ethanol was not measured in the studies reported here. During the last week of ethanol administration, the daily intake of ethanol was 16.0 ± 0.9 g/kg/day for the low-fat males and 16.0 ± 0.6 g/kg/day for the high-fat males. The low-fat females consumed 18.1 ± 0.6 g/kg/day, and the high-fat females ingested 14.5 ± 0.8 g/kg/day. The values for the low-fat males have been corroborated in another laboratory which uses the same type of animals and the same diet.* These animals were conscious and very quiescent; they remained in the rear of their cages.

Table 1 compares the effects of chronic ethanol ingestion on the four marker enzymes of lipogenic activity in male and female rats fed the low-fat diet. Note that glucose 6-phosphate dehydrogenase activity was decreased 42 per cent in the males as a result of ethanol feeding, whereas in the females ethanol had no effect. In the controls, the activity of this enzyme was elevated 2-fold greater in the female than in the male. This difference in activity increased almost 4-fold in the female compared to the male in the ethanol-treated animals. The increase in the difference between the sexes was the result of the ethanol-induced decrease in the male. When this enzyme was compared in males and females as a function of feeding a high-fat diet (Table 2), it was found that ethanol ingestion depressed the activity of the enzyme in the males by 39 per cent, and as with the low-fat diet, no change was observed in the females as a result of ethanol feeding. Thus, ethanol affected the activity of this enzyme only in the male, and increasing the dietary levels did not alter the relative effects of ethanol. It also will be observed that there was no difference between the male and female controls with the high-fat diet. Further, the high-fat diet resulted in a 65 per cent decrease in activity in control males and an

* Personal communication from Dr. Frank R. Ciofalo, Dept. of Pharmacology, University of Nevada, School of Medical Sciences.

Table 1. Effects of chronic ethanol ingestion on lipogenic marker enzymes in male and female rats fed a low-fat diet

Enzyme	Controls*	Ethanol*	% Change	P
ATP citrate lyase				
Male	15.4 ± 2.5	9.8 ± 1.4	−37.0	<0.01
Female	13.1 ± 0.9	18.5 ± 2.3	+41.2	<0.01
NADP malic dehydrogenase				
Male	6.0 ± 1.4	2.6 ± 0.4	−56.6	<0.01
Female	5.1 ± 0.9	2.4 ± 0.6	−52.9	<0.01
NADP isocitrate dehydrogenase				
Male	122.9 ± 2.4	110.6 ± 4.3	−10.0	NS†
Female	158.3 ± 12.7	169.3 ± 13.4	+6.9	NS
Glucose 6-phosphate dehydrogenase				
Male	65.7 ± 8.1	38.1 ± 4.1	−42.0	<0.01
Female	145.0 ± 17.9	146.1 ± 35.3	+0.8	NS

* Expressed as nmoles/min/mg of protein ± S. E. M. of five to twelve animals. The enzymes were assayed as described in Materials and Methods.
† Not significant.

Table 2. Effects of chronic ethanol ingestion on lipogenic marker enzymes in male and female rats fed a high-fat diet

Enzyme	Controls*	Ethanol*	% Change	P
ATP citrate lyase				
Male	9.7 ± 0.7	5.1 ± 0.8	−47.4	<0.01
Female	8.6 ± 1.2	7.1 ± 1.1	−17.0	NS†
NADP malic dehydrogenase				
Male	5.7 ± 0.6	1.5 ± 0.2	−73.7	<0.01
Female	2.5 ± 0.7	0.8 ± 0.2	−68.0	<0.01
NADP isocitrate dehydrogenase				
Male	131.1 ± 10.7	153.5 ± 15.4	+17.1	<0.05
Female	153.8 ± 12.2	170.8 ± 18.3	+11.2	NS
Glucose 6-phosphate dehydrogenase				
Male	23.1 ± 1.8	14.0 ± 1.1	−39.0	<0.01
Female	23.4 ± 2.0	24.2 ± 2.0	+0.3	NS

* Expressed as nmoles/min/mg of protein ± S. E. M. of five to twelve animals. The enzymes were assayed as described in Materials and Methods.
† Not significant.

84 per cent decrease in control females when compared to the low-fat diet. This is consistent with the findings of Leveille [30] who observed that high dietary fat lowers the activity of this enzyme.

When isocitrate dehydrogenase activity was measured, ethanol ingestion had small and variable effects on this enzyme regardless of whether the low-fat diet (Table 1) or the high-fat diet (Table 2) was used. As a result of low-fat feeding (Table 1), the effect of ethanol on either males or females was not significant statistically. However, when males were compared to females, it was observed that the activity of the enzyme was 28.8 per cent higher in control females compared to control males. After ethanol treatment, the difference in activity between females and males had increased to 53 per cent. Thus, there was a 25 per cent increase in the difference between females compared to males as a direct result of ethanol in the diet.

When the high-fat diet was used, the activity of isocitrate dehydrogenase was not changed in either the control or the ethanol-treated females. However, there was about 7 per cent increase in activity in the control males and a 39 per cent increase in the ethanol-treated males. These effects of ethanol feeding were statisically significant in the male animals; 17 per cent increase was observed. Thus, the effects of both ethanol ingestion and high-fat feeding on isocitrate dehydrogenase were observed to be significant only in males.

NADP-malate dehydrogenase activity was decreased 57 per cent in males and 53 per cent in females fed the low-fat diet containing ethanol, and no differences were observed between either control males and females or between ethanol-treated males and females with respect to the activity of this enzyme. When the high-fat diet was used, the decrease resulting from ethanol ingestion was more pronounced (74 and 68 per cent in males and females, respectively). Further, when control males and control females were compared, there was a 56 per cent decrease in activity in the females. When the ethanol-treated males and females were compared, there was a 47 per cent decrease in activity in the females. Thus, the use of the high-fat diet affected the activity of this enzyme mainly in the female; however, high dietary fat did potentiate the effect of ethanol in both males and females.

Ethanol affected ATP-citrate lyase quite differently in the two sexes. In the group of animals fed the low-fat diet (Table 1), ethanol ingestion resulted in a decrease

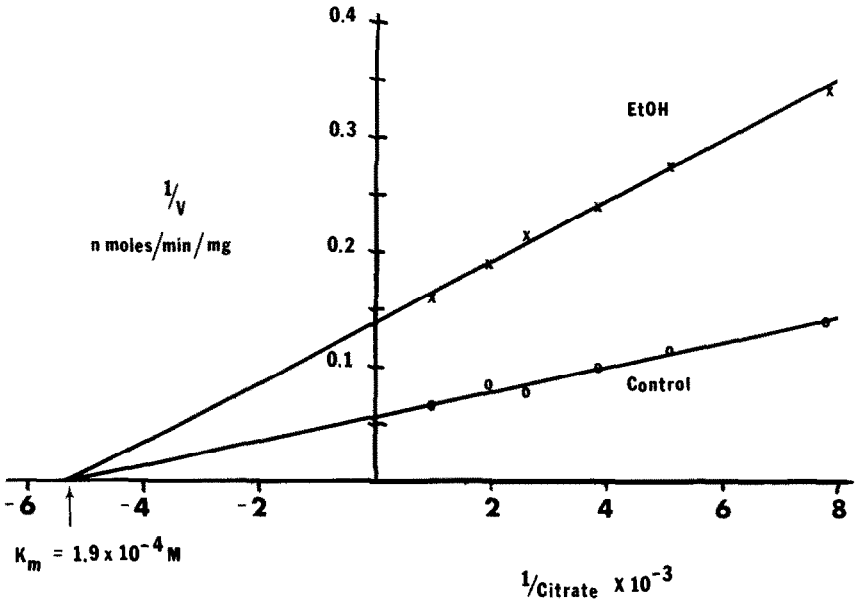

Fig. 1. Lineweaver–Burk plot of ATP-citrate lyase activities for males fed the low-fat diet. The enzyme was assayed according to the procedure of Ochoa [26]. Citrate concentration was varied from 1.3 × 10^{-4} M to 10.0 × 10^{-4} M.

in activity of this enzyme in the male which corroborates the findings of other investigators [31, 32]. In the female, activity of this enzyme was elevated 41 per cent as a result of chronic ethanol feeding. When fed a high-fat diet, the enzyme activity was depressed significantly by ethanol only in the males. It also was observed that the high-fat diet decreased the activity of the control males and control females by 37 and 34 per cent, respectively. When ethanol was introduced into the diet, the activity of this enzyme was decreased 48 per cent in males and 61 per cent in females as a result of the high-fat feeding. Thus, increasing the dietary fat decreased the activity of this enzyme in both sexes as reported by others [31]. Further, the effect of increasing dietary fat completely removed the effect of ethanol in the female.

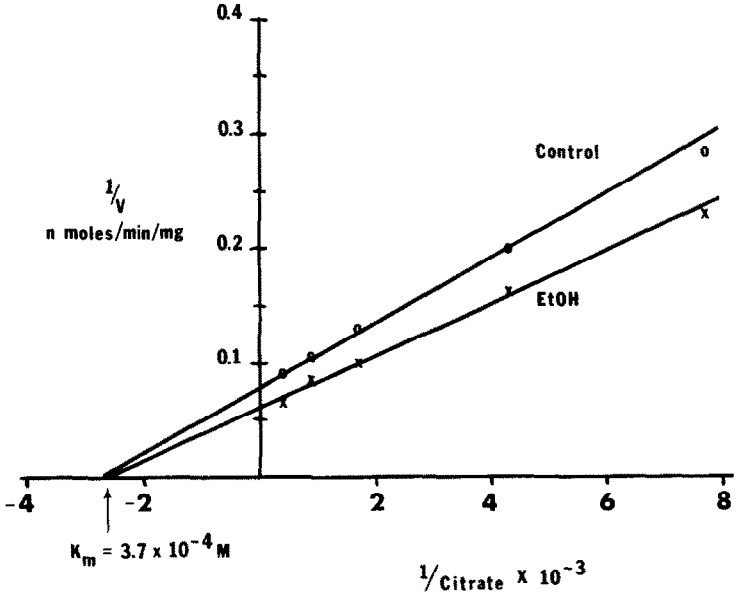

Fig. 2. Lineweaver–Burk plot of ATP-citrate lyase activities for females fed the low-fat diet. The enzyme was assayed according to the procedure of Ochoa [26]. Citrate concentration was varied from 1.3 × 10^{-4} M to 25.0 × 10^{-4} M.

These striking differences between males and females with respect to the activity of ATP-citrate lyase were studied further by kinetic analysis. Figures 1 and 2 show the Lineweaver–Burk plots for the data obtained from the low-fat fed males (Fig. 1) and females (Fig. 2). It is quite obvious from these figures that ethanol ingestion has affected only the V_{max} of the enzyme. In the males (Fig. 1), the V_{max} was 7.2 nmoles/min/mg in the ethanol-treated animals compared to 17.5 in the controls. In the females (Fig. 2), the relationship was reversed; the ethanol-treated animals exhibited a V_{max} value of 16.3 nmoles/min/mg compared to 14.9 in the controls. Thus, these data corroborate the data shown in Tables 1 and 2. The K_m for citrate was about 1.9×10^{-4} M in the males (Fig. 1) compared to 3.7×10^{-4} M in the females, and ethanol ingestion did not alter these values. Because the females fed the high-fat diet did not show any significant difference after ethanol ingestion, only the males fed the high-fat diet were studied kinetically. The data from this study were almost identical to those shown in Fig. 1. The K_m for citrate was 2.3×10^{-4} M in both controls and ethanol-treated. The V_{max} was 9.1 nmoles/min/mg in the controls and 3.45 nmoles/min/mg in the ethanol-treated. These data may indicate that chronic ethanol ingestion has altered the content of enzyme, a pattern that would be consistent with the nature of adaptive enzymes. Thus, the females fed the low-fat diet would have increased amounts of enzyme while the males would have decreased amounts. Another possible interpretation is that the K_{cat} has been altered by ethanol. However, to more fully explain these differences, additional data must be gathered.

DISCUSSION

Because the enzymes chosen are integrated into a rather complex web of metabolic pathways, it was the purpose of this study to attempt to identify general loci where ethanol effects may vary as a function of sex and to relate these findings to alterations observed as a result of normal sex differences.

With respect to glucose 6-phosphate dehydrogenase, our findings with male rats fed the low-fat diet were almost in perfect agreement with those reported by Markku *et al.* [31]. In their study using male rats, ethanol substitution for carbohydrates in a low-fat liquid diet resulted in a 46 per cent decrease in the activity of this enzyme. This figure compares favorably to the 42 per cent decrease observed in our experiment using males (Table 1). This inhibition of the first catalytic step in the hexose monophosphate shunt (HMPS) may be related to the inhibition by ethanol ingestion of a number of dehydrogenases that use NADP or NAD as an oxidizing agent [31–34]. Inhibition would be expected as a result of the increase in reduced pyridine nucleotides that accompanies chronic ethanol metobolism [35, 36]. Unlike males, virtually no differences were observed as a function of ethanol ingestion in female rats. The failure of prolonged ingestion of ethanol to alter this enzyme in the females suggests that a more oxidized state of the pyridine nucleotides exists in the female than in the male. If this is the case, then the female may have a greater dependency on HMPS activity during chronic ethanol metabolism. A low NADPH/NADP ratio in females would be consistent with the findings of Lieber and DeCarli [37] that chronic ethanol feeding increases the so-called microsomal ethanol oxidizing system in females by 138 per cent compared to 48 per cent in males. Because this membrane system uses NADPH, the HMPS may not be depressed in females, allowing the female to compensate for the greater loss of NADPH that would occur if the microsomal ethanol oxidizing system were in fact behaving in accord with the observations of Lieber and DeCarli. Another possibility is that the differential effects of ethanol on HMPS activity may be mediated by androgenic hormones. This hypothesis has credence in light of previous observations that ethionine induced fatty livers in female and castrated male rats [38] but not in normal males or females pretreated with testosterone [39].

Finally, that the observed differences in glucose 6-phosphate dehydrogenase in the controls fed low-fat diets may be related to a modulation of gene dose phenomena is enticing. It is well established that glucose 6-phosphate dehydrogenase is a sex linked protein with the gene located on the X chromosome [40]; however, it also has been demonstrated that there is no sex difference in the activity of the human erythrocyte enzyme [41]. Failure to display gene dosage effects has been postulated by Lyons [42] to be related to an inactivation of the gene on one of the X chromosomes in early embryonic development. However, other investigators have presented data to challenge this [43]. Russell [44] has suggested that the X chromosome in the mouse may be only partially inactivated in early embryonic life. Because it has been suggested that both Xg alleles are expressed in cells of heterozygous females [45], both alleles also may be expressed in males. Whether nutritional conditions exert influence on the gene inactivation by a repressor–depressor mechanism resulting in alterations of a gene dosage effect remains to be elucidated, and our data suggest that such studies may be heuristic.

Even though ethanol ingestion did not induce statistical differences in isocitrate dehydrogenase in either males or females fed the low-fat diet, it was extremely interesting to note that the activity of the enzyme was 25 per cent higher in the control females compared to the control males and that this difference increased to 53 per cent after ethanol was added to the diet. Because palmitoyl-CoA levels have been reported to be elevated significantly in response to chronic ethanol ingestion [46], this effect of ethanol may be related to the inhibition of isocitrate dehydrogenase by palmitoyl-CoA [47]. This suggestion is supported by the data in Table 2 which show that the high-fat diet minimized the male–female differences. It should be pointed out that the level of palmitoyl-CoA required to inhibit isocitrate dehydrogenase was such that the inhibition probably resulted from detergent effects of the palmitoyl-CoA.

That nutritional factors modulate the rate of hepatic fatty acid synthesis has been indicated by increased rates of lipogenesis in animals fed low-fat diets and decreased rates in animals fed high-fat diets [48]. Further, it is generally accepted that depressed ATP-citrate lyase and NADP-malic enzyme activities reflect a depression in hepatic lipogenesis [19, 20]. A comparison of Tables 1 and 2 demonstrates that an increase in dietary fat under the conditions of our experiments did result in a decreased activity of ATP-citrate lyase in the

controls from both sexes; however, a decrease in NADP-malic enzyme was seen in the females only. When ethanol was added to the diet, a marked decrease in the activity of the NADP-malic enzyme was observed in both groups of males and females. On the other hand, ethanol decreased the activity of ATP-citrate lyase in both groups of males and increased its activity in the low-fat females. No change was observed in the high-fat females. This is interesting from the standpoint that previous investigators have demonstrated a high correlation between ATP-citrate lyase and NADP-malic enzyme activities after feeding both chow [21] and liquid diets with and without ethanol [31, 32]. Our data show a reasonable correlation in the relative alterations of these two enzymes in the male, but in females fed the low-fat diet, the relationship was inverse, i.e. with increasing ATP-citrate lyase activity a corresponding decrease in malic enzyme activity occurred. These data suggest a difference in substrate flow due to differences between males and females in the rate of acetyl-CoA production via oxidation of ethanol. Lieber and DeCarli [37] have reported a 21 per cent decrease in the activity of alcohol dehydrogenase in females after chronic ethanol ingestion; no change was observed in males. Therefore, after chronic ethanol ingestion, there may be a decrease in the synthesis of acetate from ethanol in females which could contribute to differences in substrate flow between the two sexes. Atkinson and Walton [49] have shown that the ATP-citrate lyase could be regulated *in vivo* by alterations in the so-called energy charge. Because French [50] has observed a decrease in total liver ATP in chronically treated males and no change in females, the difference in the energy charge which exists between the sexes also should contribute to the opposite effects of ethanol on the ATP-citrate lyase observed in Table 1.

Four mechanisms were postulated in the introduction as plausible explanations for the accumulation of lipids in the liver. Regardless of which mechanism one chooses to accept as a basis for hypothesis, the lipids that accumulate can originate from three main sources: dietary lipids, adipose tissue lipids and lipogenesis [7, 8]. Decreased ATP-citrate lyase activity as a result of chronic ethanol ingestion has been used to negate enhanced lipogenesis as a source of lipid accumulation during fatty liver development [31, 32]. Our findings with the male would support these conclusions. However, it should be emphasized again that females fed a low-fat diet displayed an elevation in the activity of this enzyme (Table 1). The kinetic data presented in Figs. 1 and 2 suggest that these alterations may be related to modifications in the quantitative content of the enzyme as indicated by the relative change in V_{max} of the enzyme without any change in the "apparent" K_m. This response is consistent with the nature of adaptive enzymes.

The activity of ATP-citrate lyase depends on the nutritional state of the animal [51]. It has been shown to be suppressed on starvation and restored on refeeding after starvation [52]. Further, the type of diet used for refeeding exerts an effect on the extent of the increase in enzyme activity [19, 53]. Lactation increased ATP-citrate lyase activity in mammary tissue [54], and genetically obese mice have an increased activity of this enzyme in the liver [54]. Because the above described alterations in nutrition, etc., have led to changes in the ATP-citrate lyase, our data may be related to previous reports which show that free fatty acids (FFA) are mobilized differently in males than in females [55, 56]. Further, the alterations in the kinetic constants may be related to the findings that free fatty acids are esterified more rapidly in livers of females than males [10]. In the females, one might postulate that the free fatty acids are esterified rapidly enough to keep the effective acyl-CoA concentration sufficiently low, whereas, in the male, the levels of acyl-CoA may be exerting inhibitory effects. If this mechanism were correct, one might expect females fed the high-fat diet to display inhibition patterns similar to those observed in males. Because FFA esterification rates have been shown to be higher in the female [14], an increased influx of FFA into the liver, contributed by the supplemental dietary lipids, may have been rapid enough to negate the effects of enhanced incorporation into neutral lipids, thereby inhibiting ATP-citrate lyase. This was, in fact, the observed effect. Thus, we conclude that the differential effects of ethanol observed between males and females fed the low-fat diet were eliminated by an increase in the influx of FFA brought about by the increase in lipid in the high-fat diet.

The data regarding sex differences and ethanol effects on the enzyme activities presented here suggest that ethanol may not increase the NADPH/NADP ratio in females as it does in males. These data also would suggest that *de novo* fatty acid synthesis may not be decreased in females as a result of ethanol ingestion. Arakawa *et al.* [57] noted that the effects of chronic ethanol ingestion on ATP-citrate lyase and NADP-malic enzyme resulted in elevated activities of these enzymes in a strain of mouse that is characterized by obese hyperglycemia and diabetes. They also presented evidence to show that these enzyme activities were correlated positively with plasma insulin levels which are modulated to a great extent by sex hormones. Thus, our data may be the result of effects of ethanol on hormonal regulation of insulin metabolism. It would be interesting to determine if our results with these lipogenic marker enzymes would be altered by exogenously introduced sex hormones. Regardless of which mechanisms are actually affected, these data very definitely suggest sex differences in the response of lipogenic enzymes to ethanol ingestion and point to the necessity of enhanced utilization of females in evaluating the metabolic responses to the ethanol that lead to the accumulation of fat in liver.

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REFERENCES

1. M. G. Horning, M. Wakabayashi and H. M. Maling, in *Biochemical Factors in Alcoholism* (Ed. R. P. Maickel), p. 139. Pergamon Press, Oxford (1967).
2. O. Hernell and O. Johnson, *Lipids* **8**, 503 (1973).
3. K.-H. Kiessling and K. Tilander, *Expl. Cell Res.* **30**, 476 (1963).
4. K.-H. Kiessling, G. Degerman and M. Skolund, *Acta chem. scand.* **17**, 2513 (1963).
5. J. A. Thompson and R. C. Reitz, *Lipids* **13**, 540 (1978).
6. C. S. Lieber, *Ann. N.Y. Acad. Sci.* **252**, 63 (1975).
7. C. S. Lieber, in *The Biological Basis of Medicine* (Ed. E. E. Britton), p. 317. Academic Press, New York (1969).

8. C. S. Lieber, *Lipids* **9**, 103 (1974).
9. C. Soler-Argilaga and M. Heimberg, *J. Lipid Res.* **17**, 605 (1976).
10. K. Breen, S. Schenker and M. Heimberg, *Gastroenterology* **69**, 714 (1975).
11. S. Otway and D. S. Robinson, *J. Physiol., Lond.* **190**, 321 (1967).
12. E. Farber, *Adv. Lipid Res.* **5**, 119 (1967).
13. F. F. Schlunk and B. Lombardi, *Lab. Invest.* **17**, 299 (1967).
14. R. O. Scow, S. S. Chernick and M. S. Brinley, *Am. J. Physiol.* **206**, 796 (1964).
15. M. Aurell, K. Cramer and G. Rybo, *Lancet* **1**, 291 (1966).
16. L. C. Filios, *Endocrinology* **60**, 22 (1957).
17. L. L. Abell and E. H. Mosbach, *J. Lipid Res.* **3**, 88 (1962).
18. H. Bar-on, O. Stein and Y. Stein, *Biochim. biophys. Acta* **270**, 444 (1972).
19. M. S. Kornacker and J. M. Lowenstein, *Biochem. J.* **94**, 209 (1965).
20. H. M. Tepperman and J. Tepperman, *Am. J. Physiol.* **206**, 357 (1964).
21. E. M. Wise, Jr. and E. G. Ball, *Proc. natn. Acad. Sci. U.S.A.* **52**, 1255 (1964).
22. M. S. Kornacker and E. G. Ball, *Proc. natn. Acad. Sci. U.S.A.* **54**, 899 (1965).
23. G. W. Winston, R. S. Pardini and C. Gilbrith, *Proc. Twenty-ninth Ann. N. W. Reg. Am. Chem. Soc.*, p. 37. Eastern Washington State College, Cheney, WA (1974).
24. G. W. Löhr and H. D. Waller, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 744. Academic Press, New York (1965).
25. P. A. Srere, *J. biol. Chem.* **234**, 2544 (1959).
26. S. Ochoa, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 1, p. 739. Academic Press, New York (1955).
27. G. Siebert, in *Methods of Enzymatic Analysis* (Ed. H. U. Burgmeyer), p. 318. Academic Press, New York (1965).
28. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
29. C. S. Lieber and L. M. DeCarli, *Am. J. clin. Nutr.* **23**, 474 (1970).
30. G. A. Leveille, *J. Nutr.* **91**, 25 (1967).
31. S. J. Markku, J. K. Hiltunen and I. E. Hassinen, *Biochem. J.* **164**, 169 (1977).
32. E. Fellenius, U. Nisbeth, L. Pilstrom and K.-H. Kiessling, *Br. J. Nutr.* **29**, 307 (1973).
33. G. Idèo, R. DeFranchis, E. DelNinno and N. Dioguardi, *Enzymologia* **43**, 245 (1972).
34. H. A. Krebs, R. A. Freedland, R. Hems and M. Stubbs, *Biochem. J.* **112**, 117 (1969).
35. C. S. Lieber, *Gastroenterology* **50**, 119 (1966).
36. C. S. Lieber and E. Rubin, *New Engl. J. Med.* **280**, 705 (1969).
37. C. S. Lieber and L. M. DeCarli, *J. biol. Chem.* **245**, 2505 (1970).
38. E. Farber, D. Koch-Weser and H. Popper, *Endocrinology* **40**, 205 (1951).
39. E. Farber and A. Segaloff, *J. biol. Chem.* **216**, 471 (1955).
40. L. E. Rosenberg, in *Duncan's Disease of Metabolism* (Eds. P. K. Bondy and L. E. Rosenberg), p. 31. W. B. Saunders Co., Philadelphia (1974).
41. M. M. Grumbach, P. A. Marks and A. Morishima, *Lancet* **1**, 1330 (1962).
42. M. F. Lyon, *Am. J. hum. Genet.* **14**, 135 (1962).
43. H. Gruneberg, *Ann. hum. Genet.* **30**, 239 (1967).
44. L. B. Russell, *Proc. Eleventh Int. Cong. of Genet.* **1**, 302 (abstr.) (1963).
45. P. J. Fialkow, R. Lisker, E. R. Giblett and C. Zavala, *Nature, Lond.* **226**, 367 (1970).
46. Ch. Bode, E. Stahler, H. Kono and H. Gobell, *Biochim. biophys. Acta* **210**, 448 (1970).
47. K. Taketa and B. M. Pogell, *J. biol. Chem.* **241**, 720 (1966).
48. R. Hill, J. M. Linazasoro, F. Chevallier and I. L. Chaikoff, *J. biol. Chem.* **233**, 305 (1958).
49. D. E. Atkinson and G. M. Walton, *J. biol. Chem.* **242**, 3239 (1967).
50. S. W. French, *Proc. Soc. exp. Biol. Med.* **121**, 681 (1966).
51. J. M. Lowenstein, in *The Metabolic Roles of Citrate* (Ed. T. W. Goodwin), p. 61. Academic Press, London (1968).
52. A. Spencer, L. Corman and J. M. Lowenstein, *Biochem. J.* **93**, 378 (1964).
53. M. S. Kornacker and J. M. Lowenstein, *Biochem. J.* **95**, 852 (1965).
54. A. F. Spencer and J. M. Lowenstein, *Biochem. J.* **99**, 760 (1966).
55. R. Ostwald, R. Okey, A. Shannon and J. Tinoco, *J. Nutr.* **76**, 341 (1962).
56. S. B. Tove and F. H. Smith, *Archs. Biochem. Biophys.* **85**, 352 (1959).
57. A. Arakawa, S. Taketomi, K. Furuno, T. Matsew, H. Iwatsuka and F. Suzuoki, *J. Nutr.* **105**, 1500 (1975).