Liver Fat and Plasma Ethanol Are Sharply Lower in Rats Fed Ethanol in
Conjunction with High Carbohydrate Compared with High Fat Diets

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ABSTRACT The effects of high fat and high carbohydrate diets on alcohol metabolism were studied on blood alcohol and liver fat concentration. In Experiment 1, rats consumed an alcohol-containing liquid diet. Blood was collected for ethanol, glucose and lactate analyses and livers were excised for lipid determination. Blood ethanol and liver fat were lower when rats consumed the high carbohydrate diet. Glucose concentrations were lower in rats fed the high fat diet compared with those fed the high carbohydrate diet when ethanol was consumed. In Experiment 2, rats consumed a high fat, ethanol-containing diet for 13 d. Half of the rats were switched to a high carbohydrate, ethanol-containing diet for an additional 11 d. The same analyses were carried out as for Experiment 1. Switching the high fat–fed rats to the high carbohydrate diet reversed the high blood ethanol and high liver fat values, even though the rats consumed significantly more alcohol with the high carbohydrate diet. In Experiment 3 the same high fat and high carbohydrate diets without ethanol were consumed for 2 wk, at which time ethanol was administered acutely, intraperitoneally, at 2 g/kg. Blood was analyzed for ethanol, glucose and lactate 30, 60 and 120 min after injection. Rats fed the high carbohydrate diet had lower blood ethanol but higher lactate at 120 min compared with those fed the high fat diet. The results suggest that the rate of ethanol elimination is slower in rats fed high fat than in those fed high carbohydrate diets, resulting in elevated blood ethanol and liver fat levels for the former. J. Nutr. 132: 2732–2736, 2002.

KEY WORDS: blood alcohol, blood glucose, blood lactate, liver fat, rats.

The Lieber-DeCarli, ethanol-containing, liquid diet (1) made it possible to study alcohol dependence in rats and produced high blood alcohol levels and substantial liver fat accumulation. Rao and associates (2–4) suggested that the fatty liver and high blood alcohol levels in rats fed the Lieber-DeCarli diet were the result of nutritional inadequacies rather than alcohol per se. Work from this laboratory (5,6) helped clarify this issue by showing that high carbohydrate, low fat diets ameliorated the effects of alcohol compared with high fat, low carbohydrate diets but did not prevent withdrawal seizures or elevated blood alcohol and liver fat levels when alcohol was provided in excess of 30% of dietary energy. Several other groups have also reported beneficial effects of high carbohydrate, low fat diets on ethanol-induced fatty livers (7–10). Some of these studies suggested that in rats consuming a low carbohydrate (high fat) diet, ethanol induces high levels of cytochrome P-450 2E1 (CYP2E1) which, in turn, generates reactive oxygen species and other free radicals from ethanol and fatty acid metabolism. These substances are then thought to be responsible for the liver damage and fat accumulation (9,10). However, Badger's group (9), as well as Tsukamoto et al. (11), concluded that high carbohydrate diets will not prevent ethanol-induced fatty liver. Questions remain concerning whether high carbohydrate diets can reverse the deleterious effects of ethanol on the liver and whether protective effects of carbohydrate might be secondary to altered absorption of alcohol or induction of metabolic enzymes.

The present experiments were undertaken to study more extensively the relationship of isocaloric high fat and high carbohydrate diets to ethanol utilization when ethanol is administered either chronically or acutely. Furthermore, the effect of switching rats from high fat to high carbohydrate diets with the uninterrupted consumption of a high level of ethanol was also studied. Liver fat and plasma ethanol, glucose and lactate were measured.

MATERIALS AND METHODS

General procedures. Male, Long-Evans rats, weighing 120–130 g, were obtained from Charles River Laboratories (Wilmington, MA). The rats were housed individually in a room at constant temperature (21–23°C) with a 12-h light:dark cycle. In all experiments in which blood was taken at the end of the experiment for ethanol, glucose and lactate analyses, the cycle was reversed, i.e., light from 1900 to 0700 h, and dark from 0700 to 1900 h. All experiments were conducted as part of an approved protocol for animal care by the Office of Research and Sponsored Programs, Rutgers University.

Experimental diet. This was an improved version of the original Lieber-DeCarli diet (1), given as a liquid suspension as previously reported by Mirovsky et al. (12). Each liter of this diet contained the

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following ingredients common to all diets (referred to as “premix” in Table 1) (g): casein, 42.0; dl-methionine, 0.6; salt mix (13), 7.3; vitamin mix (13,14), 2.1; choline bitartrate, 0.4; cellulose, 1.0; xanthan gum, 3.0; sodium saccharin, 0.254; sucrose, 25.0. These ingredients, common to the four diets used in these experiments, were combined with varying amounts of corn oil, maltodextrin, ethanol and water as shown in Table 1. Fresh mixtures of liquid diet were prepared daily and were offered in glass or plastic bottles designed to minimize spillage.

Experiment 1. In this experiment, 8 rats per treatment group, were fed high carbohydrate, low fat and high fat, low carbohydrate diets with or without alcohol for 2 wk. Measurements after killing included plasma ethanol, glucose and lactate and liver fat. The rats were given fresh food at 0700 h (when the lights went off) on the final day. This ensured food and alcohol consumption before killing 2 h later.

Experiment 2. In this experiment, 16 rats consumed the high fat, low carbohydrate, ethanol-containing diet for 13 d. Of the 16 rats, 8 continued to consume this diet, and the other 8, of similar body weight, were switched to the low fat, high carbohydrate, ethanol-containing diet for 11 d. Plasma and liver metabolites in the 2 groups were compared.

Experiment 3. Rats were fed ethanol-free, high carbohydrate, low fat and high fat, low carbohydrate diets and were administered a single, acute, intraperitoneal injection of ethanol (2 g/kg body) at the end of a 2-wk feeding period and 30, 60 and 120 min before killing.

Blood and liver analyses. Trunk blood was taken after decapitation of the rats for the determination of plasma alcohol, glucose and lactate concentrations. For alcohol and lactate, blood was collected into heparinized capillary tubes which were then centrifuged at 500 × g for 25 min. Alcohol was determined in plasma by an enzyme assay (Sigma 333-UV, St. Louis, MO), and lactate was measured using Sigma procedure 735. Glucose was measured in whole blood applied to the One-Touch II Blood Glucose Monitor from Lifescan (Milpitas, CA).

The whole liver was removed, blotted to remove blood, weighed and frozen in liquid nitrogen and stored at −70°C until fat analysis. Duplicate pieces of 0.25 g frozen liver were cut up and homogenized with 10 mL of a 2:1 chloroform/methanol solution. The homogenate was vortexed for 15 s every 5 min for a total of 30 min. The homogenate was then filtered and the filter paper and filtrate washed with 5 mL chloroform/methanol solution. Then, 0.84 g/L KCl (0.2 × volume of solution) was added, followed by further vortexing and subsequent resting for 30 min. After removal of the top (water) layer, the bottom layer was poured into aluminum dishes for evaporation of solvent and weighing of the remaining fat.

Statistical analyses. Two-way ANOVA was used to compare the data for Experiments 1 and 3, followed by post-hoc comparison of individual means by Fisher’s partial least square difference. Experiment 2 was analyzed by one-way ANOVA. Results are means ± SEM. Differences were considered significant at P ≤ 0.05. In Experiment 3, because the lactate values for the 30- and 60-min groups within each diet treatment did not differ, they were combined for analysis to yield a larger sample size.

RESULTS

Experiment 1. Daily energy intake by rats consuming ethanol was less than in their diet-matched controls; this was reflected in sharply lower weight gains (Table 2). The high fat, low carbohydrate, ethanol-fed group actually lost 12 g during the 2-wk experimental period.

Plasma ethanol concentration was lower in rats fed the high carbohydrate, low fat, ethanol-containing diet, than in those fed the high fat, low carbohydrate, ethanol-containing diet. Plasma glucose was lower in rats ingesting the high fat, low carbohydrate, ethanol diet compared with all other groups. The high carbohydrate, low fat, ethanol-fad rats had blood glucose levels not different from those fed the high fat or low fat ethanol-free diets. Plasma lactate levels were not affected by diet or ethanol.

The liver fat concentration was highest in the high fat, low carbohydrate, ethanol-fed group, and lowest in the high carbohydrate, low fat, ethanol-free controls. The high carbohydrate, low fat, ethanol-supplemented rats had liver fat levels that did not differ from the rats fed the high fat, low carbohydrate, ethanol-free diet.

Experiment 2. Energy and ethanol intakes and weight gain were greater in rats fed the high carbohydrate, low fat diet for the last 11 d of the experiment than in rats fed the high fat, low carbohydrate diet for the full 24 d (Table 3). The plasma ethanol concentration was lower in the rats that had the diet switched compared with the rats fed the high fat, low carbohydrate, ethanol-containing diet throughout. The liver fat concentration of the switched group was lower (P = 0.0001) than that of the rats that had been fed the high fat, low carbohydrate, ethanol-containing diet throughout. The importance of these findings is accentuated by the fact that the rats switched to the high carbohydrate, low fat diet consumed significantly more alcohol during the 11 d that they consumed this diet than the rats that continued to be fed the high fat, low carbohydrate diet.

Experiment 3. The rats fed the high fat, low carbohydrate diet consumed less energy than those fed the high carbohydrate diet, and frozen in liquid nitrogen and stored at

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TABLE 1

Composition of the four diets used in Experiments 1–3 of this study

<table>
<thead>
<tr>
<th>Diet components</th>
<th>High fat Low carbohydrate</th>
<th>High fat Low carbohydrate Ethanol</th>
<th>Low fat High carbohydrate</th>
<th>Low fat High carbohydrate Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>g/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premix</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>—</td>
<td>—</td>
<td>204</td>
<td>120</td>
</tr>
<tr>
<td>Corn oil</td>
<td>90</td>
<td>53</td>
<td>—</td>
<td>48</td>
</tr>
<tr>
<td>Ethanol</td>
<td>48</td>
<td>48</td>
<td>—</td>
<td>48</td>
</tr>
<tr>
<td>Water</td>
<td>818</td>
<td>807</td>
<td>704</td>
<td>740</td>
</tr>
<tr>
<td>Diet energy distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet energy, total, kJ/kg</td>
<td>4960</td>
<td>4906</td>
<td>4985</td>
<td>4906</td>
</tr>
<tr>
<td>Carbohydrate, % energy</td>
<td>67</td>
<td>67</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Fat, % energy</td>
<td>77</td>
<td>77</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>Ethanol, % energy</td>
<td>27</td>
<td>27</td>
<td>—</td>
<td>77</td>
</tr>
</tbody>
</table>

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TABLE 2

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Ethanol intake g/(kg·d)</th>
<th>Energy intake kJ/d</th>
<th>Body weight change g/14 d</th>
<th>Ethanol mmol/L</th>
<th>Glucose mmol/L</th>
<th>Lactate mmol/L</th>
<th>Liver g/100 g body weight</th>
<th>Liver fat g/100 g wet liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>High</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.9 ± 0.3b</td>
<td>5.7 ± 0.9</td>
<td>4.8 ± 0.1b</td>
<td>9.1 ± 0.8b</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>8.8 ± 0.5b</td>
<td>139 ± 4d</td>
<td>12 ± 3c</td>
<td>54 ± 8a</td>
<td>4.2 ± 0.2c</td>
<td>6.1 ± 1.2</td>
<td>5.7 ± 0.3a</td>
<td>12.0 ± 0.8a</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>—</td>
<td>294 ± 16a</td>
<td>84 ± 7a</td>
<td>—</td>
<td>6.9 ± 0.3a</td>
<td>4.4 ± 0.4</td>
<td>4.2 ± 0.2b</td>
<td>3.7 ± 0.2c</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>11.0 ± 0.6a</td>
<td>172 ± 8c</td>
<td>16 ± 5b</td>
<td>32 ± 6b</td>
<td>6.2 ± 0.2a,b</td>
<td>5.8 ± 0.4</td>
<td>5.3 ± 0.2a</td>
<td>8.2 ± 1.1b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. There were no significant interactions. Significant effect of diet, P < 0.0001; significant effect of alcohol, P < 0.001.
2 Means in a column with different superscript letters differ, P < 0.05 (Fisher’s partial least square difference test).

DISCUSSION

The present experiments confirmed our earlier observation (5) and that of others (3), that alcohol-consum ing rats have much lower blood alcohol levels when given high carbohydrate diets than isocaloric, high fat diets. Sankaran et al. (15) suggested that lower blood alcohol levels were essentially the result of an increase in total nonalcoholic energy. Although this may be a contributing factor, the acute injection study (Table 4) showed that a difference in metabolism due to type of diet is the main factor. The ethanol injections could not have induced the changes observed after only 2 h. They must have been produced by the preinjection consumption of the high carbohydrate and high fat diets.

This study extended our earlier observations to liver fat of chronically ethanol-administered rats. Liver fat was very low in rats fed high carbohydrate, low fat compared with high fat, low carbohydrate diets. Remarkably, in Experiment 1, the high carbohydrate, ethanol-fed rats had liver fat concentrations no higher than those in rats fed a high fat, low carbohydrate, control diet without ethanol. The results of Experiment 2 further accentuate the positive role of high carbohydrate intake in chronically alcohol-fed rats. The deleterious high blood ethanol and elevated liver fat values were lowered within 11 d of switching from a high fat to a high carbohydrate diet, even while consuming more alcohol after the switch (Table 3).

A protective effect of carbohydrate vis-à-vis fatty liver disease has been reported by several research teams (7,9,10).

TABLE 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Ethanol intake g/(kg·d)</th>
<th>Energy intake kJ/d</th>
<th>11-d body weight change g</th>
<th>Ethanol mmol/L</th>
<th>Glucose mmol/L</th>
<th>Lactate mmol/L</th>
<th>Liver fat g/100 g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.1 ± 0.2</td>
<td>277 ± 12</td>
<td>62 ± 6</td>
<td>51 ± 4</td>
<td>5.4 ± 0.2</td>
<td>4.4 ± 0.7</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>15.7 ± 0.3*</td>
<td>328 ± 8*</td>
<td>81 ± 4*</td>
<td>36 ± 5*</td>
<td>5.8 ± 0.2*</td>
<td>3.8 ± 0.3</td>
<td>5.4 ± 0.2*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8.
2 Group 1: This group received the high fat, low carbohydrate, ethanol diet throughout the 24-d experimental period; the ethanol intake, energy intake and body weight change results are for the last 11 d only. Group 2: This group received the high fat, low carbohydrate, ethanol diet for the first 13 d, followed by receiving the low fat, high carbohydrate, ethanol diet for 11 d.
3 Ethanol intake for the 8 rats switched to the low fat, high carbohydrate diet was not different from that of the 8 rats that continued to consume the high fat, low carbohydrate diet to the end of the experiment.
4 Different from group 1, P < 0.05.
and was also reviewed by Lieber (16). Much of the focus in the cited studies concerns the role played by CYP2E1, an enzyme system that appears to be strongly induced by ethanol in animals fed low carbohydrate diets. The liver damage has been proposed to be caused by the CYP2E1 metabolism of ethanol, which yields reactive oxygen species and other free radicals. Support for this comes from a study by Morimoto et al. (17), who observed that CYP2E1 inhibitors partially ameliorated changes in hepatic fatty acid composition of rats that chronically consumed ethanol and high fat diets. On the other hand, Kono et al. (18) recently reported in CYP2E1 knockout (-/-) mice that CYP2E1 is not involved in early, alcohol-induced liver injury. Our findings, although not directly related to CYP2E1, do cast some doubt on the importance of this hypothesis. It would be hard to explain the reversal in liver fat accumulation and blood ethanol level upon switching from the high fat to the high carbohydrate diet. In particular, the sharp reduction in blood ethanol in rats fed the high carbohydrate diet only 2 h after a single alcohol injection is not likely due to the induction of CYP2E1 by ethanol.

Tsutsumi and Takase (19), in reviewing possible causes of alcohol-induced fatty liver, mention a mechanism proposed by Lieber, in which ethanol becomes "a preferred fuel for the liver and displaces fat as a source of energy, contributing to fat accumulation." The present study casts doubt on this explanation because the rats with the fatty livers also had much higher blood alcohol levels when fed high fat rather than high carbohydrate diets (Experiment 1, Table 2).

Blood glucose levels were significantly lower in rats fed the high fat, ethanol-containing diet (Table 2), whereas rats fed the high fat diet after ethanol injection had lower lactate levels (Table 4). These results suggest that ethanol administration exacerbates a diet-related inhibition of glucose metabolism that may be related to the accumulations of ethanol in blood and, in turn, fat in the liver.

The current measures of glucose and lactate in plasma, although indirect indications of liver function, are generally consistent with more direct measures. Walker and Gordon (20) found a profound depletion of liver glycogen in rats after chronic ethanol feeding, a finding that is reflected in altered expression of glucose transporters (21). In the current study, the lowered levels of blood glucose after chronic ethanol intake are consistent with the low availability of liver glycogen. Chronic feeding of ethanol to rats has previously been found to lower levels of lactate and pyruvate in subsequently isolated hepatocytes (22). A likely explanation for this is an inhibition of glycolysis. A decrease in glycolytic compared with gluconeogenic enzyme activity has been demonstrated in rats following acute ethanol administration (23). In the current study, the differences in steady-state lactate production in liver were not apparent in blood, possibly due to other compensatory mechanisms. However, lactate was lowered after acute ethanol injection.

In summary, this study has advanced our understanding of alcohol-induced fatty liver in rats by showing the following: 1) an ethanol-containing high carbohydrate, low fat diet produces liver fat levels no higher than those in rats fed an isocaloric, high fat diet without ethanol; 2) the elevated liver fat accumulation in rats fed a high fat, ethanol-containing diet can be reversed by switching to a high carbohydrate diet without altering the ethanol intake of the rats; 3) blood ethanol, after a single intraperitoneal injection of ethanol, was reduced by more than half within 2 h in rats prefed a high carbohydrate, low fat compared with a high fat, low carbohydrate diet. This last finding should put to rest explanations involving a differential ethanol absorption rate by rats fed such diets. It also makes it unlikely that the differences are due to an ethanol effect on enzyme induction within such a short time after ethanol ingestion.

**LITERATURE CITED**


**TABLE 4**

Effect of acute ethanol administration to rats receiving high fat, low carbohydrate (HF LC) or low fat, high carbohydrate (LF HC) diets for 2 wk on plasma ethanol, glucose and lactate levels (Experiment 3)

<table>
<thead>
<tr>
<th>Time after ethanol injection (min)</th>
<th>Plasma ethanol (mmol/L)</th>
<th>Plasma glucose (mmol/L)</th>
<th>Plasma lactate (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF HC&lt;sup&gt;3&lt;/sup&gt;</td>
<td>HF LC&lt;sup&gt;3&lt;/sup&gt;</td>
<td>LF HC</td>
</tr>
<tr>
<td>30</td>
<td>48 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>60</td>
<td>40 ± 2&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>41 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>120</td>
<td>19 ± 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.3 ± 0.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. There were no significant interactions. Significant effect of diet, P < 0.0001 for lactate and P = 0.02 for ethanol; means for a variable with different superscript letters differ, P < 0.05 (Fisher’s partial least square difference test). Significant effect of time, P < 0.0001 for both lactate and ethanol.
2 All rats were injected intraperitoneally with 2 g ethanol/kg body; they were killed 30, 60 and 120 min later.
3 The 30 + 60 minute values, when combined, differed between the two diet groups, P < 0.05 (Fisher’s partial least square difference test).


