Diet and Risk of Ethanol-Induced Hepatotoxicity: Carbohydrate–Fat Relationships in Rats

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Nutritional status is a primary factor in the effects of xenobiotics and may be an important consideration in development of safety standards and assessment of risk. One important xenobiotic consumed daily by millions of people worldwide is alcohol. Some adverse effects of ethanol, such as alcohol liver disease, have been linked to diet. For example, ethanol-induced hepatotoxicity in animal models requires diets that have a high percentage of the total calories as unsaturated fat. However, little attention has been given to the role of carbohydrates (or carbohydrate to fat ratio) in the effects of this important xenobiotic on liver injury. In the present study, adult male Sprague-Dawley rats (8–10/group) were infused (intragastrically) diets high in unsaturated fat (25 or 45% total calories), sufficient protein (16%) and ethanol (38%) in the presence or absence of adequate carbohydrate (21 or 2.5%) for 42–55 days (d). Animals infused ethanol-containing diets adequate in carbohydrate developed steatosis, but had no other signs of hepatic pathology. However, rats infused with the carbohydrate-deficient diet had a 4-fold increase in serum ALT levels (p < 0.05), an unexpectedly high (34-fold) induction of hepatic microsomal CYP2E1 apoprotein (p < 0.001), and focal necrosis. The strong positive association between low dietary carbohydrate, enhanced CYP2E1 induction and hepatic necrosis suggests that in the presence of low carbohydrate intake, ethanol induction of CYP2E1 is enhanced to levels sufficient to cause necrosis, possibly through reactive oxygen species and other free radicals generated by CYP2E1 metabolism of ethanol and unsaturated fatty acids.

Key Words: ethanol; diet; carbohydrates; risk assessment; CYP2E1; hepatotoxicity; total enteral nutrition; hepatic necrosis; steatosis.

It is well established that the effects of xenobiotics on phase I and phase II metabolic systems are dependent upon several important factors such as age, gender and endocrine status (Alterman et al., 1995; Larssen et al., 1994; Madra et al., 1996; Ronis and Cuny, 1993; Webster et al., 1996). More recently, it has become apparent that nutritional status and diet are two variables that influence cellular responses to toxins (Duffy et al., 1997). Ethanol is a xenobiotic that is known to be associated with beneficial and adverse biological responses, depending upon all the factors mentioned above as well as the dose, frequency of intake, duration of exposure, and pattern of consumption.

It is not uncommon for xenobiotics to influence food consumption to such a degree as to result in poor nutritional status. Ethanol is a substance to which most laboratory rats have an aversion such that, if given a choice between pure water and dilute ethanol, most rats will prefer the water. Even when alcohol is mixed into liquid diets, rats will not typically consume the diet in levels necessary to either maintain body weight gains consistent with controls, or to achieve blood ethanol concentrations sufficient to produce the pathological effects typically observed in alcoholics. These changes in nutritional status can make data interpretation difficult for researchers studying the mechanisms associated with alcohol-induced hepatotoxicity, especially during chronic-exposure studies necessary to determine the long-term effects of ethanol.

One method to study the effects of any substance that affects dietary consumption is to control the amount of diet intake. In our laboratory, we use the common clinical nutrition procedures of total enteral nutrition (TEN) and total parenteral nutrition to deliver nutrients at levels recommended by the National Research Council for animals to study xenobiotic/diet interactions that affect endocrine and metabolic systems (Badger, 1985; Badger et al., 1979, 1993a,b,c, 1995; National Research Council, 1978; Ronis et al., 1991). We have used TEN to study the effects of chronic ethanol intake on cytochrome P450-dependent monoxygenases, growth hormone and ethanol metabolism (Badger et al., 1993a,b,c, 1995; Ronis et al., 1991). However, ethanol-induced liver damage was not detected in any of these chronic ethanol studies. Analysis of published studies from laboratories that use these nutritional delivery systems revealed that diets producing ethanol-induced liver damage had high levels of protein (20–22% total calories), fat (35–40%), and ethanol (40–47%), leaving very little room in the diet for carbohydrate calories (French, 1993; Tsukamoto et al., 1986; Nanji et al., 1989; 1995). Preliminary
studies in our laboratory suggested that one important factor in the hepatotoxic effects of chronic ethanol intake is low carbohydrate/fat ratio.

In the present studies, we investigated the hepatic effects of chronic intragastric ethanol administration using two diets differing only in the carbohydrate to fat ratio. Our results suggest that low dietary intake of carbohydrates may be an important factor in the rapid development of alcohol-induced liver damage previously reported to occur in the intragastric rat model. Furthermore, the mechanism by which ethanol-induced liver injury develops in this carbohydrate deficient model appears to be associated with an enhanced CYP2E1 induction. These data suggest that diet is an important factor in toxicity mediated by xenobiotic inducible P450s. In the case of CYP2E1, dietary carbohydrates appear to be important in its induction by ethanol.

MATERIAL AND METHODS

Animals. Virus-free adult male Sprague-Dawley rats (approximately 320 g) were purchased from Harlan Industries (Indianapolis, IN). Rats were kept in an American Association for Advancement of Laboratory Animal Care-approved facility with lights on between 0600 h and 1800 h. Each rat was conditioned by twice daily gentle handling for at least 7 days prior to surgically implanting a single gastric cannula, as previously described (Badger et al., 1993c). Rats were randomly assigned to treatment groups and one intragastric (IG) cannula was surgically implanted under Nembutal anesthesia, with care taken to monitor reflexes to assure proper surgical anesthetic levels. Water was infused IG at the rate of 1 ml/hr during a 14-day surgical recovery period in which they had ad libitum access to standard rat food and water. Experiments were approved by the Institutional Animal Care and Use Committee and all procedures had been previously standardized to avoid unnecessary pain and suffering of animals.

Diets. The total enteral nutrition (TEN) diets were formulated to meet National Research Council’s (NRC) requirement for the growing rat, when infused at the recommended level of calories (National Research Council, 1978). There were two basic diets employed in these studies. The TEN diet composition in Experiment 1 was identical to that published previously (Badger et al., 1993c), except that the protein content was increased from 15% to 16% and the source of dietary fat was changed to corn oil with the level decreased from 35 to 25% of total calories, as illustrated in the left panel of Figure 1. This resulted in an increase in the carbohydrate content from 50 to 59% of total calories. To study the effects of ethanol on liver function using this basic diet, ethanol was added at a level of 36.5% to 38% of total calories by substituting ethanol for a portion of the carbohydrate, resulting in a decrease in carbohydrate from 59 to 21% of total dietary carbohydrates, as illustrated in the left side of Figure 1. Thus, the ethanol-containing diet was isocaloric with the non-ethanol-containing diet, and infused at a rate of 187 kcal/kg/d. This level of total caloric infusion was based on published NRC data suggesting that growing rats require between 150 and 225 kcal/kg/d as well as on data collected in our lab from several experiments standardizing the weight gains of rats on the TEN system with those of rats fed standard rat food. A second diet was formulated to study the effects of chronic ethanol on liver function during carbohydrate deficiency in Experiment 2. The level of dietary carbohydrate was reduced from 21% to 2.5% of the total calories by substituting additional fat for carbohydrates, thus increasing the fat calories from 25% to 45%, as depicted in the right side of Figure 1. The control diet for Experiment 2 was formulated to be isocaloric by substituting carbohydrate calories for ethanol calories. These diets were infused at 187 kcal/kg/d.

Ethanol. Ethanol was introduced at 8–10 g/kg/d on day one, progressively increased to 13 g/kg/d (Experiment 1) or 12.5 g/kg/d (Experiment 2) over approximately 10 days, and remained at that level thereafter. The ethanol dose was carefully titrated to produce only mild signs of intoxication at peak blood ethanol concentrations (BECs).

Experiment 1. Sprague-Dawley rats were randomly assigned to one of two TEN diets, one with ethanol or one without. The diet used in this experiment is shown in Figure 1, where the carbohydrate to fat ratio was 0.84 in the ethanol group. The effects of chronic ethanol on liver pathology were studied after 55 days of continuous diet infusion.

Experiment 2. Sprague-Dawley rats were randomly assigned to one of two groups (control or ethanol-treated). The extremely low carbohydrate diet (2.5% of total calories) of the ethanol-treated rats is illustrated in Figure 1. This ethanol-containing diet had a carbohydrate to fat ratio of 0.022, nearly 38 times lower than the ethanol-containing diet in Experiment 1. The effects of chronic ethanol on liver pathology were studied after 42 days of continuous diet infusion.

Histological analysis. A thin slice of each hepatic lobe was fixed in 1% buffered formalin for histological examination. The histologic sections were routinely processed, cut and stained with hematoxylin and eosin (H and E). The examination was performed by a pathologist (SK) who had no prior knowledge of the treatment groups. Selected cases were reviewed by a second pathologist (JW). The presence or absence of fibrosis was confirmed by Masson’s trichrome and reticulin stain (special stain for collagen and reticulin). Fatty steatosis was scored as the percentage of liver cells containing fat according to Nanji et al. (7): <25% = 1; 25–50% = 2; 50–75% = 3; and <75% = 4. The presence of inflammatory
reaction (infiltration by polymorphonuclear leukocytes and mononuclear cells) was evaluated. The presence of hepatocyte necrosis was evaluated and scored 1 to 4 as follows: occasional (<1%) necrotic hepatocytes = 1; frequent (5–10%) necrotic hepatocytes = 2; small foci of necrosis (clusters greater than 10 necrotic hepatocytes) = 3; and extensive areas of necrosis (over 25% of the lobular unit) = 4. A total pathology score was calculated by summing the scores for steatosis and necrosis.

**Urine ethanol concentrations.** Daily urine ethanol concentrations (UECs) were measured as previously described (Badger *et al.*, 1993a; 1995). Daily UECs are excellent predictors of BECs as previously reported (Badger *et al.*, 1993b).

**Alanine aminotransferase (ALT: EC 2.6.1.2).** Serum ALT was assessed by a commercial kit according to the manufacturer’s instructions (Sigma Chemical, St. Louis, MO).

**Western blot analysis.** Isolated hepatic microsomes were assayed by Western blotting to determine the levels of CYP2E1, a previously described procedure (Badger *et al.*, 1995).

**Statistical analysis.** All data were analyzed by Student’s *t*-test and *p* ≤ 0.05 was considered statistically significant. The results are expressed as mean ± SEM.

**RESULTS**

**Experiment 1**

Continuous intragastric infusion of diets resulted in steady body weight gains in control and ethanol-treated rats. The mean body weights for Experiment 1 are presented in the top panel of Figure 2. The mean body weight gain of rats infused with our standard TEN diet without ethanol was 3.88 ± 0.6 g/d, and these body weight gains are very similar to the 3.25 ± 0.43 g/d of this age rats having *ad libitum* access to standard rat food and water in our laboratory (Badger *et al.*, 1993a,b,c, Ronis *et al.*, 1991). Ethanol infused rats in this experiment gained weight also, but at a lower rate of 2.0 ± 0.4 g/d (*p* ≤ 0.05). Some attrition (20%) occurred in both groups throughout the 55-day experiment, due to a combination of mechanical problems related to intragastric tubing malfunctions or to unknown causes, possibly ethanol toxicity.

UECs were cyclic (pulsatile) in nature, as reported previously (Badger *et al.*, 1993a,b,c, 1995; Tsukamoto *et al.*, 1985b) despite the continuous infusion of ethanol. Figure 2 (lower half) illustrates the pulsatile urine ethanol concentrations (UECs) of two representative rats per group between days 20 and 40 of continuous infusion of ethanol containing diets. The mean pulse frequency (6.12 ± 0.21 days), the mean peak UECs (469 ± 15), and the mean UEC (307 ± 9) are represented by the bars on the right side of this figure for all rats (10 rats per group) were similar to our previously reported data (Badger *et al.*, 1993a).

The mean liver weight of the ethanol-treated group at the end of the experiment was greater (*p* ≤ 0.05) than that of the control group (Table 1). Whereas steatosis was not observed in control rats of either experiment, all samples from ethanol-treated rats in this study had significant (*p* < 0.005) microvesicular and macrovesicular steatosis as compared to control rats, but necrosis did not occur (Table 1). No other signs of pathology were observed in the ethanol-treated rats. CYP2E1 was induced by ethanol approximately 9-fold, and the mean serum ALT concentrations (25 ± 3.4 vs. 41 ± 12.2 SF units/ml) did not differ between groups.

**Experiment 2**

Figure 3 illustrates the mean body weight gains and UECs of rats in this experiment. Mean body weight gains of control and ethanol-treated rats in Experiment 2 were similar to those in Experiment 1, with ethanol-infused rats having lower mean weight gains than controls (*p* ≤ 0.05). Some attrition (15%) occurred in both groups throughout the 42 day experiment due to a combination of mechanical problems related to intragastric tubing malfunctions or to unknown causes, possibly ethanol toxicity.

The UECs of two representative rats per group between days 20 and 40 of continuous infusion of ethanol-containing diets are presented on the lower left, and the bars on the lower right are the mean (± SEM) UEC, peak concentration, and frequency for 8–10 rats per group. When UECs from Experiment 1 were compared with Experiment 2, no statistically significant differences were found in these measures.
The mean liver weight of the ethanol-treated group was greater ($p < 0.01$) than that of control group (Table 2). In addition, livers from ethanol-treated rats in Experiment 2 were perceptively lighter in color than those from Experiment 1 and had a tendency to easily break apart at necropsy when compared with livers from control rats. Similar to Experiment 1, the overall architecture was preserved in both ethanol and control rats. Steatosis was not observed in control rats, but all samples from ethanol-treated rats in this experiment had significant ($p < 0.005$) microvesicular and macrovesicular steatosis as compared to control rats (Table 2). The severity of fatty

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight $^{a,b}$</th>
<th>Steatosis score</th>
<th>Necrosis score</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.1 ± 0.1</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>18.6 ± 0.5$^{c}$</td>
<td>3.4 ± 0.8$^{c}$</td>
<td>1.0 ± 0.0</td>
<td>4.4 ± 0.4$^{c}$</td>
</tr>
</tbody>
</table>

Note. Values are means (± SEM) of 8 to 10 rats per group.
$^{a}$ Liver weight in grams/rat.
$^{b}$ Represents 3.2% and 4.3% total body weight for control and ethanol-treated groups, respectively.
$^{c}$ $p \leq 0.05$ when compared with the respective control.

The severity of necrosis was greater ($p < 0.05$) when compared with the respective control. The UECs for two representative rats are presented in the lower portion of the figure and the bars represent the means (± SEM) of the UEC average of the 20-day period ($288 \pm 11$) and peak ethanol concentrations ($476 \pm 9$) for all rats/group ($n = 8/10$).

![Graph](Image)

**FIG. 3.** Body weights and urine ethanol concentrations (UECs) in experiment 2. The mean (± SEM) body weights of control (open symbols) and ethanol (closed symbols) in Experiment 2. The mean weights of ethanol-treated Sprague-Dawley rats were lower ($p \leq 0.05$) than the control, as indicated by the asterisks. The UECs for two representative rats are presented in the lower portion of the figure and the bars represent the means (± SEM) of the UEC average of the 20-day period ($288 \pm 11$) and peak ethanol concentrations ($476 \pm 9$) for all rats/group ($n = 8/10$).

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight $^{a,b}$</th>
<th>Steatosis score</th>
<th>Necrosis score</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.6 ± 0.9</td>
<td>1.0 ± 0.0</td>
<td>1.6 ± 0.6$^{d}$</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19.9 ± 1.0$^{e}$</td>
<td>4.1 ± 0.2$^{e}$</td>
<td>3.2 ± 0.4$^{e}$</td>
<td>7.3 ± 0.4$^{e}$</td>
</tr>
</tbody>
</table>

Note. Values are means (± SEM) of 8 to 10 rats per group.
$^{a}$ Liver weight in grams/rat.
$^{b}$ Represents 2.4% and 4.1% total body weight for control and ethanol-treated groups, respectively.
$^{c}$ $p \leq 0.05$ when compared with the respective control.
$^{d}$ One rat had a small focal necrotic lesion.

| DISCUSSION |

Nutrition, diet composition and the level of dietary intake affect an animal’s response to various xenobiotic challenges (Alterman et al., 1995; Larsen et al., 1994; Madra et al., 1996; Ronis et al., 1991; Webster et al., 1996). We have developed a nutritional delivery system for animals (Ronis et al., 1991) that allows controlled dietary intake via intragastric (IG) infusion of liquid diets. Using this system, we studied the effects of varying the carbohydrate to fat ratio on the ability of one
xenobiotic, ethanol, to induce CYP2E1 and produce hepatotoxicity.

This study demonstrated significant liver damage with chronic intragastric infusion of diets containing high concentrations of ethanol and unsaturated fat, only when the carbohydrate to fat ratio was extremely low. It is interesting that in Experiment 1, rats were infused with slightly greater ethanol doses than in Experiment 2 (13 g/kg/d and 12.5 g/kg/d, respectively) and for a longer period (55 and 42 days, respectively), but liver damage only occurred in Experiment 2 where the carbohydrate-to-fat ratio was low. This suggests that in addition to sustained high BECs and high dietary levels of unsaturated fat, a low ratio of carbohydrate-to-fat can be an important factor in the rapid development of alcohol-induced liver damage. These data further suggest that this low carbohydrate-to-fat ratio may predispose the liver to the harmful effects of chronic ethanol, or that a greater carbohydrate to fat ratio may provide some protective effect against such tissue damage. A similar conclusion was reached by Sankaran et al., who suggested that “severe liver pathology occurred in this model (intragastric model) only when rats consumed high amounts of both alcohol and fat and low amounts of carbohydrate”, and further suggested “that intake of adequate amounts of nutrients can abate the effects of a toxic alcohol dose administered to rats.” (Sankaran et al., 1994, p. 115).

Using the Lieber DiCarli diet, Sankaran et al. also reported that the level of dietary carbohydrate altered blood alcohol concentrations and the extent of steatosis in alcohol-treated rats (Sankaran et al., 1991, 1992). In the present study, we assessed both blood ethanol concentrations (BECs) and urine ethanol concentrations (UECs) during chronic ethanol infusions. Since previous studies have demonstrated that UECs accurately track BECs in the intragastric model (Badger et al., 1993a,b,c, 1995) and because urine collection has far fewer technical problems.

**FIG. 4.** Effects of ethanol on hepatic pathology in experiment 2. This H and E micrograph is from the liver of a representative ethanol-treated Sprague-Dawley rat in Experiment 2. Panel A, an original × 40, shows preserved overall architecture with diffuse microvesicular steatosis and focal mononuclear infiltrate. The insert delineates the area magnified × 100 in Panel B and × 400 in panel C. The arrows in Panel C highlight mononuclear inflammatory cells. Panel D, original magnification × 200, shows individual cell necrosis of the hepatocyte marked by the large arrow in addition to mononuclear inflammatory cells marked by the small arrow.
than daily blood collection in chronic experiments, UECs were relied upon more than BECs. Our analysis found that in this chronic model, there were no differences in mean UEC, pulse frequency, or pulse amplitude between Experiments 1 and 2, even though substantial differences in carbohydrate intake existed. Similar results were obtained in a fewer number of spot serum samples (data not presented). However, the rats receiving low carbohydrate diets were infused 12.5 g/kg/d, whereas, rats having high carbohydrate intake were infused 13 g/kg/d. Although the difference of 0.5 g/kg/d ethanol seems almost too little to be important, increasing the ethanol intake from 12.5 to 13 g/kg/d in Experiment 2 would have been sufficient to cause ethanol toxicity. Thus, the level of carbohydrate intake may have influenced ethanol metabolism such that the high levels BECs and UECs were maintained at slightly lower ethanol doses.

Other investigators have suggested that diets high in carbohydrate prevented ethanol-induced steatosis (Sankaran et al., 1994; Yonekura et al., 1989). However, the present study, as well as in published observations (Tsukamoto et al., 1985a,b) and other unpublished studies from our laboratory, demonstrate that fatty liver occurs with chronic ethanol even when diets are high in carbohydrate, suggesting that high carbohydrate intake will not prevent ethanol-induced fatty liver. Thus, it appears that ethanol-induced steatosis is quite variable and does not necessarily correlate with either dietary fat or carbohydrate levels.

In the present study, diets were kept isocaloric, and the level of ethanol and protein were essentially the same in all diets. There are two obvious consequences of reducing the carbohydrate-to-fat ratio while keeping ethanol and protein levels the same: (1) an increase in total fat calories from 25% to 45%; and (2) carbohydrate deficiency. When two variables change simultaneously within a given experiment, it is difficult to determine the mechanisms of resulting effects. For example, the dramatic differences in ethanol-induced hepatic pathogenesis observed between Experiments 1 and 2 could have been due primarily to carbohydrate deficiency, elevated fat, or the ratios of calories derived from any of the energy sources (carbohydrate:fat, carbohydrate:protein, carbohydrate:ethanol, fat:ethanol, or fat: protein).

We suspect that the carbohydrate component of the low carbohydrate-to-fat ratio is the most important, for two reasons. First, published evidence suggests that unsaturated fatty acids alone do not induce CYP2E1 to levels as high as we observed in the current studies. For example, Yoo et al. (1991) demonstrated that CYP2E1 induction was linear in response to unsaturated fatty acids (UFA), such that when 25% and 45% of the total calories were composed of UFA, CYP2E1 induction was less than 2-fold and 4-fold, respectively. Thus, the Yoo et al. data would suggest that although CYP2E1 is significantly induced by UFA, the increase in UFA from 25% in Experiment 1 to 45% in Experiment 2 of the current study is relatively minor in terms of CYP2E1 induction and is not the primary reason for the 34-fold CYP2E1 induction in Experiment 2. CYP2E1 induction by chronic ethanol in the TEN model appears to be limited to 3- to 9-fold when diets have CHO levels greater than an as yet to be established threshold (i.e. 10% of total calories). Under this scenario we would postulate that when the CHO levels of our ethanol-containing TEN diets drop below this threshold, ethanol induction of CYP2E1 is increased to levels of 27- to 34-fold, suggesting that CHO may be repressing CYP2E1 expression. Further studies are required to resolve these issues and to determine the mechanisms underlying these effects.

The second reason we suspect that the level of dietary carbohydrate intake is of primary importance in ethanol-induced hepatotoxicity from diets having a low carbohydrate-to-fat ratio resides in suggestions of Sankaran et al. that the level and source of total energy intake are important determinants of the adverse effects of chronic ethanol intake (Sankaran et al., 1991; 1992; 1994). Since carbohydrate is a major energy source, the increased ethanol-induced hepatic pathology in carbohydrate-deficient rats may be related to energy metabolism during cellular stress created by high levels of ethanol. Thus, the high carbohydrate diet may have protected against necrosis because the carbohydrate level was sufficient to provide the needed energy to prevent damage, energy that could not be supplied by fat as an energy source.

It is interesting that ethanol-induced liver injury in the presence of a low carbohydrate-to-fat ratio was associated
with the highest CYP2E1 induction we have ever observed in our laboratory. Such elevated levels of CYP2E1 in the presence of high concentrations of two CYP2E1 substrates, unsaturated fatty acids and ethanol, could generate damaging reactive oxygen species and oxidative stress sufficient to produce an inflammatory response and hepatic necrosis. Support for CYP2E1 involvement in the ethanol-induced liver damage is also provided by experiments in which liver damage was reported blocked by inhibition of CYP2E1 in rats treated chronically with ethanol (Morimoto et al., 1995).

Further research is necessary to determine if a similar relationship between the levels of dietary carbohydrate, fat and ethanol exists in the human alcoholic prior to developing alcoholic liver disease, and if it does, what levels of carbohydrate are necessary for such effects. This is important, because the very low carbohydrate level used in the intragastric rat model is unlikely to be present in most human diets.

In summary, we have studied the effects of chronic ethanol infusion on ethanol-induced liver pathology using two diets (high and low carbohydrate-to-fat ratio). We found that carbohydrate deficiency and CYP2E1 induction were associated with rapid development of alcohol-induced macrophage infiltration and hepatic necrosis; whereas neither were observed in rats fed higher levels of dietary carbohydrate. These data suggest that low-dietary-carbohydrate or low carbohydrate-to-fat ratios may predispose rats to the liver damaging effects of chronic ethanol and the mechanism may be enhanced by CYP 2E1 induction. Thus, results from this study demonstrate the significance of diet composition on the effects of at least one xenobiotic on a key phase I enzyme involved in hepatotoxicity and add further support for the assertion that diet and nutritional status are important factors to consider in risk assessment.

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