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HEPATIC GAMMA-GLUTAMYLTRANSFERASE ACTIVITY: ITS INCREASE FOLLOWING CHRONIC ALCOHOL CONSUMPTION AND THE ROLE OF CARBOHYDRATES

ROLF TESCHKE and ALEXANDER S. PETRIDES
Medical Department D, University of Düsseldorf, Moorenstr. 5, 4000 Düsseldorf, West Germany

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Abstract—Prolonged feeding of diets containing ethanol leads to a significant increase of hepatic gamma-glutamyltransferase (GGT) activity which has been ascribed either to ethanol itself or to dietary imbalance with respect to carbohydrates. Hepatic GGT activity was therefore determined in Sprague–Dawley rats fed for five weeks liquid diets containing various amounts of protein, fat and vitamins. Compared to the normal control diet containing 47% of total calories as carbohydrates, a hypocaloric diet with 11% of total calories of the control diet as carbohydrates failed to result in major alterations of hepatic GGT activity (0.27 ± 0.04 Units/g liver wet weight vs 0.35 ± 0.06; N.S.). Similarly, hepatic GGT activity remained virtually unchanged under a hypercaloric carbohydrate rich diet. However, hepatic GGT activity was strikingly enhanced by a diet in which carbohydrates were replaced to the extent of 36% of total calories by ethanol to achieve a carbohydrate content of 11% (0.66 ± 0.12 Units/g liver; $P < 0.005$), indicating that alcohol itself is capable of increasing hepatic GGT activity. However, alcohol given with a high carbohydrate diet was shown to be incapable of increasing the hepatic activity of GGT. These data therefore indicate that upon chronic intake ethanol itself enhances hepatic GGT activity provided that the carbohydrate content of the diet is low, whereas such an effect could not be observed with ethanol in a high carbohydrate diet.

Gamma-glutamyltransferase (GGT; EC 2.3.2.2) is a component of the gamma-glutamyl cycle responsible for the metabolism of glutathione and the transfer of amino acids through plasma membranes [1, 2]. Enzymic activity of GGT can be detected in a variety of organs including kidney [3–5], pancreas [5], intestinal tract [5] and liver [5–10].

Prolonged intake of alcohol leads to an increase of hepatic GGT activity both in man [8, 11–13] and experimental animals [5, 6, 9, 10, 13–15], suggesting that hepatic enzyme induction rather than liver cell injury may be the primary event leading to increased hepatic activities. On the other hand, it has been speculated that enhanced hepatic GGT activities observed following experimental alcohol feeding might exclusively be due to dietary imbalance with respect to carbohydrates rather than to ethanol itself [16]. Indeed, in the alcohol diets commonly used for experimental studies for the effects of alcohol parts of the carbohydrates have to be substituted for by alcohol in order to achieve isocaloric pair-feeding [5, 6, 9, 10]. The present experiments were therefore undertaken to elucidate the mechanism leading to increased GGT activities in the liver following the administration of alcohol containing diets and to study the respective roles of alcohol and carbohydrates.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats with a starting body weight of 130–150 g were obtained from Zentralinstitut für Versuchstierzucht (Hannover). They were fed Altrumin-R chow and tap water *ad libitum* until they reached a body weight of 170–190 g. The animals were then placed in individual wired-bottom cages and group-fed for five weeks with liquid diets containing various amounts of carbohydrates and, when indicated, also ethanol. The liquid diets were offered to the animals in drinking tubes as the only source of food and water.

Preparation and composition of the diets. For the preparation of the various liquid diets the method of DeCarli and Lieber [17] was followed. The control diet (Table 1) was nutritionally adequate and contained dextrin-maltose as carbohydrates (47% of total calories), protein (18% of total calories), fat (35% of total calories) as well as sufficient amounts of vitamins and trace elements [17]. The corresponding regular alcohol diet exhibited the same composition as the control diet, except that carbohydrates were isocalorically replaced by ethanol to the extent of 36% of total calories (Table 1). Both the control diet and the regular alcohol diet contained 1.0 kcal/ml corresponding to 4.2 kJ/ml.

The hypocaloric diet contained all nutrients, vitamins and essential trace elements as the control diet but carbohydrates only to the extent of 11% rather than 47% of total calories of the control diet (Table 1). Since the carbohydrates deleted in the hypocaloric diet were not replaced by ethanol, the hypocaloric diet exhibited a caloric value of only 0.64 kcal/ml corresponding to 2.7 kJ/ml.

The hypercaloric alcohol diet had the same dietary composition as the control diet, except that it was supplemented by ethanol to the same extent as the regular alcohol diet (Table 1). Finally, the compo-

Table 1. Composition of liquid diets

Diet	Dietary components carbohydrate ethanol protein fat (per cent of total calories of the control diet)				Dietary caloric value kcal/ml	kJ/ml
Control	47	—	18	35	1.0	4.2
Regular alcohol	11	36	18	35	1.0	4.2
Hypocaloric	11	—	18	35	0.64	2.7
Hypercaloric alcohol	47	36	18	35	1.36	5.7
Hypercaloric	83	—	18	35	1.36	5.7

The caloric value of the control diet was 1.0 kcal/ml (4.2 kJ/ml) and set as 100%. All diets exhibited the same amount of vitamins and trace elements. Dextrin-maltose was used as the source for carbohydrates.

sition of the hypercaloric diet was identical to the hypercaloric alcohol diet, except that alcohol was isocalorically replaced by additional carbohydrates. The caloric value of both the hypercaloric alcohol diet and the hypercaloric diet was 1.36 kcal/ml corresponding to 5.7 kJ/ml.

Feeding regimen. The rats were divided into five groups each consisting of eight animals. One of the groups received one of the liquid diets described above. The diets were given daily between 8 and 9 a.m. During the 24 hr preceding the killing of the rats, the diets were given in three divided doses at approximately 8 hr intervals. The various diets were freshly prepared every two to three days and stored until further use at 4°.

In preliminary experiments it was shown that animals fed the regular alcohol diet exhibited the lowest dietary intake with respect to volume. To ensure that each animal of one group received the same volume of the particular diet as the corresponding animals of the other groups, the volume of these diets was therefore determined according to the dietary volume consumed the day before by the animals fed the regular alcohol diet.

Preparation of liver homogenates. After feeding the liquid diets for five weeks, the animals were killed by decapitation. Their livers were perfused *in situ* with ice-cold 0.15 M KCl through the portal vein, excised and chilled. The livers were then homogenized in three volumes of 0.15 M KCl using a glass homogenizer with a Teflon pestle to obtain a 25% liver homogenate.

Enzyme assays and biochemical analyses. The activity of gamma-glutamyltransferase (GGT) was measured in liver homogenates by spectrophotometric assay at 405 nm according to the method of Szasz [18] in an incubation medium containing gamma-L-glutamyl-*p*-nitranilide at a final concentration of 4 mM, 40 mM glycylglycine and 1.85 mM Tris buffer, pH 8.25. Glutamate oxalacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were measured according to Bergmeyer and Bernt [19, 20]. Alkaline phosphatase (ALP) activity was determined according to Hausamen *et al.* [21] with natrium-*p*-nitrophenyl-phosphate as substrate. Glutamate dehydrogenase (GDH) activity was assessed according to the method of Schmidt [22]. All kits for enzyme assays were obtained from Boehringer Corp. (Mannheim, West Germany). The protein determination was performed according to the method of Lowry *et al.* [23], using crystalline bovine albumin as standard.

Statistical analysis. Each measurement was carried out at least in duplicate. The results obtained are expressed as means (± S.E.M.), and the significance of differences was assessed by Student's *t*-test for groups.

RESULTS

Dietary intake, weight gain, liver weight and liver protein

The daily volume of dietary intake was similar in all experimental groups (Table 2). Moreover, in the two alcohol groups fed either the regular alcohol diet or the hypercaloric alcohol diet the alcohol intake was similar with respect to average daily consumption. Although the daily dietary as well as caloric intake was similar in the groups fed either the control diet or the regular alcohol diet, the average daily body weight gain was much more pronounced in the former group (Table 2). The latter group exhibited a daily weight gain similar to that observed in animals fed the hypocaloric diet. As expected, a major weight gain was achieved with the hypercaloric diet. However, this was not the case with the hypercaloric alcohol diet (Table 2). The liver weights in all experimental groups showed no major alterations (Table 2). However, the hepatic protein content was considerably higher in both the regular alcohol and hypercaloric alcohol diets when compared to the other diets devoid of alcohol.

Gamma-glutamyltransferase (GGT)

Prolonged administration of the regular alcohol diet resulted in a striking rise of hepatic gamma-glutamyltransferase activity by 144% ($P < 0.005$) when the activity was compared with the data obtained following the application of the control diet and expressed per gram of liver wet weight (Table 3). The increase persisted when GGT activity was calculated per gram of hepatic protein or per 100 g of body weight. These data raise the question of whether the increase can be ascribed to ethanol itself or to the decreased carbohydrate content of the regular alcohol diet due to isocaloric replacement of carbohydrates by ethanol.

To eludicate the mechanism of increased hepatic GGT activity due to alcohol containing diets, GGT activity was determined after prolonged intake of

Table 2. Dietary intake and effect of various liquid diets on body weight, liver weight and liver protein

	Diets				
	Control	Regular alcohol	Hypocaloric	Hypercaloric alcohol	Hypercaloric
Daily dietary intake (ml/day)	53.2 ± 0.4	50.1 ± 1.2	53.3 ± 0.7	50.3 ± 1.2	53.0 ± 0.6
Daily caloric intake (kcal/day)	53.2 ± 0.4	50.1 ± 1.6	35.0 ± 0.4	69.5 ± 1.5	71.5 ± 1.0
Daily ethanol intake (g/day)	—	2.8 ± 0.1	—	2.7 ± 0.1	—
Body weight gain (g/day)	0.75 ± 0.01	0.25 ± 0.01	0.18 ± 0.01	0.70 ± 0.01	0.95 ± 0.01
Liver wet weight (g)	7.3 ± 0.3	6.3 ± 0.4	6.0 ± 0.4	7.2 ± 0.4	8.5 ± 0.4
Liver weight (g/100 g of body weight)	3.20 ± 0.05	3.39 ± 0.09	3.01 ± 0.07	3.25 ± 0.05	3.62 ± 0.09
Liver protein (mg/g of liver wet weight)	138.0 ± 14.0	165.2 ± 23.0	134.9 ± 7.5	156.7 ± 3.0	139.9 ± 13.6

Female Sprague–Dawley rats were fed for five weeks with various liquid diets. The composition of the diets are shown in Table 1. The data are given as means ± S.E.M. of 8 animals of each experimental group.

diets containing various amounts of carbohydrates and/or alcohol (Table 1). Compared to the regular alcohol diet, the administration of the hypocaloric diet exhibiting the same amount of carbohydrates (11%) as the regular alcohol diet but no alcohol resulted in a significantly lower activity of hepatic GGT by 47% ($P < 0.005$) (Table 3). Moreover, the hypocaloric diet had little if any effect on hepatic GGT activity when compared to the control diet and there was no significant change of hepatic GGT activity following the administration of the hypercaloric diet when compared with the control diet (Table 3).

Of particular interest was the finding that ethanol exerts its effect on hepatic GGT activity only in alcohol diets with a low but not with a high carbohydrate content. Indeed, the administration of the hypercaloric alcohol diet, which contains the same constituents as the regular alcohol diet but with 47% instead of 11% of total calories as carbohydrates, had little if any effect on hepatic GGT activity when compared with the values obtained for the control diet (Table 3).

Since changes of the diet with respect to the ethanol and carbohydrate content resulted in striking alterations of hepatic GGT activity when expressed per gram of liver protein, the question arose whether similar data could be obtained considering the activity per gram of liver wet weight or per unit body weight. The calculation shows that the various diets employed in this study exhibit similar results for GGT activity irrespective of whether the activity was assessed per gram of liver wet weight, per gram of hepatic protein or per 100 g of body weight (Table 3).

Alkaline phosphatase (ALP)

The administration of the regular alcohol diet and the hypercaloric alcohol diet failed to significantly alter the hepatic ALP activity, irrespective of whether the activity was expressed per gram of liver wet weight, per gram of liver protein or per 100 g of body weight (Table 4). On the other hand, the hypocaloric diet with a low carbohydrate content significantly increased the hepatic ALP activity in comparison with the control diet, whereas the opposite effect could be demonstrated for the hypercaloric diet rich in carbohydrates, when the activities are given per gram of liver wet weight (Table 4).

Glutamate pyruvate transaminase (GPT)

The administration of the regular alcohol diet

Table 3. Effect of alcohol and carbohydrates on the hepatic activity of the gamma-glutamyltransferase (GGT)

Diet	Gamma-glutamyltransferase (GGT)		
	U/g of liver wet weight	U/g of liver protein	U/100 g of body weight
Control	0.27 ± 0.04	1.95 ± 0.28	0.91 ± 0.14
Regular alcohol	0.66 ± 0.12*	4.92 ± 1.21*	2.49 ± 0.48†
Hypocaloric	0.35 ± 0.06‡	2.59 ± 0.48‡	1.09 ± 0.18‡
Hypercaloric alcohol	0.29 ± 0.03‡	1.89 ± 0.24‡	0.97 ± 0.12‡
Hypercaloric	0.25 ± 0.03‡	1.79 ± 0.20‡	0.89 ± 0.13‡

* $P < 0.005$; † $P < 0.01$; ‡ N.S.

Female Sprague–Dawley rats were fed for five weeks various liquid diets as shown in Table 1. The enzyme activities were determined in the 25% liver homogenates. The values are derived from 8 animals in each group and are given as means ± S.E.M.

Table 4. Effect of alcohol and carbohydrates on the hepatic activity of alkaline phosphatase (ALP), glutamate pyruvate transaminase (GPT), glutamate oxalacetate transaminase (GOT) and glutamate dehydrogenase (GDH)

Assay	Diets				
	Control	Regular alcohol	Hypocaloric	Hypercaloric alcohol	Hypercaloric
ALP					
U/g of liver wet weight	2.30 ± 0.09	2.53 ± 0.37§	2.85 ± 0.12‡	2.47 ± 0.28§	2.07 ± 0.12*
U/g of liver protein	17.3 ± 1.4	18.0 ± 3.8§	21.5 ± 2.0*	16.6 ± 2.6§	15.8 ± 1.8§
U/100 g of body weight	7.76 ± 0.16	8.99 ± 1.3§	8.95 ± 0.43§	8.49 ± 0.96§	7.65 ± 0.38§
GPT					
U/g of liver wet weight	155 ± 17	229 ± 20†	203 ± 14*	151 ± 10§	138 ± 14§
U/g of liver protein	1200 ± 190	1620 ± 220*	1510 ± 150*	1000 ± 10§	1110 ± 210§
U/100 g of body weight	524 ± 59	815 ± 77†	640 ± 50§	516 ± 34§	509 ± 53§
GOT					
U/g of liver wet weight	703 ± 31	870 ± 79*	704 ± 29§	658 ± 28§	682 ± 31§
U/g of liver protein	5340 ± 560	5660 ± 410§	5130 ± 230§	4280 ± 250*	5220 ± 630§
U/100 g of body weight	2370 ± 95	3107 ± 210†	2208 ± 96§	2249 ± 99§	2513 ± 89§
GDH					
U/g of liver wet weight	1275 ± 48	1438 ± 75*	1480 ± 96*	1425 ± 59*	1301 ± 81§
U/g of liver protein	9460 ± 960	9650 ± 840§	10870 ± 830§	9340 ± 640§	9910 ± 1210§
U/100 g of body weight	4302 ± 153	5109 ± 283*	4693 ± 343*	4885 ± 247§	4795 ± 243§

* $P < 0.05$; † $P < 0.005$; ‡ $P < 0.001$; § N.S.

Female Sprague–Dawley rats were fed for five weeks various liquid diets as shown in Table 1. The enzyme activities were determined in the 25% liver homogenates. The data were expressed as U/g of liver wet weight, U/g of liver protein, and U/100 g of body weight. The values are derived from 8 animals of each group and represent means ± S.E.M.

resulted in a striking increase of hepatic GPT activity compared with the control diet (Table 4), raising the question of whether this effect is due to the action of ethanol itself or to the low carbohydrate content of the regular alcohol diet. The hypercaloric alcohol diet with a high carbohydrate content failed to significantly alter the hepatic GPT activity compared with animals fed the control diet or even the hypercaloric diets, ruling out the possibility that alcohol itself is capable of increasing hepatic GPT activity. This was substantiated by the increase of hepatic GPT activity which was similar with both the hypocaloric diet as well as the regular alcohol diet (Table 4) when expressed per gram of liver wet weight or per gram of liver protein. The two diets exhibit an identical carbohydrate content, and the failure of the latter diet to further enhance the activity of hepatic GPT shows that ethanol itself has little effect on hepatic GPT activity.

Glutamate oxalacetate transaminase (GOT)

The hepatic GOT activity remained virtually unaffected by the administration of hypocaloric and hypercaloric diets when compared with the control diet (Table 4), indicating that carbohydrates alone are incapable of influencing the hepatic levels of GOT activities. However, ethanol in the regular alcohol diet but not in the hypercaloric alcohol diet resulted in a striking increase of hepatic GOT activity when expressed per gram of liver wet weight or per 100 g of body weight. The effect of ethanol was completely suppressed by carbohydrate rich diets.

Glutamate dehydrogenase (GDH)

Compared to the control diet, hepatic GDH activity was significantly increased following the administration of the regular alcohol diet (Table 4) when expressed per gram of liver wet weight or per 100 g of body weight. This enhancement, however, is entirely due to the low carbohydrate content of the regular alcohol diet, since the hypocaloric diet itself with the same carbohydrate content but devoid of alcohol has a similar inductive effect. On the other hand, with the hypercaloric alcohol diet an induction of hepatic GDH activity could be observed when compared with the control diet or the hypercaloric diet (Table 4) when expressed per gram of liver wet weight but not per gram of liver protein or per 100 g of body weight.

DISCUSSION

In the present experiments the effect of alcohol and carbohydrates on the activity levels of hepatic gamma-glutamyltransferase was assessed. It has been shown that hepatic GGT activities are strikingly enhanced after prolonged administration of diets in which part of the carbohydrates were isocalorically replaced by ethanol, provided that the carbohydrate content of the alcohol diets was low (Table 3). However, the enhancing effect of alcohol on hepatic GGT activity could completely be prevented by alcohol diets rich in carbohydrates. It is concluded from these results that the increase of hepatic GGT activity due to ethanol is dependent upon the amount of carbohydrates consumed during alcohol ingestion.

There has been considerable debate concerning the effects of ethanol on the hepatic activity of GGT. Previous studies have shown that alcohol consumption leads to an increase of hepatic GGT activities both in rats [5, 6, 9, 10, 13–15] and in man [8, 11–13], but in another experimental study it has been pointed out that ethanol depresses rather than enhances rat liver GGT activity [24]. The statement

of Singer and Kaplan [24] was based upon the finding that the zero time values for hepatic GGT activity were considerably higher than the subsequent values obtained following the administration of liquid alcohol diets. However, data on hepatic GGT activity in a control group fed the liquid control diet for the same period of time were not published, and a comparison between the two experimental groups was therefore not possible. Moreover, it should be pointed out that the zero time values of hepatic GGT activity published by Singer and Kaplan [24] are extremely high compared to other reports [5, 15, 25].

Of major concern was the question of the mechanism underlying the enhancement of hepatic GGT activities as a consequence of prolonged intake of diets containing alcohol. Some groups have suggested that increased hepatic activities of GGT can be ascribed to the action of ethanol itself [5, 14, 15], whereas Mørland *et al.* [16] strongly opposed this view and claimed that the enhancement of hepatic GGT activities may merely reflect the low carbohydrate content of the commonly used alcohol diets rather than the action of ethanol itself. The latter thesis was mainly based upon the observation that carbohydrates are capable of suppressing some hepatic functions [26], and it has therefore been concluded that diets with a low carbohydrate content may exert opposite effects [16]. That this may not apply for the hepatic activity of GGT could be clarified in the present study. Indeed, compared with the control diet with 47% of total calories as carbohydrates, the hypocaloric diet restricted with respect to carbohydrates to the extent of 36% of total calories failed to significantly increase the hepatic activity of GGT, and the hypercaloric diet rich in carbohydrates had no significant suppressive effect on hepatic GGT activity (Table 3). These data clearly show that the carbohydrate content has little if any influence on the activity levels of GGT in the liver, thereby opposing other viewpoints [16]. Evidence against a major role of carbohydrates in the activity levels of hepatic GGT was also provided by the fact that the chow pellet diet exhibits a carbohydrate content similar to or even higher than the commonly used liquid control diet [5, 15, 16]. Thus, the higher activities of hepatic GGT in animals fed the chow diet compared with those fed the liquid control diet [16] cannot be attributed to dietary imbalance with respect to carbohydrates. A similar conclusion has been reached in a more recent study by the group of Mørland [25]. Similarly, other studies have shown that the enhancement of GGT activity after ethanol were not due to decreased carbohydrate content under experimental conditions of carbohydrate substitution by fat [14]. Finally, a simple carbohydrate repression theory is weakened by the fact that the zero time control rats had been fed a pellet diet in which carbohydrates constituted at least the same percentage as carbohydrates in the control diet [16].

Although carbohydrates alone exhibit no major effects on the activity levels of hepatic GGT, they may substantially modify the alcohol mediated influence on hepatic GGT activity. Indeed, the administration of the regular alcohol diet had a striking effect on hepatic GGT activity, when compared either with the control diet or the hypocaloric diet (Table 3). Since the latter diet exhibits the same carbohydrate content as the regular alcohol diet (Table 1), the increase of the hepatic GGT activity following the administration of the regular alcohol diet can be ascribed primarily to the action of ethanol itself rather than to a carbohydrate effect, thereby opposing views to the contrary [16]. On the other hand, alcohol may fail to exert its increasing property on hepatic GGT activity when administered as hypercaloric alcohol diet (Table 3), since high carbohydrate diets are incapable of supporting the rise of hepatic GGT activity due to ethanol. These data therefore show the dependency of the alcohol-mediated increase of hepatic GGT activity on the amount of carbohydrates consumed during alcohol ingestion, but the exact reason for this phenomenon remains to be established.

On a subcellular level, GGT activity has been found to be increased following chronic alcohol consumption both in the microsomal fraction [5, 6, 14] and in plasma membranes [9, 10] of the hepatocytes. The enhancement was observed in plasma membranes free from bile canaliculi as well as in those rich in bile canaliculi [9, 10]. It is reasonable to assume that the hepatic increase of GGT activity after ethanol administration occurs primarily at the site of the endoplasmic reticulum [6, 9, 10]. The enzyme could then be translocated from the site of its synthesis to plasma membranes, possibly by means of the Golgi apparatus. However, the possibility cannot be ruled out that part of the GGT activity recovered in the microsomal fraction is due to contamination by plasma membrane GGT [9, 10].

The present study shows that the activity levels of other hepatic enzymes such as ALP, GPT, GOT and GDH are influenced by the carbohydrate content of the diets (Table 4). Hepatic ALP activity increases following the application of the hypocaloric low carbohydrate diet and decreases with the hypercaloric high carbohydrate diet. Moreover, the hepatic activities of GPT and GDH are enhanced after the application of the hypocaloric diet with a low carbohydrate content when compared with the control diet. The enhancement of hepatic GPT activity following the regular alcohol diet was not found with the hypercaloric alcohol diet rich in carbohydrates, and similar results were obtained with GOT and GDH. However, the enhancing effect of ethanol on hepatic GPT and GOT activities was completely suppressed by diets rich in carbohydrates.

This investigation confirms and extends previous reports on the effect of alcohol on body weight gain [27, 28]. It has been demonstrated before that compared to the respective control diet the weight gain of experimental animals was much less with alcohol diets exhibiting 36% of total calories as ethanol and 11% as carbohydrates [27], a finding confirmed in this study (Table 2). It is noteworthy that in our study rats pair-fed the regular alcohol diet only grow at 33% of the controls (Table 2) whereas even lower values have been reported by Gadeholt *et al.* [25]. In addition, in the present report a weight gain lower than with the regular alcohol diet was observed in rats fed the hypocaloric diet (Table 2) which exhibits the same nutrients as the former except that ethanol was omitted. These data clearly demonstrate that

ethanol *per se* has little if any effect regarding body weight gain due to an ineffective utilization of calories derived from ethanol. This is substantiated by the failure of the hypercaloric alcohol diet to achieve a significant weight gain compared with the regular control diet which apart from an additional ethanol content exhibits the same nutrients as the former diet (Table 2). The failure of ethanol of energy conservation may be due at least in part to the increased microsomal oxidation of ethanol, a process which is not linked to ATP production [28].

In conclusion, the present study shows that ethanol itself is capable of increasing hepatic GGT activity. This effect occurs, however, only when the carbohydrate content of the alcohol diets is low, whereas alterations of the carbohydrate composition by itself have little if any effect on the hepatic level of GGT activity.

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