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Adverse Reversible Effects of Chronic Ethanol Intake on Carbohydrate Metabolism (39416)

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(Introduced by W. C. Buchbinder)

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Several studies have shown that ethanol per se does not alter insulin secretion (1, 2). However, the data on the effects of ethanol on glucose-mediated insulin release and glucose tolerance are conflicting. For example, ethanol has been reported to delay or inhibit glucose-mediated insulin response as well as to induce glucose intolerance in humans and experimental animals (3-5). In contrast, other studies have shown an improvement in glucose tolerance and potentiation of glucose-mediated insulin release after ethanol pretreatment (6, 7). Metz *et al.* (6) have speculated about the use of ethanol as a therapeutic agent for the treatment of diabetes. The present study was designed to determine changes in carbohydrate metabolism after daily ingestion of ethanol for a period of 15, 30, and 60 days.

Materials and methods. Male Albino mice, weighing 20-25 g, were housed in conditions of controlled temperature, humidity, light cycle, and were fed Purina chow and tap water *ad libitum*. One week later, the mice were weighed, divided into three groups, with each group to receive one of the following solutions as the only drinking source: Group A, 10% v/v ethanol; Group B, 30% v/v ethanol; and controls, Group C, tap water for 15, 30, or 60 days. In order to check ethanol ingestion, blood ethanol levels were determined (8) in the fed state of randomly selected animals at different time intervals. Mean ± SE blood ethanol levels (mg/100 ml) were 32 ± 1.4 (n = 30) and 36 ± 1.8 (n = 30), respectively, in animals in Groups A and B. These values were not statistically different.

Oral Glucose Tolerance Test (GTT) was performed at the end of 15, 30, and 60 days of ethanol treatment. Mice were fasted for 18 hr except for water. In Groups A and B, ethanol was replaced by water to avoid the presence of ethanol in blood during the performance of GTT. A glucose load of 0.75 g/kg body weight dissolved in 0.2 ml of water per 10 g body weight was given by intragastric intubation. Some animals died during intubation resulting in different number of animals in each group. Blood samples were obtained from the orbital sinus before (0 time), and 30, 60, and 120 min after glucose administration. Whole blood was used for glucose measurements. For plasma insulin measurements, the microtubes were kept at 4° for 1 hr and then centrifuged for 3 min in a Beckman Spinco Microfuge. Plasma was separated and stored in plastic tubes at −20° for subsequent insulin assay. Blood glucose was determined on a Technicon Auto-Analyzer by the Hoffman ferricyanide method (9). Plasma immunoreactive insulin was determined by the double-antibody method of Morgan and Lazarow as modified by Blanks and Gerritsen (10). Plasma samples which showed hemolysis were discarded. Bovine insulin was used as a standard. Glucose increment ΔG, expressed as mg/100 ml/hr above its fasting value, and insulin increment ΔI, expressed as μU/ml/hr above its fasting value, were calculated. The insulinogenic index was determined by dividing ΔI by ΔG.

Three days after the performance of the GTT, peripheral insulin sensitivity was assessed in the same animals by determining the fall in blood sugar following the ip injection of insulin (0.2 U/kg) after an overnight fast. Blood samples for glucose estimation were obtained from the orbital sinus just before (0 time) and 30, 60, and 120 min after insulin injection.

To study whether the influence of chronic ethanol ingestion on carbohydrate metabolism was transient or permanent, 15 mice were given 10% ethanol as the only drink-

ing solution for 30 days and subsequently tap water for another 30 days. An equal number of control animals received tap water. At the end of 60 days, GTT and insulin sensitivity tests were done on both groups of animals, as described above. The data was analyzed statistically by Student's *t* test.

Results. Figure 1 shows the influence of daily ethanol ingestion for 60 days on body weights of mice. There was not statistical difference in body weights of control and 10% ethanol-fed animals as determined at weekly intervals during the entire study period. Mice receiving 30% ethanol lost weight for the first 15 days and then gained weight, but to a significantly lesser degree ($P < 0.001$ at all points) than either the control or the 10% ethanol-treated animals.

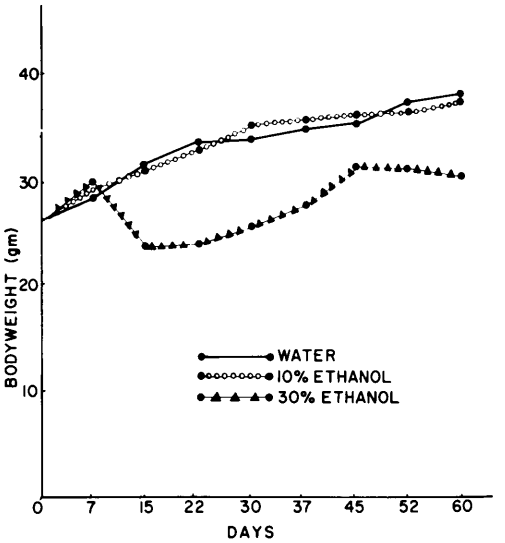

FIG. 1. Mean body weights of normal mice during ingestion of 10% and 30% ethanol and tap water (controls) for 60 days. Thirty animals were used in each group.

Effects of ethanol on oral glucose tolerance test. Fifteen days of daily ingestion of either 10 or 30% ethanol produced no discernible change in GTT as compared to control values (data not shown). Figure 2 shows the mean blood glucose response to oral GTT in mice fed 10% or 30% ethanol for 30 days. Fasting blood glucose values in ethanol-treated animals were not significantly different from those of the control animals. The respective values were 95 ± 3 ($n = 24$), 96 ± 4 ($n = 26$), and 86 ± 3 ($n = 23$) in control, 10% ethanol-, and 30% ethanol-treated animals. However, both groups of mice receiving ethanol showed abnormal glucose tolerance. Blood sugar values at 30, 60, and 120 min in the 10 and 30% mice were significantly different ($P < 0.01$) from the corresponding control values. Figure 2 also shows the effects of ethanol treatment for 60 days on GTT. The fasting blood sugar levels were not significantly different but the glucose tolerance tests were definitely abnormal in both ethanol-treated groups.

Effects of ethanol on plasma insulin. Figure 3 shows the mean plasma insulin response to oral glucose administration in control and ethanol-treated animals ($n = 15$ for each group of animals). Daily ingestion of 10 or 30% for 30 days did not exert any

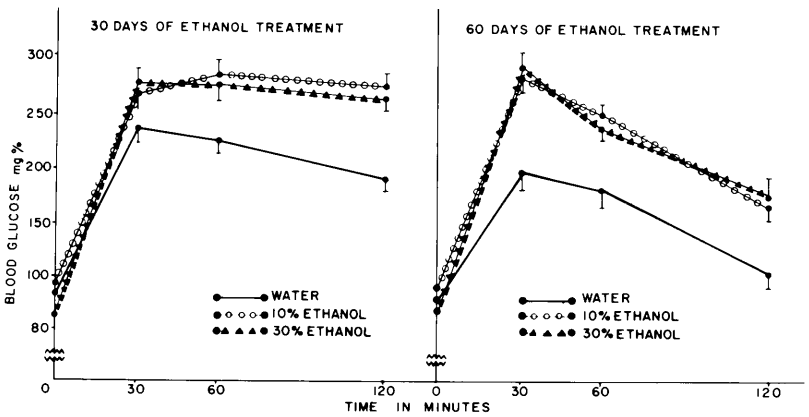

FIG. 2. Blood glucose values during oral glucose tolerance test in ethanol-fed and control mice. Means ± SE values are shown, and the number of animals in each group is as given in the text.

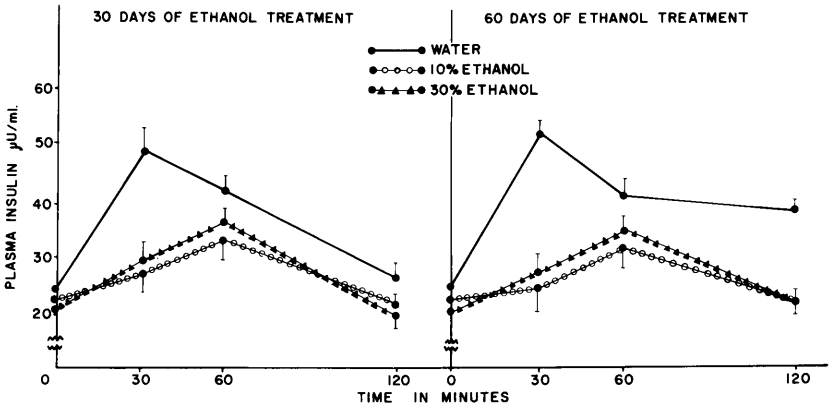

FIG. 3. Plasma insulin values during oral glucose tolerance test in ethanol-fed and control mice. Means ± SE values are shown and the number of animals in each group is as given in the text. Bovine insulin was used as standard for the plasma insulin determinations.

discernible effect on fasting plasma insulin levels. A significant decrease ($P < 0.01$ at all points) in plasma insulin response was observed at 30, 60, and 120 min in both ethanol-treated groups as compared to the respective control values. Peak insulin response to glucose was observed at 60 min in ethanol-fed animals and at 30 min in control animals. Figure 3 also shows that 60 days of 10 or 30% ethanol ingestion production essentially similar effects on plasma insulin levels. To see if the observed differences in insulin and glucose responses were interrelated, plasma insulin and blood glucose increments above the respective fasting values and the insulinogenic index were calculated (Table I). The insulinogenic index was significantly smaller ($P < 0.01$) in both groups of animals that received ethanol for 30 days than in the control animals (Table I). Similar changes in insulinogenic index were observed in animals that received ethanol for 60 days.

Effects of ethanol on insulin sensitivity test. Figure 4 shows the mean blood glucose response to insulin injection in control and ethanol-treated animals for 30 and 60 days. Both groups of animals that received ethanol were significantly ($P < 0.001$) less sensitive to insulin than the corresponding control animals. The magnitude of decreases in insulin sensitivity in the 10% ethanol-treated animals was not statistically different from that of the 30% ethanol-treated ones.

Effects of ethanol withdrawal on blood glucose and insulin levels. Figure 5 shows the mean blood glucose and plasma insulin responses to oral GTT in mice that were given 10% ethanol as the only drinking solution for 30 days and subsequently tap water for 30 days. In contrast to adverse effects of ethanol on GTT described above (Figs. 2 and 3), withdrawal of ethanol resulted in slight but significantly improved GTT as compared to that of control animals. Blood glucose increments were 124 ± 13 and 87 ± 3, $P < 0.01$ for the control and ethanol groups, respectively. There was no statistical difference in plasma insulin response between the two groups and the insulin increments were 24 ± 1 and 22 ± 1 μU/ml, respectively. Insulin sensitivity responses were also not statistically different as maximum decrease in blood glucose values were 29.1 ± 0.9 and 26.9 ± 1.1% of the respective baseline values in the ethanol-treated and control animals.

Discussion. Acute ethanol ingestion has been shown both to impair (3–5) and improve (6, 7) glucose tolerance in humans and experimental animals. The present study shows that chronic ethanol intake resulted in significant changes in glucose metabolism which include glucose intolerance, decreased and delayed glucose-mediated insulin response, as well as decreased peripheral insulin sensitivity. These changes were observed in animals that ingested ethanol as the only drinking solution for 30 or 60 days,

TABLE I. Effect of Daily Ethanol Ingestion for 30 Days on Insulinogenic Index in Normal Mice.

Treatment	Blood glucose increment (mg/100 ml/hr)	Plasma insulin increment (μU/ml/hr)	Insulinogenic index $\frac{\Delta\text{Insulin}}{\Delta\text{Glucose}}$
Control	158 ± 13	22 ± 2	0.15 ± 0.02
10% Ethanol (15)[a]	246 ± 15*	7 ± 2*	0.03 ± 0.01*
30% Ethanol (15)	271 ± 11*	12 ± 3*	0.04 ± 0.01*

[a] Number of animals given in parentheses.

* $P < 0.01$ vs control. Results (Mean ± SE) represent increments of blood glucose and plasma insulin above the respective fasting values. Glucose tolerance test was done as described in the text.

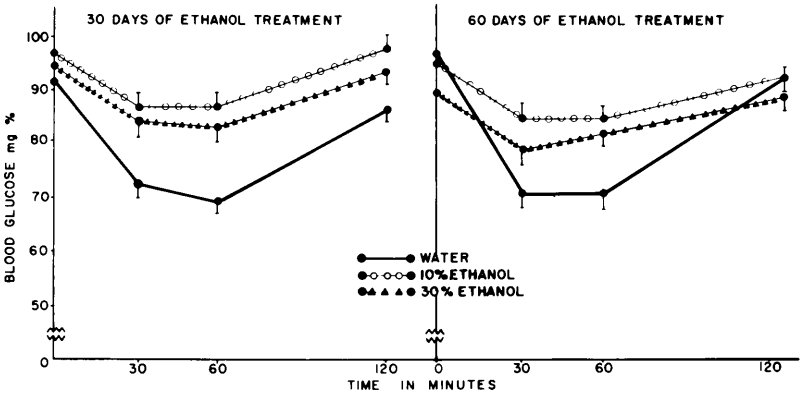

Fig. 4. Blood glucose values during insulin sensitivity test in ethanol-fed and control mice. Bovine insulin (0.2 U/kg) was given by ip injection. Means ± SE values are shown and number of animals in each group is as for Fig. 2 and given in the text.

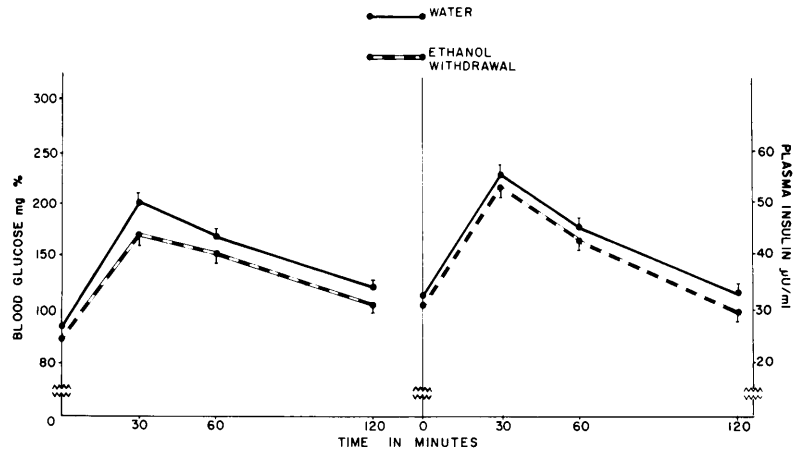

Fig. 5. Blood glucose and plasma insulin values during oral glucose tolerance test in 15 mice given 10% ethanol as the only drinking solution for 30 days and subsequently tap water for next 30 days. Age-matched 15 control mice received tap water for 60 days. Means ± SE values are shown. Bovine insulin was used as standard for the plasma insulin determinations.

but not when ethanol was given for 15 days. These experiments also indicate that the above-mentioned metabolic derangements were not permanent as withdrawal of ethanol improved or reversed various parameters to normal levels.

The data suggest that some of the factors (i.e., a decrease in glucose-mediated insulin

response as well as in peripheral insulin sensitivity) may be responsible for ethanol-induced glucose intolerance. However, the underlying mechanisms by which ethanol influences insulin secretion or peripheral insulin sensitivity are unclear. First, ethanol might exert direct effect on β-cells. *In vitro* experiments performed with pancreas of rats, rabbits, and golden hamsters have shown ethanol inhibition of glucose-mediated insulin release (11, 12). Malaisse *et al.* (13) reported that ethanol interferes with the functions of the microtubular system of β-cells. Secondly, during a single transhepatic circulation, the liver normally retains approximately 50% of the insulin presented. An enhancement of insulin degradation by the liver because of ethanol might be responsible for the diminished plasma insulin response. However, except for the possibility that the amount of insulin reaching the liver itself affects the insulin uptake by liver (14), other factors which might influence the retention of insulin by the liver are not well established. Ethanol-induced hepatic damage might be another reason for glucose intolerance as derangement in carbohydrate metabolism occurs in chronic liver disease. Liver morphology was not studied in the present investigations. Another mechanism might be an increase in blood levels of catecholamines that can result from ethanol ingestion. Catecholamines are known to inhibit insulin secretion and action. Finally, ethanol might alter insulin secretion via one or more gut hormones that influence the islets of Langerhans (15).

The significant decrease in peripheral insulin sensitivity in ethanol-treated animals is concordant with the observations of Lochner *et al.* (16) in dogs and Kreisberg (17) in man, that ethanol has an inhibitory effect on peripheral glucose utilization. Ethanol influence might be indirect via elevated free fatty acids, adrenal corticosteroids and catecholamines. Blood levels of these substances are known to increase after ethanol ingestion (18–20). Whether adverse effects of ethanol on glucose metabolism are the results of ethanol per se or one or both of its breakdown products (acetaldehyde and acetate) cannot be ascertained from the present data. Nonetheless, the observation that peripheral insulin sensitivity and diminished glucose-mediated insulin response improved or were restored to normal levels after ethanol withdrawal, suggests that the underlying mechanisms are reversible.

The findings of the present study may have implications concerning the use of ethanol in diabetic subjects. Some subjects have reported an improvement in glucose tolerance and potentiation of glucose-mediated insulin response after ethanol pretreatment (6, 7). This led to speculation about the use of ethanol as a therapeutic agent in diabetic humans. The present study showed adverse influence of chronic ethanol intake on glucose metabolism, albeit in normal mice. Daily ingestion of 10% and 30% ethanol induced essentially similar effects on glucose metabolism although the animals fed 30% ethanol lost weight as compared to those ingesting 10% ethanol.

Summary. The influence of chronic ethanol intake on carbohydrate metabolism was studied in mice. The results showed that daily ingestion of 10% or 30% ethanol for 30 or 60 days induced glucose intolerance, decreased and delayed glucose-mediated insulin response, as well as decreased peripheral insulin sensitivity. However, these metabolic derangements were not permanent as withdrawal of ethanol improved or restored these parameters to normal.

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