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Short Communication

Alterations in Glucose Transporter Proteins in Alcoholic Liver Disease in the Rat

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We used the intragastric feeding rat model for alcoholic liver disease to investigate alterations in glucose transporter isoforms GLUT 1 and GLUT 2 in response to different dietary fats and ethanol. Six groups of rats (three rats/group) were fed ethanol or dextrose with either saturated fat, corn oil, or fish (menhaden) oil. All control animals were pair fed the same diets as ethanol-fed rats except that ethanol was isocalorically replaced by dextrose. In all animals, the following were assessed: pathological changes in the liver, immunohistochemical and Western blot analysis of GLUT 1 and GLUT 2 isoforms, and glycogen distribution. The most severe pathological changes were seen in fish oil/ethanol fed rats, moderate changes were seen in the corn oil/ethanol group and no changes were observed in the dextrose-fed or saturated fat/ethanol groups. In the groups of rats showing pathological liver injury (corn oil/ethanol and fish oil/ethanol), the depletion in liver glycogen was accompanied by decreased GLUT 2 expression and increased GLUT 1 expression. A decrease in glycogen and GLUT 2 expression was also seen in the fish oil/dextrose-fed rats. We hypothesize that the shift in glucose transporters from GLUT 2 to GLUT 1 probably reflects a compensatory response to attenuated gluconeogenic activity and to meet the increased intracellular demand for glucose. This demand for glucose in the presence of depleted glycogen may serve to provide a source for ATP synthesis in the centrilobular zone where hypoxia occurs secondary to ethanol metabolism. (Am J Pathol 1995, 146:329–334)

The effects of alcohol on glucose metabolism are complex and sometimes contradictory.[1,2] These contradictions can in large part be explained on the basis of the nutritional status of the individual or experimental animal at the time of ethanol administration.[1] Alcohol provokes a reduction in liver glycogen content and decreased incorporation of glucose into glycogen.[1,2] High ethanol concentrations also inhibit gluconeogenesis.[3] The effects of ethanol in chronic ethanol-fed rats can also be compounded by the concomitant effects of endotoxemia.[4,5] Increased endotoxin levels have been described in both humans and experimental animals.[4–6]

Although several investigators have evaluated the role of ethanol on glucose metabolism,[3,7,8] much less is known about the regulation of glucose transporters in ethanol-fed rats. In liver, glucose is transported into parenchymal cells mainly through a facilitative transport mechanism. Within this family of facilitative glucose transporters that have been detected so far,[9–11] the low affinity liver type glucose transporter GLUT 2 is responsible for the symmetrical transport of glucose. In contrast to GLUT 2, the GLUT 1 glucose transporter isoform is localized in rat liver to a subset of hepatocytes that form the first row of cells around the terminal hepatic venule.[12] GLUT 1 is expressed mainly by cells oxidizing glucose in which the direction of glucose transport is unidirectional, ie, out to in.

The present study was designed to investigate alterations in the liver glucose transporters in the intragastric feeding model for alcoholic liver disease.[13,14] We attempted to relate the alterations in glucose transporters to severity of liver injury and type of dietary fat. On the basis our epidemiological observations in which we showed a relationship between type of dietary fat and alcoholic liver dis-

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ease,[15,16] we carried out additional studies in the intragastric feeding rat model in which we confirmed the relationship between type of dietary fat and alcoholic liver injury. Rats fed saturated fat and ethanol were protected against liver injury whereas rats fed corn oil and ethanol developed fatty liver, necrosis, inflammation, and fibrosis.[17,18] Recently, French[19] has shown that rats fed ethanol and fish oil develop extremely severe liver injury. We therefore used these three dietary models (ethanol with saturated fat, corn oil, or fish oil) to investigate the relationship between alterations in glucose transporters in the liver and severity of liver injury and type of dietary fat.

Materials and Methods

Animals

The experimental animals were male Wistar rats weighing between 225 and 250 g. Six groups of rats (three rats/group) were studied: saturated fat plus dextrose (SD), saturated fat plus ethanol (SE), corn oil plus dextrose (CD), corn oil plus ethanol (CE), fish oil plus dextrose (FD), and fish oil plus ethanol (FE). All animals were sacrificed at 1 month, and livers we assessed to pathological changes, immunohistochemical analysis of GLUT 1 and GLUT 2, and Western blot analysis for GLUT 1 and GLUT 2.

All animals were pair fed by continuous infusion of liquid diets through permanently implanted gastric tubes as described previously.[17,20] The diet contained either saturated fat, corn oil, or fish oil as the source of fat. The percentage of calories derived from fat was 25% of total calories. The fatty acid composition of the diets is shown in Table 1. Proteins, carbohydrates, minerals, and vitamins were administered as described previously.[17,20] The percentage composition of diets was such that the animals in all dietary groups consumed the same amount of calories, proteins, and vitamins. All control animals were pair fed the same diet as ethanol-fed rats except that ethanol was isocalorically replaced by dextrose. All diets were prepared fresh daily. In particular, the fish oil diet was stored in airtight containers filled with nitrogen and kept in a cold room at 4 C. Malondialdehyde content of the diets was <5 nmol/g of sample, suggesting that very little auto-oxidation was taking place. The amount of ethanol was initially started at 8 g/kg/day and gradually increased as tolerance developed. Blood alcohol levels, measured by an alcohol dehydrogenase method (Sigma Chemical Co., St. Louis, MO), were maintained between 150 and 350 mg/dL. All animals were sacrificed at 1 month after the start of feeding. All animals received humane care in compliance with the National Institutes of Health criteria for care of laboratory animals.

Table 1. *Fatty Acid Composition (Percentage by Weight) of Saturated Fat, Corn Oil, and Fish (Menhaden) Oil Diets*

Fatty acids	Saturated fat (%)	Corn oil (%)	Fish (menhaden) oil (%)
Octanoic (C8:0)	52.5	0	0
Decanoic (C10:0)	33.2	0	0
Lauric (C12:0)	0.5	0	0
Myristic (C14:0)	0	0.1	11.6
Palmitic (C16:0)	1.4	11.3	13.0
Palmitoleic (C16:1 w7)	0	0	13.3
Stearic (C18:0)	1.0	1.9	2.1
Oleic (C18:1 w9)	1.0	25.5	6.7
C18:1 w7	0	0	3.3
Linoleic (C18:2 w6)	1.5	59.0	1.1
Arachidonic acid (C20:4 w6)	0	0	0.7
(C20:4 w3)	0	0	1.9
Eicosapentaenoic acid (C20:5 w3)	0	0	17.3
(C22:5 w6)	0	0	0.4
(C22:5 w3)	0	0	2.0
Docosahexenoic acid (C22:6 w3)	0	0	8.2

Histological Analysis

A small sample of liver was obtained at sacrifice and formalin fixed. Hematoxylin and eosin stain was used for light microscopy. The examination was carried out by a pathologist who had no previous knowledge of the treatment groups. The liver pathology was scored as follows[20]: for steatosis (the percentage of liver cells containing fat), 1+ = <25%, 2+ = 26 to 50%, 3+ = 51 to 75%, and 4+ = >75% of cells containing fat; and for inflammation and necrosis, 1+ = one focus/lobule and 2+ = two or more foci/lobule.

Western Blot Analysis of GLUT 1 and GLUT 2

Frozen liver specimens were homogenized in TS solution (10 mmol/L Tris, pH 7.5, 250 mmol/L sucrose, and 0.1% phenolmethylsulfanil fluoride), and 200 µg of protein were separated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to a nitrocellulose filter. Gels were stained with Coomassie blue to verify transfer. The membranes were immunoblotted with polyclonal antibodies to GLUT 1 and GLUT 2 (Biodesign International, Kennebunk, ME).

Light Microscopic Immunohistochemistry

Frozen sections (7 µ thick) were air dried for 20 minutes, fixed in acetone for 1 minute at room temperature, and washed in phosphate-buffered saline, pH

7.4. The sections were preincubated for 10 minutes in 5% (v/v) normal serum. Subsequently, the sections were incubated with the primary antibodies for 30 minutes. After three washes with phosphate-buffered saline for 5 minutes each, the sections were incubated with the biotinylated secondary antibody for 30 minutes, washed again with phosphate-buffered saline, and then incubated with a strepavidin-biotin-horseradish peroxidase complex (Vector Laboratories, Burlingame, CA). After washes with phosphate-buffered saline, the horseradish peroxidase was developed with 3,3′-diaminobenzidene (Sigma). Sections were counterstained with methyl green. Negative controls for each section were also performed.

Glycogen

The periodic acid Schiff stain was used for glycogen in formalin-fixed tissue.

Results

In each of the groups, the rats increased their body weights at a constant rate. Although the average weight gain in ethanol-fed rats was lower compared with the respective dextrose-fed controls, the differences between the groups was not significant. The range of blood alcohol levels in the ethanol-fed rats was between 150 and 350 mg/dL; there was no significant difference between the groups. The pathological changes in the rats in the different groups is shown in Table 2. The most severe pathological changes were seen in the FE rats, moderate changes occurred in the CE rats, and no significant pathological changes were seen in the SE rats and dextrose-fed controls.

In the liver parenchyma of control rats, glycogen was uniformly distributed in the perivenular and periportal regions of the liver lobule (Figure 1). In ethanol-fed rats, a marked decrease in glycogen was

Table 2. *Pathological Changes in Rats in Different Experimental Groups**

Experimental group	Rat No.	Pathological changes		
		Fatty liver	Necrosis	Inflammation
CE	1	2+	1+	1+
	2	3+	0	1+
	3	3+	1+	1+
FE	1	3+	2+	2+
	2	4+	1+	2+
	3	4+	1+	1+

*None of the rats in the remaining groups (SD, SE, CD, and FD) developed significant pathological changes.

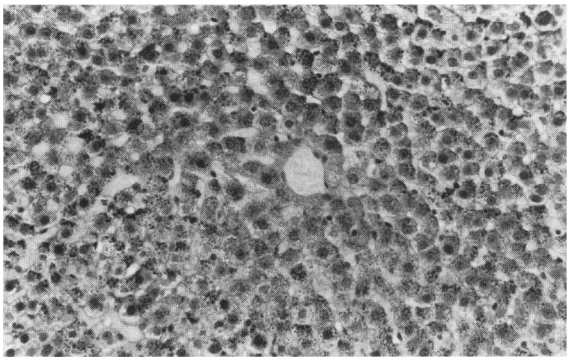

Figure 1. *Section of liver from a rat fed corn oil and dextrose showing uniform staining for glycogen (periodic acid Schiff reaction). Rats fed saturated fat and dextrose, fish oil and dextrose and saturated fat and ethanol (no liver injury) showed a similar pattern of glycogen distribution. Magnification, ×200.*

seen in the rats showing evidence of pathological liver injury (Figure 2). In the saturated fat groups (both SD and SE) and CD rats, GLUT 2 occurred as a basolateral protein in all hepatocytes (Figure 3). In the FD group, a uniform decrease in staining intensity for GLUT 2 was seen. In the CE and FE groups, the decrease in staining intensity for GLUT 2 was particularly evident in the centrilobular region (Figure 4). The reduction in glycogen content and decreased expression of GLUT 2 occurred in the same areas in the CE and FE groups. The decreased intensity of GLUT 2 staining in the FD, CE, and FE groups was corroborated by Western blot analysis (Figure 5).

In normal rats, GLUT 1 was localized to the first row of hepatocytes around the terminal hepatic venule. In the ethanol-fed rats, GLUT 1 was expressed in several rows of hepatocytes around the terminal hepatic venule. Figure 6 depicts the expression of GLUT 1 in the livers of CE and FE rats. Positive staining for GLUT 1 was also seen in sinusoidal lining cells in the CE and

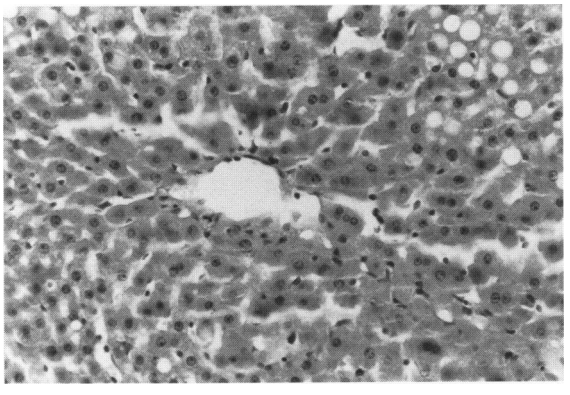

Figure 2. *Section of liver from a rat fed fish oil and ethanol. Glycogen depletion was fairly uniform across all lobules. The fat appears to be midzonal in distribution. A negative control (no added antibody) showed no positive staining. Magnification, ×400.*

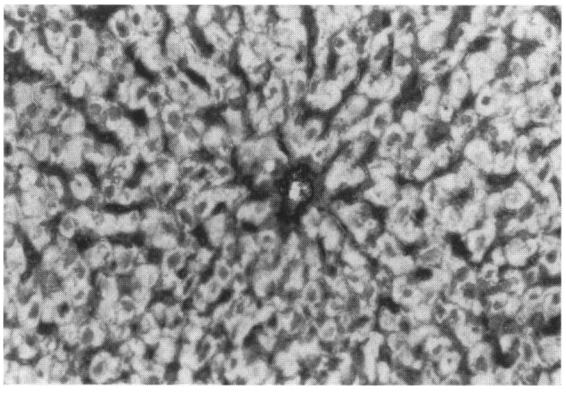

Figure 3. *Section of liver from a rat fed corn oil and dextrose showing the distribution of GLUT 2. Note that every hepatocyte is stained for GLUT 2. Note also the presence of GLUT 2 in the hepatocyte membranes lining the sinusoids. Magnification, ×400.*

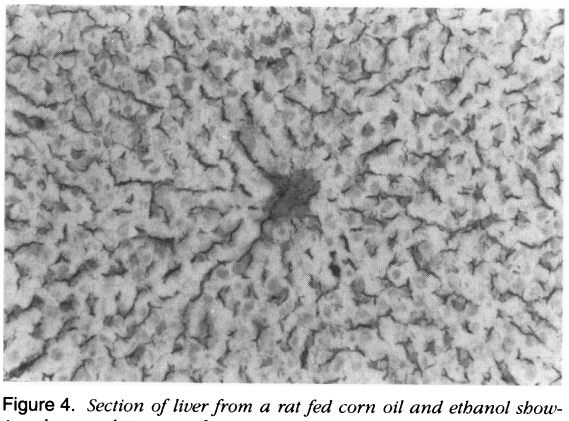

Figure 4. *Section of liver from a rat fed corn oil and ethanol showing decreased intensity for GLUT 2 staining. The decreased staining for GLUT 2 is especially evident around the central vein. Magnification, ×200.*

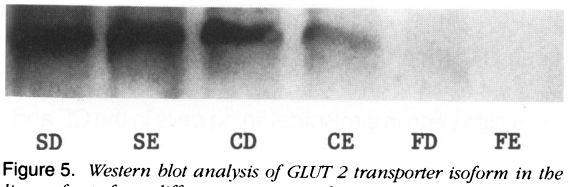

Figure 5. *Western blot analysis of GLUT 2 transporter isoform in the livers of rats from different experimental groups. A total of 200 μg of protein was loaded onto each lane. A typical result is depicted from three independent experiments.*

FE rats (Figure 6). The increased presence of GLUT 1 in these rats was confirmed by Western blot analysis (Figure 7).

Discussion

In ethanol-fed rats that develop pathological liver injury (CE and FE), the reduction in glycogen in perivenular hepatocytes was accompanied by increased expression of GLUT 1 and decreased expression of GLUT 2. GLUT 2 is the predominant glucose transporter in the liver and is expressed in the basolateral

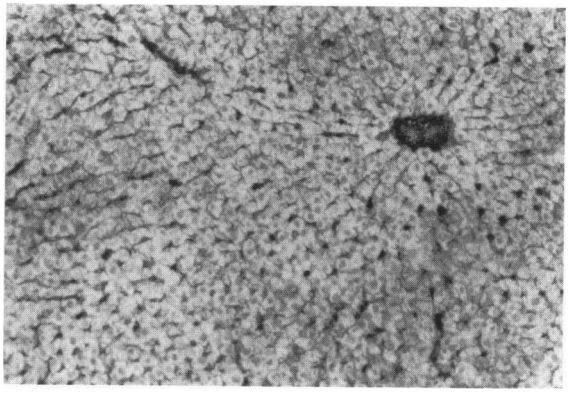

Figure 6. *Section of liver from a rat fed corn oil and ethanol showing localization of GLUT 1 to the plasma membranes of hepatocytes that are in several rows around the central vein. Many of the sinusoids also showed intense staining for GLUT 1, suggesting enhanced expression of GLUT 1 by endothelial and Kupffer cells. Sections from livers of rats fed fish oil and ethanol showed a similar pattern of staining. Magnification, ×100.*

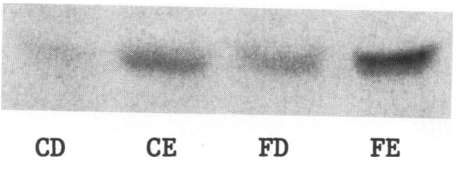

Figure 7. *Western blot analysis of GLUT 1 protein in livers from rats in different experiment groups. A total of 200 μg of protein was loaded onto each lane. A typical result is depicted from three independent experiments. No GLUT 1 was detectable by Western blot analysis in SD and SE rats.*

blood-facing plasma membrane of every hepatocyte.[9–11] The high K_m for glucose of GLUT 2 (15 to 20 mmol/L) allows glucose influx or efflux to respond passively to changes in extracellular glucose. In contrast to GLUT 2, the GLUT 1 glucose transporter isoform is localized in rat liver to a subset of hepatocytes that form the first row of cells around the terminal hepatic venule.[12]

The decreased expression of GLUT 2 and increased GLUT 1 expression in the centrilobular area is consistent with the known effects of ethanol on liver cells. Ethanol causes hepatotoxicity mainly in the perivenular hepatocytes,[21,22] and the shift in glucose transporter expression from bidirectional glucose transport (GLUT 2) to unidirectional transport into hepatocytes (GLUT 1) may be necessary to meet the intracellular demand for glucose. One potential advantage of increasing GLUT 1 content may be related to the kinetic asymmetry property of this isoform; the net influx K_m for glucose by GLUT 1 is approximately 1.6 mmol/L, which is significantly lower than the net efflux K_m value.[9–11] This asymmetry allows GLUT 1 to function effectively under conditions in which the intracellular demand for glucose is high, eg, during endotoxemia or increased oxidative stress. Increased

expression of GLUT 1 is also seen in cells that use glucose as a carbon source via glycolysis; GLUT 2 expression is seen mainly in gluconeogenic cells.[9–11]

The effects of alcohol on glucose metabolism are complex and sometimes contradictory.[1] These contradictions in large part can be explained on the basis of the nutritional state of the experimental animal at the time of ethanol administration. In the intragastric feeding rat model for alcoholic liver disease, the additional role for endotoxemia should also be considered. We have previously shown that rats fed corn oil and ethanol have increased endotoxin levels and rats fed saturated fat and ethanol have much lower circulating endotoxin.[4,5] We have recently shown that rats fed fish oil and ethanol also have high plasma endotoxin levels (unpublished observations). Spolarics et al[23] showed that endotoxin administration results in decreased GLUT 2 and increased GLUT 1 expression in liver parenchymal cells. These investigators also showed that the increase in glucose utilization by liver nonparenchymal cells was accompanied by an increased GLUT 1 protein content of these cells. Our results in CE and FE rats are similar to those seen with endotoxin administration.[23] An increase in GLUT 1 expression by sinusoidal lining cells (endothelial and Kupffer cells) was also seen in rats showing evidence of pathological liver injury. It is probable that these changes in glucose transporter expression in both endotoxemic and ethanol-fed rats serve as a compensatory mechanism against attenuated gluconeogenic activity or hypoxia seen after both ethanol and endotoxin administration. Increased GLUT 1 expression has also been observed *in vitro* in the presence of hypoxia[24] and tumor necrosis factor.[25] Hypoxia, particularly of the centrilobular area, is considered to be important in the pathogenesis of alcoholic liver disease.[26] It is probable that increased GLUT 1 expression in response to hypoxia leads to increased glucose transport in the centrilobular area, thus providing an energy source for ATP synthesis. Decreased concentrations of ATP have been observed in response to chronic ethanol feeding in the livers of rats fed via the intragastric route.[27,28] The enhanced provision of glucose in the presence of depleted glycogen stores may reduce the likelihood of necrosis. Tumor necrosis factor, also important in the pathogenesis of alcoholic liver disease,[29] is increased in the livers of rats fed corn oil and ethanol but not in those fed saturated fat and ethanol.[30]

The decreased expression of GLUT 2 in rats fed fish oil and dextrose is also of interest. In rats fed fish oil, a diffuse decrease in glycogen and GLUT 2 expression was seen. A similar effect of fish oil on hepatic glycogen has been observed previously.[31] A decrease in intestinal glucose uptake is observed in rats fed fish oil.[32] These investigators proposed that the influence of fatty acids on membrane phospholipids could affect the function of membrane-associated proteins such as glucose transporters.[32,33] A decrease in glucose uptake in the liver, similar to that seen in the intestine, may occur secondary to decreased GLUT 2 protein and account for the diminution in hepatic glycogen in rats fed fish oil and dextrose. Although we did not observe any pathological changes in the FD fed rats, these animals can, after long-term feeding, develop centrilobular fibrosis.[19] Thus the decreased expression of GLUT 2 may have pathogenetic relevance to the development of fibrosis.

In summary, we have shown that in rats that develop pathological liver injury, the decrease in glycogen content of the liver is accompanied by decreased GLUT 2 and increased GLUT 1 expression in the centrilobular areas. These findings probably reflect a shift in glucose transport (GLUT 2) to preferred unidirectional transport (GLUT 1) to meet the increased intracellular demand for glucose.

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