Acute inhibition by ethanol of intestinal absorption of glucose and hepatic glycogen synthesis on glucose refeeding after starvation in the rat

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1. Intragastric administration of ethanol (75 mmol/kg body wt.) at 1 h before glucose refeeding of 24 h-starved rats inhibited hepatic glycogen deposition (by 69%) and synthesis (by approx. 70%), but was without significant effect on muscle glycogen deposition and synthesis. 2. Treatment of ethanol-administered rats with methylpyrazole (an inhibitor of alcohol dehydrogenase) did not significantly diminish the inhibitory effect of ethanol on hepatic glycogen deposition after glucose refeeding, suggesting that the inhibition was not dependent on ethanol metabolism. 3. Ethanol delayed and diminished intestinal glucose absorption, at least in part by delaying gastric emptying. 4. At a lower dose (10 mmol/kg body wt.), ethanol inhibited hepatic glycogen repletion and synthesis without compromising intestinal glucose absorption. Ethanol inhibited glycogen deposition (by 40%) in hepatocytes from starved rats provided with glucose + lactate + pyruvate as substrates, consistent with it having a direct effect to diminish hepatic glycogen synthesis by inhibition of gluconeogenic flux at a site(s) between phosphoenolpyruvate and triose phosphate in the pathway. 5. It is concluded that ethanol acutely impairs hepatic glycogen repletion by inhibition at at least two distinct sites, namely (a) intestinal glucose absorption and (b) hepatic gluconeogenic flux.

INTRODUCTION

There is now good evidence that in starved animals (including man) which are refed with carbohydrate the major pathway of hepatic glycogen synthesis involves the conversion of glucose into C_3 derivatives (e.g. lactate) before the incorporation of its carbon skeleton via the gluconeogenic pathway into glycogen [1–10]. Consequently hepatic glycogen deposition on glucose refeeding after starvation is sensitive to inhibition by 3-mercaptopicolinate (an inhibitor of phosphoenolpyruvate carboxykinase; see [3,8]). The roles of the absorbed glucose are therefore considered to be (a) a source of C_3 precursors for glycogenesis and (b) a signal to the liver to suppress glucose production and divert glucose 6-phosphate into glycogen synthesis [11].

It is well documented that excessive ethanol intake acutely impairs carbohydrate and lipid metabolism [12–14]. In part this impairment is thought to involve inhibition of the intestinal absorption of nutrients [see 15,16]. Ethanol is mainly metabolized in the liver via alcohol dehydrogenase (EC 1.1.1.1) and acetaldehyde dehydrogenase (EC 1.2.1.3), which oxidize ethanol via acetaldehyde to acetate. Ethanol oxidation via this pathway is NAD^+ -dependent and generates NADH in the cytosol. The cytosolic (and mitochondrial) $NAD^+/NADH$ ratio is thereby decreased. The ethanol-induced perturbation in the redox state is thought to play a role in the genesis of many of the acute metabolic abnormalities associated with excessive alcohol intake (reviewed in [14]). The abnormal redox state may result in inhibition of hepatic gluconeogenesis and may

precipitate hypoglycaemia in the starved state (see [17]).

Acute and chronic ethanol consumption markedly decrease liver glycogen stores [18,19]. Given that ethanol inhibits hepatic gluconeogenesis, and that continued gluconeogenic flux makes an important contribution to hepatic glycogen synthesis, the present paper addresses the question of whether ethanol inhibits hepatic glycogen deposition in response to glucose refeeding after starvation through decreased hepatic conversion of C_3 intermediates into glucose 6-phosphate and glycogen.

MATERIALS AND METHODS

Materials

Sources of materials were as in [8,9].

Treatment of animals

Male albino Wistar rats (100–120 g) were maintained at approx. 17 °C in a 12 h-light/12 h-dark cycle (lighting from 08:00 h). The rats were starved for 24 h (in grid-bottomed cages) before the experiments, which were commenced before 09:00 h. Ethanol [normally 75 mmol/kg body wt., equivalent to 0.69 ml of 50% (v/v) aq. soln./100 g body wt.] or an equivalent volume of water was administered intragastrically under light diethyl ether anaesthesia. In rats receiving other doses of ethanol (10, 20, 40, 60 or 100 mmol/kg body wt.), the concentration of the administered ethanol soln. was adjusted [to 6.7, 13.3, 26.7, 40 or 66.7% (v/v) respectively] such that the volume administered to each rat was 0.69 ml/100 g body wt. At 1 h after intragastric ethanol,

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rats received glucose (2 mmol/100 g body wt.; 2 M soln.) or water (1 ml/100 g body wt.) intragastrically or by intraperitoneal injection under light ether anaesthesia. Animals were sampled (under ether anaesthesia) at time intervals thereafter (as specified in text). At 1 h before sampling, rats were injected with $^3\text{H}_2\text{O}$ (5 mCi: 0.5 ml in 0.9% NaCl; injected intraperitoneally) or $\text{NaH}^{14}\text{CO}_3$ (approx. 100 $\mu\text{Ci}/100\text{ g body wt.}$ intravenously: soln. in sterile water containing 0.5 mCi and 0.8 mg of NaHCO_3/ml). Certain rats received 4-methylpyrazole (2 mmol/kg body wt.; 2 M soln. in 0.9% NaCl) by intraperitoneal injection 10 min before ethanol administration (75 mmol/kg body wt.) and again 1 h thereafter.

Measurement of glycogen synthesis and blood metabolites

Glycogen content (calculated as glucose equivalents) [8,9] and the rate of glycogen synthesis as measured by incorporation into glycogen of either ^3H from $^3\text{H}_2\text{O}$ [20] or ^{14}C from ^{14}C bicarbonate [2] were determined in freeze-clamped tissue.

Arterial blood from the left ventricle was collected into heparinized syringes. Samples (approx. 0.5 ml) for blood metabolite assays were added to 2 ml of 5% (w/v) HClO_4 and treated as described elsewhere [8,9]. Blood ethanol was determined enzymically [21]. At the time of sampling, the oesophagus and terminal ileum were clamped to allow excision and removal of the intact stomach and small intestine without fluid loss. The fluid volume in the stomach was measured before gastrointestinal glucose extraction and determination [22].

Hepatocytes

Hepatocytes were prepared by the method of Katz *et al.* [23] from 24 h-starved rats. The cells isolated were incubated in media containing 10 mM-glutamine [24,25] and 10 mM-glucose alone or in combination with 20 mM-dihydroxyacetone or 18 mM-lactate + 2 mM-pyruvate. Incubations were carried out in groups of six and the means taken. Glycogen was determined as in [8].

Statistics

Results are presented as means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Statistical analysis was by Student's unpaired *t* test.

RESULTS

Tissue glycogen deposition after glucose refeeding

Intragastric glucose administration to 24 h-starved rats led to marked glycogen deposition in liver and skeletal muscle (Table 1). This deposition was accompanied by increased incorporation of ^3H from $^3\text{H}_2\text{O}$ into glycogen, a measure of glycogen synthesis *de novo* from all available precursors. ^3H incorporation from $^3\text{H}_2\text{O}$ is an established technique to estimate glycogen synthesis [13], ^3H being incorporated from $^3\text{H}_2\text{O}$ into products of reactions that lead to formation of new C-H bonds by protonation of carbanions. Glycogen glucose contains seven carbon-bound hydrogen atoms, two of which (at C-2 and C-5) become labelled with ^3H from $^3\text{H}_2\text{O}$ on glycogen synthesis from glucose and approx. 90% of which become labelled on synthesis from C_3 precursors [see 7,13]. The intragastric administration of ethanol (75 mmol/kg body wt.) before glucose refeeding decreased hepatic glycogen deposition (by 69%) and synthesis (by 72%) in response to glucose, but was without significant effect on skeletal-muscle glycogen deposition and synthesis. Ethanol had no significant effect on hepatic glycogen and muscle content in control (water-fed) rats.

Glycogen synthesis using precursors metabolized via oxaloacetate can be estimated by the incorporation of $^{14}\text{CO}_2$ from ^{14}C bicarbonate, which labels a carboxyl carbon atom of oxaloacetate in the pyruvate carboxylase reaction [2]. To determine whether the inhibition of hepatic glyconeogenesis by ethanol was associated with decreased flux from C_3 precursors, ^{14}C incorporation from ^{14}C bicarbonate into hepatic glycogen was investigated in rats treated with glucose or glucose plus ethanol. The impairment of net glycogen deposition was accompanied by parallel inhibition (by 67%: $P < 0.001$) of incorporation of ^{14}C from ^{14}C bicarbonate into glycogen: ^{14}C incorporation from ^{14}C bicarbonate into liver glycogen (d.p.m. $\times 10^3/\text{g wet wt.}$) was 121.0 ± 10.4 (6) in glucose-refed rats and 39.8 ± 6.9 (6) in glucose-refed rats treated with ethanol. The finding suggested that ethanol inhibited hepatic glycogen synthesis at a site(s) proximal to oxaloacetate formation. This site(s) may be extrahepatic and/or intrahepatic in location.

Table 1. Effects of intragastric ethanol (75 mmol/kg) on hepatic and muscle glycogen content and synthesis (measured as ^3H incorporation from $^3\text{H}_2\text{O}$ into glycogen) on oral glucose refeeding (2 mmol/100 g body wt.) after starvation

Rats starved for 24 h were administered ethanol or an equivalent volume of water 1 h before refeeding with glucose or water. $^3\text{H}_2\text{O}$ was given 1 h thereafter and animals were sampled after a further 1 h. For experimental details see the text. Statistically significant effects of ethanol administration are shown as *** $P < 0.001$.

Treatment	Liver glycogen ($\mu\text{mol as glucose}/$ g wet wt.)	^3H incorporation from $^3\text{H}_2\text{O}$ into liver glycogen ($\mu\text{g-atoms of } ^3\text{H}/\text{h}$ per g wet wt.)	Muscle glycogen ($\mu\text{mol as glucose}/$ g wet wt.)	^3H incorporation from $^3\text{H}_2\text{O}$ into muscle glycogen ($\mu\text{g-atoms}$ of $^3\text{H}/\text{h}$ per g wet wt.)
Water-fed				
Control	1.2 ± 0.3 (12)	11.9 ± 1.5 (12)	6.5 ± 0.5 (12)	2.5 ± 0.2 (12)
+ Ethanol	0.5 ± 0.1 (12)	10.1 ± 1.0 (12)	7.9 ± 0.6 (12)	3.3 ± 0.4 (12)
Glucose-fed				
Control	91.1 ± 5.5 (23)	151.5 ± 10.2 (6)	23.2 ± 2.4 (11)	10.5 ± 0.8 (12)
+ Ethanol	28.5 ± 3.6 (28)***	41.7 ± 5.4 (17)***	19.1 ± 2.1 (21)	9.2 ± 1.2 (12)

Fig. 1. Effects of oral administration of ethanol (75 mmol/kg body wt.) on the time course of intestinal glucose absorption, glycogen repletion and associated metabolic changes after glucose refeeding (2 mmol/100 g body wt.)

Ethanol or an equivalent volume of water was administered 1 h before glucose refeeding. Animals were sampled at time intervals thereafter, in all instances receiving $^3\text{H}_2\text{O}$ 1 h before sampling. For experimental details see the Materials and methods section. The amount (% of administered dose) of glucose remaining in the stomach and small intestine (a), the blood concentrations of glucose (b) and lactate (c) and hepatic glycogen content (d) and synthesis (e; measured as ^3H incorporation from $^3\text{H}_2\text{O}$ into liver glycogen) were determined in ethanol-treated rats (Δ) and control (water-fed) rats ($\circ$). Blood ethanol concentrations in ethanol-treated rats ($\square$) are shown in (b): there was no detectable ethanol in blood from water-fed rats. Statistically significant effects of ethanol treatment are shown as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. The vertical bars denote S.E.M.

Effects of 4-methylpyrazole

4-Methylpyrazole inhibits alcohol dehydrogenase and thereby impairs ethanol oxidation *in vivo* [26]. Treatment with methylpyrazole delayed blood ethanol clearance such that its concentration in blood at 3 h after an oral dose of ethanol (75 mmol/kg body wt.) was increased > 2-fold [from 34 ± 4 mM (8) to 72 ± 6 mM (6); $P < 0.001$]. Despite this increase, methylpyrazole administration did not block the inhibition by ethanol of hepatic glycogen deposition and synthesis in response to glucose refeeding (results not shown). The results suggested that ethanol metabolism was not necessary for inhibition of hepatic glycogen deposition.

Ethanol and intestinal glucose absorption

The results shown in Fig. 1(a) demonstrate that ethanol at a dose of 75 mmol/kg body wt. impairs the intestinal absorption of glucose. Whereas control (water-fed) rats absorbed > 80% of an oral glucose load within 90 min, ethanol pretreatment caused a marked delay in absorption. Less than 1% of the glucose load was absorbed in the first 1 h after its administration, and approx. 40% remained unabsorbed even after 3 h. Presumably as a consequence of the delayed intestinal absorption of glucose, the glycaemic response to an oral glucose load was decreased in ethanol-treated rats (by 10%, based on the difference in areas underneath the respective glucose tolerance curves: Fig. 1b), and the shape of the glucose tolerance curve was abnormal in that the blood glucose concentration continued to increase beyond 2 h after oral glucose. It is noteworthy that the dose (75 mmol/kg body wt.) and route (oral) of ethanol administration led to elevated blood ethanol concentrations that continued to increase throughout the 3 h period after oral glucose (Fig. 1b). Impaired glucose tolerance in ethanol-treated rats was also associated with changes in the response of blood lactate. Concentrations of lactate did not increase significantly until 2 h after glucose administration (Fig. 1c), whereas in control (water-fed) rats blood lactate concentrations rose rapidly. On the basis of estimation of the areas underneath the

blood lactate-response curves (Fig. 1c), it was calculated that ethanol treatment decreased the lactaemia associated with glucose refeeding by 28%. Ethanol treatment not only delayed the rise in blood lactate concentration in response to an oral glucose load, but also altered the blood [lactate]/[pyruvate] ratio: at 1 h after oral glucose the ratio was 12.1 ± 0.5 (6) in ethanol-treated rats, compared with 8.2 ± 0.5 (6) in control rats ($P < 0.001$ for difference).

Given that ethanol impairs intestinal glucose absorption and results in abnormal glucose tolerance, it follows that hepatic glycogen deposition (Fig. 1d) and synthesis (Fig. 1e) should be compromised by ethanol treatment. At 3 h after glucose administration, however, the rate of glycogen synthesis in both ethanol-treated and control rats approached basal values. What is notable is that, despite impaired glucose absorption, muscle glycogen repletion is not acutely compromised by ethanol treatment. It must be inferred therefore that optimal rates of muscle glycogen deposition are achieved even under conditions of a diminished glycaemic response.

Dose-dependent effects of ethanol

It can be concluded from the preceding series of experiments that inhibition of intestinal glucose absorption on glucose refeeding makes a major contribution to the impairment of hepatic glycogen deposition by ethanol at an intragastric dose of 75 mmol/kg body wt. The second series of experiments addressed the question of whether malabsorption is the sole mechanism whereby intragastric ethanol administration perturbs hepatic glycogen deposition. The inhibition by ethanol of intestinal glucose absorption at 1 h after glucose refeeding was dependent on the dose of ethanol administered (Table 2): at an ethanol dose of 10 mmol/kg body wt., there was no significant inhibition of intestinal glucose absorption (approx. 30% of administered glucose was unabsorbed at 1 h in both experimental and control rats). At a dose of 20 mmol/kg body wt., however, approx. 60% of the oral glucose load remained unabsorbed at 1 h after administration, and at doses of

Table 2. Effects of increasing oral doses of ethanol on intestinal glucose absorption and hepatic and muscle glycogen repletion

Ethanol (0.69 ml/100 g body wt., at the different concentrations specified in the Materials and methods section) or water was administered by intragastric intubation to give the doses specified. Ethanol (or water) was administered 1 h before glucose refeeding (2 mmol/100 g body wt.) and $^3\text{H}_2\text{O}$ administration (5 mCi; intraperitoneally) and animals were sampled after a further 1 h. For experimental details, see the Materials and methods section. Statistically significant effects of ethanol compared with water-fed rats are denoted as ** $P < 0.01$ and *** $P < 0.001$. Each value is the mean for at least six animals: N.D., not determined.

Dose of ethanol (mmol/kg body wt.)	Glucose absorbed (% of administered dose) in 1 h	Gastric fluid volume (ml)	Liver glycogen ($\mu\text{mol/g}$ wet wt.)	Muscle glycogen ($\mu\text{mol/g}$ wet wt.)	^3H incorporation from $^3\text{H}_2\text{O}$ into liver glycogen (μg -atoms of $^3\text{H}/\text{h}$ per g wet wt.)
0	71 ± 3	1.0 ± 0.1	74.4 ± 3.2	32.7 ± 2.6	136.7 ± 5.0
10	70 ± 5	0.9 ± 0.2	$42.2 \pm 4.0^{**}$	26.6 ± 2.1	$107.9 \pm 4.7^{**}$
20	$39 \pm 4^{***}$	$1.6 \pm 0.2^{**}$	$49.2 \pm 2.9^{**}$	29.8 ± 2.6	$61.9 \pm 2.6^{***}$
40	$19 \pm 4^{***}$	$2.1 \pm 0.1^{***}$	$21.7 \pm 2.9^{***}$	30.0 ± 2.0	$26.7 \pm 3.8^{***}$
60	$21 \pm 8^{***}$	$2.0 \pm 0.2^{***}$	$29.2 \pm 4.9^{***}$	25.7 ± 2.0	$39.2 \pm 7.7^{***}$
75	$20 \pm 4^{***}$	$1.9 \pm 0.1^{***}$	$16.5 \pm 4.0^{***}$	29.2 ± 2.0	$36.6 \pm 6.0^{***}$
100	$14 \pm 5^{***}$	N.D.	$10.4 \pm 5.0^{***}$	26.7 ± 2.3	$17.2 \pm 5.5^{***}$

Table 3. Effects of increasing oral doses of ethanol on blood metabolites

For explanation, see the legend to Table 2. Statistically significant effects of ethanol compared with water-fed rats are denoted as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; N.D., not determined.

Dose of ethanol (mmol/kg body wt.)	Blood ethanol (mM)	Blood glucose (mM)	Blood lactate (mM)	Blood [lactate]/ [pyruvate] ratio
0	0	9.5±0.5	2.6±0.2	10.3±0.8
10	0	8.6±0.4	3.1±0.2*	10.8±0.9
20	0	6.9±0.9	5.6±0.7***	42.2±6.4***
40	8.0±1.5***	6.6±0.1***	4.3±0.8**	38.3±7.3***
60	13.6±1.3***	6.6±0.1***	1.4±0.1**	13.5±2.1
75	37.4±3.2***	7.2±0.6*	2.3±0.5	17.2±3.3*
100	N.D.	5.6±0.6***	1.2±0.3**	10.7±0.7

Table 4. Effects of intragastric administration of ethanol (75 mmol/kg body wt.) on glycogen deposition after intraperitoneal glucose administration (2 mmol/100 g body wt.) to 24 h-starved rats

Rats were administered ethanol or an equivalent volume of water 1 h before glucose injection. $^3\text{H}_2\text{O}$ or [^{14}C]bicarbonate was given 1 h thereafter, and rats were sampled after a further 1 h. For experimental details see the Materials and methods section. Statistically significant effects of ethanol are denoted as *** $P < 0.001$.

Treatment	Liver glycogen ($\mu\text{mol/g}$ wet wt.)	Muscle glycogen ($\mu\text{mol/g}$ wet wt.)	^3H from $^3\text{H}_2\text{O}$ ($\mu\text{g-atoms/h}$ per g)	^{14}C from [^{14}C]- bicarbonate (d.p.m./h per g)
Control	82.1±7.6 (12)	27.3±0.9 (12)	50.3±6.0 (6)	413±31 (6)
+ Ethanol	42.8±4.1 (12)***	24.4±2.2 (12)	10.8±0.8 (6)***	175±17 (6)***

≥ 40 mmol/kg body wt. approx. 80 % of the administered glucose remained unabsorbed. There was a reciprocal relationship between glucose absorbed (%) and gastric volume, implying that delayed gastric emptying may be the major reason underlying the malabsorption of glucose.

The inhibition by ethanol of hepatic glycogen repletion and synthesis (measured as ^3H incorporation from $^3\text{H}_2\text{O}$ into liver glycogen) was also dose-dependent (Table 2), increasing with increasing dose. An important finding was that, at a dose at which there was no inhibition of intestinal glucose absorption (10 mmol/kg body wt.), there was significant ($P < 0.01$) inhibition of hepatic glycogen deposition (by 43 %). Muscle glycogen repletion in response to glucose refeeding was unimpaired by ethanol, irrespective of the dose administered (Table 2). At doses of ≥ 40 mmol/kg body wt., ethanol administration decreased the blood glucose concentration (Table 3), presumably via the inhibition of intestinal glucose absorption. At doses of ethanol of 20 or 40 mmol/kg body wt., blood lactate concentrations were increased, presumably as a consequence of redox imbalance, as indicated by the increased blood [lactate]/[pyruvate] ratios. It is noteworthy that at doses of ≥ 60 mmol/kg body wt. ethanol did not increase blood lactate or the [lactate]/[pyruvate] ratio.

Hepatic glycogen synthesis on intraperitoneal glucose administration

Glucose refeeding of 24 h-starved rats via the intraperitoneal route avoids the problems inherent in oral

administration. Intestinal absorption is not involved and therefore theoretically hepatic glycogen deposition should not be limited by the rate of glucose absorption. Ethanol administered intragastrically nonetheless inhibited liver glycogen repletion (by 48 %) and synthesis, measured either as ^3H incorporation from $^3\text{H}_2\text{O}$ (by 78 %) or as ^{14}C incorporation from [^{14}C]bicarbonate (by 58 %) into liver glycogen on glucose administration by intraperitoneal injection (Table 4). This finding indicates that ethanol has a direct effect on hepatic glycogen synthesis in addition to, and secondary to, glucose malabsorption. There was no inhibition of muscle glycogen deposition (Table 4) or synthesis measured on the basis of ^3H incorporation from $^3\text{H}_2\text{O}$ into muscle glycogen (results not shown). Blood glucose concentrations were similar in control and ethanol-treated rats [10.4 ± 0.8 mM (6) and 9.7 ± 0.7 mM (6) respectively]. By contrast blood lactate concentrations were lower (by 36 %; $P < 0.05$) in ethanol-treated rats, i.e. control 1.24 ± 0.26 mM (6) and ethanol-treated 0.80 ± 0.10 mM (6).

Glycogen synthesis in isolated hepatocytes

In isolated rat hepatocytes, efficient glycogen synthesis requires not only glucose but a gluconeogenic substrate, and is stimulated by glutamine [24,25]. Ethanol (20 mM) had no inhibitory effect on glycogen synthesis by hepatocytes from 24 h-starved rats provided with either glucose alone or glucose + dihydroxyacetone as substrates (Table 5). By contrast, it decreased hepatocyte glycogen synthesis (by 40 %) on provision of glucose +

Table 5. Effects of addition of 20 mM-ethanol on glycogen deposition by isolated hepatocytes from 24 h-starved rats

Hepatocytes were incubated in Krebs-Henseleit bicarbonate buffer containing 10 mM-glucose and 10 mM-glutamine plus gluconeogenic substrate as specified.
* $P < 0.05$ for statistically significant effects of ethanol.

Gluconeogenic substrate	Hepatocyte glycogen (μmol as glucose/100 mg of protein)	
	Control	+ 20 mM-ethanol
None	0.23 ± 0.06 (5)	0.18 ± 0.08 (5)
20 mM-Dihydroxyacetone	5.18 ± 0.68 (4)	4.77 ± 0.15 (4)
18 mM-Lactate + 2 mM-pyruvate	2.81 ± 0.36 (5)	1.70 ± 0.08 (4)*

lactate + pyruvate as substrates. The implications are (1) that ethanol has a direct hepatic site of action to inhibit glycogen repletion and (2) that the locus of this inhibition is between phosphoenolpyruvate and triose phosphate in the gluconeogenic pathway.

GENERAL DISCUSSION AND PHYSIOLOGICAL IMPLICATIONS

The results demonstrate that ethanol ingestion inhibits hepatic glycogen deposition on glucose refeeding after starvation by at least two distinct mechanisms. Firstly, at high doses (≥ 20 mmol/kg body wt.), ethanol inhibits intestinal glucose absorption. Adverse effects of ethanol on nutrient absorption which may lead to malabsorption and the development of malnutrition have been reported (see [15,16]), including inhibitory effects on carrier-mediated jejunal glucose transport *in vitro* [27]. However, in our experiments, the inhibition of intestinal glucose absorption was associated with a reciprocal increase in gastric fluid volume, implying that delayed gastric emptying was the primary mechanism in the gastric malabsorption produced by ethanol. In the rat, ethanol administered simultaneously with glucose is reported to impair insulin secretion and decrease glucose tolerance [28]. Hitherto the mechanism of this ethanol-induced oral glucose intolerance has been identified as a functional impairment in insulin secretion (coupled with decreased peripheral glucose utilization?). The present study, however, suggests that an important factor in this glucose intolerance is glucose malabsorption. At ethanol doses of ≥ 20 mmol/kg body wt., the impairment by ethanol of intestinal glucose absorption is clearly the major factor underlying the inhibition of hepatic glycogen repletion. This inhibition is not dependent on ethanol oxidation itself, which probably accounts for the finding that inhibition is not lessened by pretreatment of rats with methylpyrazole. Administration of glycerol in combination with glucose partially relieves the inhibition by ethanol of hepatic glycogen repletion (results not shown), presumably by compensating for glucose malabsorption and the diminished supply of C_3 metabolites by the provision of an alternative glycogenic precursor.

Three lines of evidence lead us to conclude that ethanol impairs hepatic glycogen repletion by a mech-

anism(s) independent of glucose malabsorption. These are: (1) that ethanol at a dose of 10 mmol/kg body wt. inhibited hepatic glycogen repletion without significantly affecting intestinal glucose absorption and gastric fluid volume; (2) that ethanol inhibited hepatic glycogen repletion on glucose refeeding via the intraperitoneal route; (3) that ethanol inhibited glycogen deposition in isolated hepatocytes *in vitro*. If these latter findings can be extrapolated to the situation *in vivo*, it is probable that ethanol has a direct hepatic action to impair hepatic glycogen synthesis. *In vitro* the specificity of this inhibition is consistent with the idea that hepatic glycogen synthesis on glucose refeeding is predominantly from C_3 metabolites (presumably mainly lactate) and is therefore sensitive to the inhibition by ethanol of gluconeogenic flux (see [6,7]). The locus of the inhibition is presumed to be between phosphoenolpyruvate and triose phosphate in the gluconeogenic sequence.

Although inhibition by ethanol of hepatic glycogen repletion may result from inhibition of hepatic gluconeogenic flux, other sites of inhibition cannot be precluded. The major pathway of hepatic glycogen synthesis from glucose involves its preliminary conversion into lactate, and this may be compromised by the inhibition by ethanol of peripheral glucose utilization [29]. Ethanol administration resulted in lower blood lactate concentrations in starved rats given glucose by intraperitoneal injection, suggesting that conversion in peripheral tissues of glucose into lactate may be decreased. Ethanol may also alter hepatic and/or peripheral carbohydrate metabolism by endocrine-mediated mechanisms, since its ingestion may increase blood concentrations of catecholamines and cortisol [30] and impair insulin secretion [31]. It therefore remains to be established to what extent (a) inhibition of gluconeogenic flux and (b) other mechanisms account for the suppression by ethanol of hepatic glycogen repletion on carbohydrate refeeding.

Nutritional implications

In man, as in the rat, hepatic glycogen resynthesis after carbohydrate refeeding is to a substantial degree from C_3 intermediates via an indirect (gluconeogenic) pathway [10]. Alcoholism may affect $> 2\%$ of the adult population in the U.K., and chronic alcohol abuse is recognized to be a contributory factor in approximately one-fifth of male acute general medical admissions. Acute metabolic abnormalities associated with excessive ethanol consumption may contribute to the pathogenesis of the diseases of chronic alcohol abuse. The liver is recognized to have a pivotal role in glucose homeostasis as a source of glucose (via glycogenolysis and gluconeogenesis) for peripheral utilization in starvation, stress and exercise. The present work indicates that not only does ethanol impair intestinal nutrient absorption but it may acutely compromise the capacity of the liver to produce glucose, not only via effects on hepatic gluconeogenesis but also potentially by limiting hepatic glycogen storage. This may be of particular importance in the context of carbohydrate refeeding after glycogen depletion caused by fasting, strenuous exercise or stress.

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