Ethanol and glycogen synthesis in cardiothoracic and skeletal muscles following glucose re-feeding after starvation in the rat

Dan XU,* Ranjana THAMBIRAJAH and T. Norman PALMER

Department of Biochemistry, University of Western Australia, Nedlands, Western Australia 6009, Australia

The pattern of glycogen deposition in individual cardiothoracic and skeletal muscles in response to oral and intraperitoneal glucose administration was examined in 40 h-starved rats. Rates of glycogen synthesis were consistently higher in oxidative muscles than in non-oxidative muscles. Intra-gastric ethanol administration was associated with an impaired glycaemic response and the almost total abolition of glycogen deposition in oxidative muscles in response to oral or intraperitoneal glucose re-feeding. This effect was dose-dependent and differential, in that ethanol produced no equivalent impairment in glycogen deposition in non-oxidative muscles. Ethanol treatment also selectively promoted glycogenolysis in oxidative muscles in the starved state. There was positive correlation ($P < 0.001$) between the decrease in glycogen levels in soleus and diaphragm muscles in response to increasing ethanol doses and blood glucose and lactate concentrations after intraperitoneal glucose administration, implying that the basis for the impairment in glycogen synthesis may be diminished glucose availability. The mechanism whereby ethanol may differentially compromise carbohydrate metabolism in oxidative muscles is discussed.

INTRODUCTION

Ethanol has acute deleterious effects on carbohydrate, lipid and protein metabolism (reviewed by Lieber, 1991; Palmer, 1990; Palmer *et al.*, 1991). Ethanol is metabolized primarily in the liver via the pathway involving alcohol dehydrogenase (EC 1.1.1.1) and aldehyde dehydrogenase (EC 1.2.1.3). This pathway oxidizes ethanol to acetate via the intermediate acetaldehyde with the concomitant production of excess reducing equivalents, mainly as cytosolic NADH. This redox imbalance is proposed to be the primary factor in the genesis of a number of acute hepatic metabolic disturbances, including the inhibition of hepatic glycogen repletion following carbohydrate re-feeding after starvation (Cook *et al.*, 1988). Up to 80 % of the ethanol oxidized by the liver appears as acetate in the hepatic venous blood and is further metabolized at extrahepatic sites, particularly skeletal muscle (Lundquist, 1962; Lundquist *et al.*, 1962, 1973).

Although muscle has little, if any, capacity to oxidize ethanol, ethanol consumption has profound effects on muscle metabolism and function. In man, ethanol acutely inhibits glucose uptake by leg muscles (Jorfeldt & Juhlin-Dannfelt, 1978), decreases the stimulatory effect of prolonged exercise on glucose uptake by the exercising leg (Juhlin-Dannfelt *et al.*, 1977), inhibits glucose conversion into lactate (Kreisberg *et al.*, 1971) and may cause acute peripheral insulin resistance and impaired glucose utilization (Shelmet *et al.*, 1988; Yki-Järvinen *et al.*, 1988). Ethanol also impairs glucose uptake by the isolated rat diaphragm (Clarke & Evans, 1960). These findings imply that ethanol consumption may diminish glucose uptake and oxidation primarily via effects on skeletal muscle. To what extent these acute metabolic effects are related to the genesis of acute (Haller & Knochel, 1984) and chronic (Peters & Preedy, 1991) alcoholic skeletal myopathy remains obscure.

Starvation is associated with the depletion of endogenous muscle glycogen reserves (Holness *et al.*, 1988). Although skeletal muscle accounts for approx. 40 % of body weight (Young, 1970) and is the primary site of glucose disposition following carbohydrate re-feeding after starvation (Cox *et al.*, 1988; Ferré *et al.*, 1985; see Sugden *et al.*, 1989), it is not homogeneous in structure

and function. Oxidative muscles, which are constantly working even in the resting state, have higher rates of glucose utilization in the fed state than do non-oxidative muscles and respond to starvation by a more marked suppression of glucose utilization (Issad *et al.*, 1987; Holness & Sugden, 1990). Rates of glycogen deposition following carbohydrate re-feeding after prolonged starvation, which may be determined primarily by the extent of glycogen depletion in starvation (Holness *et al.*, 1988), account for most of the glucose phosphorylated in individual oxidative and non-oxidative muscles, at least in the initial period following chow re-feeding (Sugden *et al.*, 1990). Previous reports (Cox *et al.*, 1988; Cook *et al.*, 1988) indicate that, whereas ethanol inhibits hepatic glycogen synthesis following glucose loading of starved rats, it does not acutely compromise glycogen deposition in mixed (thigh and calf) skeletal muscles. The recognition that patterns of glucose utilization are different in oxidative and non-oxidative muscles makes it important to re-examine whether ethanol has different effects on glycogen deposition in different muscle types. The present study focuses on the acute effects of ethanol on glycogen deposition in individual oxidative and non-oxidative muscles following glucose administration to starved rats.

MATERIALS AND METHODS

Materials

Sources of materials were as in Cook *et al.* (1988).

Treatment of animals

Male albino Wistar rats (80–100 g) were maintained on a 12 h-light/12 h-starved dark cycle (light from 07:30 h) and fed *ad libitum* on a standard laboratory chow diet (supplied by Glen Forest Stockfeeders, Glen Forest, W.A. 6071, Australia: 55 % digestible carbohydrate, 19 % protein, 5 % lipid and 21 % non-digestible residue, by weight). Animals had free access to water. In initial experiments the protocol described by Cook *et al.* (1988) was used to assess the acute metabolic effects of ethanol. Briefly, 40 h-starved rats were given ethanol [75 mmol/kg body wt., 0.69 ml of 50 % (v/v) aq. soln./100 g body wt.]

* To whom correspondence and requests for reprints should be addressed.

or an equivalent amount of water by intragastric intubation under fluothane anaesthesia. After 1 h, rats (under fluothane anaesthesia) received glucose (2 mmol/100 g body wt.; 2 M soln.) or an equivalent volume of water (1 ml/100 g body wt.) intragastrically or via intraperitoneal injection. At time intervals thereafter animals were sampled under fluothane anaesthesia: blood and individual muscles (tibialis anterior, extensor digitorum longus, soleus, plantaris, gastrocnemius, diaphragm and heart) were frozen *in situ* in aluminium clamps precooled in liquid nitrogen. The fibre compositions (fast oxidative glycolytic:fast glycolytic:slow oxidative) of the hind-limb muscles selected (see Ariano *et al.*, 1973; Maltin *et al.*, 1989) were: tibialis anterior (66:32:2), extensor digitorum longus (59:28:2), soleus (16:0:84), plantaris (53:41:6) and gastrocnemius (37:58:5). The sampling procedure took no more than 6 min. Arterial blood samples were collected into non-heparinized syringes and either deproteinized in 6% (v/v) HClO_4 or stored as plasma at -20°C .

In experiments to assess the dose-dependency of the acute effect of ethanol on glycogen deposition following glucose re-feeding, 40 h-starved rats were given ethanol at doses of 10, 20, 40, 60 or 75 mmol/kg body wt. by oral administration of 0.69 ml of 6.7%, 13.3%, 26.7%, 40% or 50% (v/v) aq. soln./100 g body wt. respectively under fluothane anaesthesia. Control rats received an equivalent volume of water. After 1 h rats received glucose (2 mmol/100 g body wt.; 4 M soln.) or an equivalent volume of water (0.5 ml/100 g body wt.) by intraperitoneal injection, and at 2 h thereafter animals were sampled under fluothane anaesthesia as described above.

Muscle glycogen and blood metabolites assay

The glycogen contents of the individual skeletal muscles and heart (calculated as μmol of glucose equivalents) were determined by the method of Palmer *et al.* (1983). Similar results were obtained by the method of Holness & Sugden (1987). It was established in preliminary experiments (see also Fig. 1) that repeated fluothane anaesthesia on up to three occasions at hourly intervals did not affect muscle glycogen concentrations. Blood concentrations of glucose, lactate and ethanol were determined as described in Cook *et al.* (1988) in KOH-neutralized HClO_4 extracts. Plasma insulin concentrations were measured with an Immo Phase Insulin kit (Corning Medical, Medfield, MA, U.S.A.).

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, unless otherwise specified.

RESULTS

Acute effects of ethanol on glycogen metabolism in individual muscles in the starved state

Starvation (40 h) was associated with muscle glycogen depletion. The extent of this depletion was independent of muscle fibre composition: the lowest glycogen concentrations in skeletal muscles after starvation were in the plantaris ($11.8 \pm 0.4 \mu\text{mol}$ of glucose equivalents/g wet wt.), diaphragm ($9.9 \pm 0.8 \mu\text{mol}$ /g wet wt.) and soleus ($13.9 \pm 0.8 \mu\text{mol}$ /g wet wt.) muscles, and the highest levels were in the gastrocnemius muscles ($18.7 \pm 0.5 \mu\text{mol}$ /g wet wt.).

Oral ethanol administration (75 mmol/kg body wt.) to starved rats, which led to a rapid rise in blood ethanol concentrations to levels $> 30 \text{ mM}$ at 1 h after administration, was not associated with any alteration in blood glucose or lactate concentrations, which remained approximately constant at 3.9 ± 0.1 and $1.0 \pm 0.1 \text{ mM}$ respectively for the initial 2.5 h after ethanol

administration. Ethanol administration resulted in no significant changes in muscle glycogen concentrations in the 1 h after oral ethanol administration to starved rats (Fig. 1). Thereafter, however, there was glycogen mobilization in oxidative skeletal muscles: rates of glycogen mobilization in the second 1 h period following ethanol administration in soleus and diaphragm muscles were 90 and 50 nmol of glucose equivalents/min per g wet wt. Ethanol treatment, by contrast, had no effect on glycogen levels in the heart and non-oxidative muscles. Over a 2 h period, glycogen levels in tibialis anterior, extensor digitorum longus, plantaris and gastrocnemius muscles remained unchanged in both ethanol-treated rats and controls (Fig. 1).

Acute effects of ethanol on glycogen deposition in individual muscles following glucose re-feeding after starvation

Intragastric administration of glucose (20 mmol/kg body wt.: see Cook *et al.*, 1988) was associated with a rise in blood glucose concentrations, which were highest at 1.5 h after re-feeding and thereafter declined. Peak insulin concentrations were in excess of $1000 \mu\text{units/ml}$. Such high insulin levels following oral glucose dosing are significantly higher (by > 20 -fold) than insulin levels following chow re-feeding in the rat (Sugden *et al.*, 1990). Oral glucose re-feeding led to rapid glycogen deposition in skeletal muscles and diaphragm, whereas cardiac glycogen levels declined (by 47% at 3 h: $P < 0.001$; results not shown) in response to re-feeding (Fig. 1) (Holness *et al.*, 1988). In agreement with Palmer *et al.* (1991), rates of glycogen deposition were consistently higher in oxidative muscles (soleus, diaphragm) and plantaris than in the other non-oxidative muscles. The initial rates of glycogen deposition in the individual muscles following oral glucose administration were 365 (diaphragm) 315 (soleus), 213 (plantaris), 160 (extensor digitorum longus), 107 (gastrocnemius) and 107 (tibialis anterior) nmol of glucose equivalents/min per g wet wt. respectively.

Intraperitoneal administration of glucose, which produced increases in blood glucose concentrations similar to those seen after oral glucose dosing (Table 1), also led to rapid glycogen deposition in skeletal muscles and diaphragm, rates of deposition being lower in soleus and plantaris muscles but otherwise higher than that after oral glucose (Fig. 2). The initial rates of glycogen deposition in the individual muscles following intraperitoneal glucose were 400 (diaphragm) 255 (soleus) and 217 (gastrocnemius) (Fig. 2) and for the other non-oxidative muscles (results not shown) 147 (plantaris), 315 (extensor digitorum longus) and 235 (tibialis anterior) nmol of glucose equivalents/min per g wet wt. respectively. Rates of cardiac glycogenolysis were similar irrespective of the route of glucose administration. The response of blood insulin concentrations to intraperitoneal glucose administration was not determined.

Oral ethanol administration (75 mmol/kg body wt.), which led to rapid increases in blood ethanol concentrations (Table 1), resulted in an impaired glycaemic response following both oral and intraperitoneal glucose administration (see also Cook *et al.*, 1988). This impairment in glycaemic response was most severe in rats administered glucose via the intraperitoneal route and was associated with parallel changes in the response of blood lactate concentrations to glucose re-feeding (results not shown). In agreement with Cook *et al.* (1988), the rise in blood lactate concentrations in response to oral, but not intraperitoneal, glucose administration was delayed in the ethanol-treated rats compared with controls.

Pre-treatment with ethanol before oral or intraperitoneal glucose administration was associated with the specific and almost total abolition of glycogen deposition in oxidative muscles, as opposed to non-oxidative muscles (Fig. 1). Whereas glycogen was deposited at initial rates of 315 and 365 nmol of

Fig. 1. Acute effects of ethanol on muscle glycogen metabolism in response to oral glucose or water administration in 40 h-starved rats

Ethanol (75 mmol/kg body wt.) was administered by intragastric intubation to 40 h-starved rats 1 h before oral administration of glucose or an equal volume of water. Animals were sampled before and at 1 h after ethanol administration and at time intervals after the administration of glucose or water (see the Materials and methods section). A control group of rats received water instead of ethanol. For details see the Materials and methods section. The time scale shown refers to time after ethanol (or water in control animals) administration, glucose (or water) being administered at 1 h. Glycogen concentrations (μmol of glucose equivalents/g wet wt.) in muscles from rats administered glucose with (●) or without (○) prior treatment with ethanol and in rats administered water with (▲) or without (△) prior treatment with ethanol in (a) soleus, (b) diaphragm, (c) plantaris, (d) tibialis anterior, (e) gastrocnemius and (f) extensor digitorum longus (EDL). Results are shown as means ± S.E.M. for a minimum of six rats. Statistically significant effects of ethanol on muscle glycogen levels in glucose- or water-refed rats are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

glucose equivalents/min per g wet wt. in soleus and diaphragm muscles following oral glucose re-feeding, pre-treatment with ethanol suppressed glycogen deposition in diaphragm muscle. Oral glucose re-feeding of ethanol-treated rats was associated with a transient phase of glycogen deposition in soleus muscle (at a rate of 15 nmol of glucose equivalents/min per g wet wt.) followed by a sustained phase of glycogenolysis (at a maximum rate of 100 nmol of glucose equivalents/min per g wet wt.). By contrast, rates of glycogen deposition following oral glucose re-feeding in the non-oxidative extensor digitorum longus, tibialis anterior, gastrocnemius and plantaris muscles were relatively unaffected by ethanol treatment. There were a few instances of impaired glycogen deposition in non-oxidative muscles, but the extent of this impairment (15.6–21.9%) was small compared with that seen in oxidative muscles (Fig. 1). Ethanol pre-treatment

did not affect the rate of cardiac glycogenolysis in response to oral glucose re-feeding.

The impairment in glycogen deposition in oxidative muscles was equally severe following intraperitoneal glucose administration (Fig. 2). Ethanol pre-treatment decreased the rate of glycogen deposition in diaphragm muscle from 400 to 95 nmol of glucose equivalents/min per g wet wt. and caused net glycogenolysis in soleus muscle. By contrast, rates of glycogen deposition following intraperitoneal glucose re-feeding in the non-oxidative gastrocnemius (Fig. 2) and other non-oxidative muscles (extensor digitorum longus, tibialis anterior and plantaris; results not shown) were consistently unaffected by ethanol treatment. Ethanol treatment was associated with lower cardiac glycogen levels at 3–4 h after intraperitoneal glucose administration (Fig. 2).

Fig. 2. Acute effects of ethanol on muscle glycogen metabolism in response to intraperitoneal glucose administration in 40 h-starved rats

Ethanol (75 mmol/kg body wt.) was administered by intragastric intubation to 40 h-starved rats 1 h before intraperitoneal administration of glucose. Animals were sampled before and at 1 h after ethanol administration and at time intervals after the administration of glucose. A control group of rats received water instead of ethanol. For details see the Materials and methods section. The time scale shown refers to time after ethanol (or water) administration, glucose (or water in control animals) being administered at 1 h. Glycogen concentrations (μmol of glucose equivalents/g wet wt.) in muscles from rats administered glucose with (●) or without (○) prior treatment with ethanol in (a) soleus, (b) diaphragm, (c) heart and (d) gastrocnemius. Results are shown as means $\pm$ S.E.M. for a minimum of six rats. Statistically significant effects of ethanol in rats administered glucose are indicated by: * $P < 0.05$; *** $P < 0.001$.

Table 1. Acute effects of ethanol on blood metabolite and insulin concentrations in 40 h-starved rats administered glucose by intragastric intubation or by intraperitoneal injection

Ethanol (75 mmol/kg body wt.) was administered by intragastric intubation to 40 h-starved rats 1 h before oral or intraperitoneal administration of glucose. Animals were sampled before and at 1 h after ethanol administration and at time intervals after the administration of glucose. A control group of rats received water instead of ethanol. For details see the Materials and methods section. The time scale shown refers to time after ethanol (or water in control animals) administration, glucose being administered at 1 h. Results are shown as means $\pm$ S.E.M. for a minimum of six rats. Statistically significant effects of ethanol are indicated by: * $P < 0.05$; ** $P < 0.01$. N/D: not determined.

Time after ethanol (h)	Ethanol (mm)	Oral glucose refeeding				Intraperitoneal glucose	
		Glucose (mm)		Insulin (μ -units/ml)		Glucose (mm)	
		Control	Ethanol-treated	Control	Ethanol-treated	Control	Ethanol-treated
0	0	3.9 $\pm$ 0.2	3.9 $\pm$ 0.2	N/D	N/D	3.9 $\pm$ 0.2	3.9 $\pm$ 0.2
1.0	32.7 $\pm$ 0.6	4.3 $\pm$ 0.2	3.9 $\pm$ 0.1	57.0 $\pm$ 10.0	N/D	3.8 $\pm$ 0.2	3.5 $\pm$ 0.3
1.5	42.9 $\pm$ 0.6	6.7 $\pm$ 0.2	5.0 $\pm$ 0.1*	70.0 $\pm$ 22.0	118.2 $\pm$ 21.0	N/D	N/D
2.0	35.2 $\pm$ 1.5	8.0 $\pm$ 0.3	6.6 $\pm$ 0.2*	344.0 $\pm$ 61.0	358.0 $\pm$ 61.0	7.5 $\pm$ 0.5	5.7 $\pm$ 0.6*
2.5	25.8 $\pm$ 0.8	10.1 $\pm$ 0.1	8.2 $\pm$ 0.2**	761.0 $\pm$ 68.0	760.0 $\pm$ 82.0	N/D	N/D
3.0	14.1 $\pm$ 0.6	7.7 $\pm$ 0.1	7.2 $\pm$ 0.2	925.0 $\pm$ 86.0	1022.0 $\pm$ 164.0	7.1 $\pm$ 0.7	4.1 $\pm$ 0.4**
4.0	9.8 $\pm$ 0.8	5.8 $\pm$ 0.1	6.0 $\pm$ 0.1	N/D	N/D	6.5 $\pm$ 0.5	4.3 $\pm$ 0.3*
5.0	20.6 $\pm$ 1.5	N/D	N/D	N/D	N/D	5.9 $\pm$ 0.5	4.6 $\pm$ 0.3*
7.0	12.5 $\pm$ 1.1	N/D	N/D	N/D	N/D	5.1 $\pm$ 0.5	3.4 $\pm$ 0.3*

Table 2. Dose-dependency of the acute effects of ethanol on blood metabolite concentrations and muscle glycogen levels in 40 h-starved rats administered glucose by intraperitoneal injection

Ethanol (at doses of 0, 10, 20, 40, 60 and 75 mmol/kg body wt.) was administered by intragastric intubation to 40 h-starved rats 1 h before intraperitoneal administration of glucose. Animals were sampled 2 h after glucose administration. For details see the Materials and methods section. Results are shown as means $\pm$ S.E.M. for a minimum of six rats. Statistically significant effects of ethanol compared with water-fed rats are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Ethanol dose (mmol/kg body wt.)	Blood metabolite concentrations (mM)			Glycogen (μ mol/g wet wt.)			
	Glucose	Lactate	Ethanol	Soleus	Diaphragm	Plantaris	Tibialis anterior
0	6.9 $\pm$ 0.5	2.2 $\pm$ 0.1	0	61.7 $\pm$ 4.9	55.2 $\pm$ 3.5	30.6 $\pm$ 2.4	30.2 $\pm$ 2.7
10	6.5 $\pm$ 0.4	1.8 $\pm$ 0.04*	13.5 $\pm$ 1.1	49.4 $\pm$ 1.4***	53.6 $\pm$ 2.9	30.4 $\pm$ 3.0	32.2 $\pm$ 1.1
20	4.9 $\pm$ 0.3*	1.7 $\pm$ 0.1**	21.3 $\pm$ 1.0	44.5 $\pm$ 2.8***	48.6 $\pm$ 2.7*	32.3 $\pm$ 1.2	32.8 $\pm$ 2.5
40	4.4 $\pm$ 0.3*	1.6 $\pm$ 0.1**	27.9 $\pm$ 1.5	37.5 $\pm$ 2.1***	38.1 $\pm$ 2.9**	32.1 $\pm$ 2.0	29.6 $\pm$ 1.3
60	3.8 $\pm$ 0.4**	1.5 $\pm$ 0.1***	33.6 $\pm$ 2.1	16.2 $\pm$ 1.5***	20.3 $\pm$ 1.7***	29.2 $\pm$ 1.9	27.7 $\pm$ 2.9
75	4.1 $\pm$ 0.4**	1.5 $\pm$ 0.13***	38.5 $\pm$ 2.0	13.3 $\pm$ 0.8***	19.0 $\pm$ 1.8***	28.9 $\pm$ 1.7	30.1 $\pm$ 2.2

Fig. 3. Relationship between glycogen levels in soleus muscle after intraperitoneal glucose administration and blood glucose and lactate concentrations

Ethanol (at doses of 0–75 mmol/kg body wt.: see Table 2) was administered by intragastric intubation to 40 h-starved rats 1 h before intraperitoneal administration of glucose, and animals were sampled 2 h thereafter. For details see the Materials and methods section. There was a positive correlation between glycogen levels (μ mol of glucose equivalents/g wet wt.) in soleus muscle and blood glucose ($\circ$: $r = 0.76$; $y = -11.8 + 9.6x$) and blood lactate ($\square$: $r = 0.71$; $y = -19.4 + 33.7x$) concentrations.

Dose-dependency of the acute effects of ethanol on muscle glycogen deposition

Glycogen levels in oxidative muscles at 2 h after intraperitoneal glucose administration were decreased in a dose-dependent manner by ethanol (Table 2): in soleus muscle, levels were decreased by 20%, 28%, 39%, 74% and 79% at ethanol doses of 10, 20, 40, 60 and 75 mmol/kg body wt. respectively, whereas in diaphragm glycogen levels were decreased by 12%, 31%, 63% and 66% at ethanol doses of 20, 40, 60 and 75 mmol/kg body wt. respectively. Ethanol, irrespective of dose, produced no decrease in glycogen levels in plantaris or tibialis anterior (Table 2) or the other non-oxidative muscles or heart (results not shown), but was associated with a dose-dependent decrease in blood glucose and lactate concentrations (Table 2).

Linear regression analysis (Fig. 3) showed strong positive correlation ($P < 0.001$) between glycogen levels in soleus muscle and blood glucose and lactate concentrations (Fig. 3). There was also positive correlation ($P < 0.001$) between glycogen levels in diaphragm muscle and blood glucose ($r = 0.81$; $y = -11.5 + 10.3x$) and blood lactate ($r = 0.63$; $y = -8.1 + 28.2x$) concentrations (results not shown).

DISCUSSION

Glucose re-feeding, whether by the oral or the intraperitoneal route, was associated with rates of glycogen deposition which were consistently higher in oxidative than in non-oxidative muscles. Our results indicate that the acute oral administration of ethanol (see Cook *et al.*, 1988) results in an impaired glycaemic response and the differential suppression of glycogen deposition in oxidative muscles, as opposed to non-oxidative muscles, in response to glucose re-feeding after starvation. Ethanol treatment also selectively promoted glycogen mobilization in oxidative muscles in the starved state. The selectivity of this acute impairment in glycogen metabolism was overlooked in previous studies (Cox *et al.*, 1988; Cook *et al.*, 1988), which examined effects of ethanol on glycogen deposition in mixed (thigh and calf) as opposed to individual muscles. These results are in agreement with other findings that indicate that ethanol may acutely impair peripheral glucose utilization via effects on glucose disposal in skeletal muscles. In man ethanol acutely inhibits glucose uptake by leg muscles (Jorfeldt & Juhlin-Dannfelt, 1978; Juhlin-Dannfelt *et al.*, 1977) and may cause acute peripheral insulin resistance and impaired glucose oxidation (Shelmet *et al.*, 1988; Yki-Järvinen *et al.*, 1988). Ethanol also decreases peripheral glucose utilization in the fasted dog (Lochner *et al.*, 1967) and impairs glucose uptake by the isolated rat diaphragm (Clarke & Evans, 1960).

The outstanding question relates to the mechanism(s) by which glucose metabolism in oxidative muscles is differentially perturbed by ethanol. Whereas others (Singh & Patel, 1976) have reported that ethanol may impair insulin secretion and cause glucose intolerance, the present study indicates that ethanol treatment was associated with a diminished glycaemic response to both oral and intraperitoneal glucose administration (Table 1), which may suggest that one factor in the impairment in glycogen deposition is decreased glucose availability. Linear regression analysis indicated positive correlation ($P < 0.001$) between glycogen levels in soleus and diaphragm muscles in

response to intraperitoneal glucose administration and blood glucose and lactate concentrations at different doses of ethanol. Ethanol may cause delayed gastric emptying (Cook *et al.*, 1988) and intestinal glucose malabsorption (see Thompson & Majumdar, 1981), at least in part via direct inhibitory effects on carrier-mediated jejunal glucose transport (Dinda *et al.*, 1975). Thus the diminution in the glycaemic response to oral glucose re-feeding may reflect effects of ethanol on gastrointestinal glucose absorption. However, ethanol treatment was also associated with a diminished glycaemic response and impaired glycogen deposition in oxidative muscles in response to intraperitoneal glucose administration. The implication is that ethanol-mediated glucose malabsorption is not a primary factor in the genesis of the impairment in glucose homeostasis and muscle glycogen deposition. Ethanol has been established to decrease rates of hepatic glucose output, primarily via inhibitory effects on gluconeogenesis (see Lieber, 1991), a process which may account for the diminished glycaemic response. It is noteworthy that ethanol pre-treatment not only promoted glycogenolysis in oxidative muscle in 40 h-starved rats but was also associated with net glycogenolysis in the latter phases of the response of oxidative muscles to oral or intraperitoneal glucose administration, despite high glucose and insulin concentrations. This may imply that ethanol pre-treatment provokes glycogenolysis, possibly via alterations in circulating catecholamine concentrations (Perman, 1961).

Skeletal muscle is recognized to have low activities of alcohol dehydrogenase and aldehyde dehydrogenase and to have little, if any, capacity to oxidize ethanol, implying that any metabolic derangement that arises secondary to ethanol oxidation must be mediated by ethanol itself, or its hepatic metabolites. Although ethanol forms esters with long-chain fatty acids that may impair membrane function (Laposata & Lange, 1986), this process is slow and unlikely to be of major importance in relation to the acute effects of ethanol. Hepatic ethanol oxidation generates acetate (Lundquist, 1962; Lundquist *et al.*, 1962, 1973) and excess reducing equivalents, the latter appearing as lactate in the hepatic venous blood. Studies in normal man suggest that neither acetate nor lactate mediates changes in glucose utilization in muscle: measurement of arterial and venous lactate/pyruvate ratios across the human forearm indicates that the cytoplasmic redox state in muscle is unchanged by ethanol administration (Lundquist *et al.*, 1973), and acetate infusion is reported to have no effects on rates of glucose disposal (Yki-Järvinen *et al.*, 1988). The question of how ethanol administration differentially impairs glycogen deposition in oxidative muscles, if it is not via the effects of ethanol and its metabolites, remains unresolved.

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