CHRONIC ALCOHOL CONSUMPTION YIELDS SEX DIFFERENCES IN WHOLE-BODY GLUCOSE PRODUCTION IN RATS

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Abstract — Aims: The effects of chronic alcohol consumption (8 weeks) on glucose kinetics, in the absence (water, 4 g/kg) and presence of an acute ethanol dose (4 g/kg), were examined in 48 h fasted male and female Wistar rats. Methods: Primed continuous infusions of [6-3H]- and [U-14C]glucose were employed to assess rates of glucose appearance (Ra), glucose disappearance (Rd), and apparent glucose carbon recycling. Results: After injecting the male and female controls with water, there were no significant alterations in glucose kinetics. Compared to controls, chronic alcohol-fed female animals (injected with water) demonstrated significantly lower: glucose Ra, blood glucose concentration, and apparent glucose carbon recycling for a majority of the experimental period. In separate groups injected with ethanol, the glucose Ra fell by 31% for male rats fed the control diet (MC), 43% for male rats fed the ethanol diet (ME), 29% for female rats fed the control diet (FC), and 42% for female rats fed the ethanol diet (FE). Further, compared to controls (MC and FC), the glucose blood concentration was significantly lower prior to and following the ethanol injection for FE. In addition, FE animals had significantly lower rates of glucose Ra and glucose carbon recycling compared to controls prior to and after the ethanol injection. ME animals demonstrated similar declines in glucose Ra (compared to FE), but only after the ethanol injection. Conversely, ME were able to match the decrease in glucose Ra with comparable declines in glucose Rd resulting in blood glucose concentrations that did not differ from controls. Conclusions: Chronic alcohol consumption results in sex differences in whole-body glucose production and glucose regulation.

INTRODUCTION

Glucose is an essential substrate for the central nervous system where the maintenance of blood glucose concentration ensures an adequate nutrient supply. The kidneys and the liver are the organs responsible for blood glucose homeostasis through glycogenolysis and gluconeogenesis. For both the kidneys and the liver, the early response to hypoglycaemia involves the release of glucose through glycogenolysis. However, as glycogen stores decline, the contribution from gluconeogenesis elevates in order to resist decrements in blood glucose concentration. In fasting states (where renal and hepatic glycogen stores are depleted), gluconeogenesis within these organs is the primary mode by which blood glucose levels are maintained. Thus, the kidneys and liver work in concert to maintain blood glucose homeostasis where any significant decrement in glucose output capacity within either organ may lead to the deleterious consequences associated with hypoglycaemia.

Freinkel et al. (1963) were among the first to report a decline in blood glucose levels after acute ethanol (hereafter and perhaps inappropriately, also referred to as ‘alcohol’) consumption. Studies by Krebs and coworkers (Krebs, 1968; Krebs et al., 1969) determined that alcohol could reduce the gluconeogenic capacity from lactate in perfused livers from fasted rats. The inhibitory effect of ethanol on glucose production capacity as observed by many investigators (Krebs, 1968; Arky and Freinkel, 1969; Krebs et al., 1969) support a possible mechanism for the prevalence of alcohol-induced hypoglycaemia. Albeit not a consistent observation, reports in fasted humans (Freinkel et al., 1963; Searle et al., 1974; Wolfe et al., 1976; Wilson et al., 1981) and fasted rats (Souza and Masur, 1981, 1982, 1984) have demonstrated a significant decline in blood glucose concentration after an acute ethanol load.

While acute ethanol ingestion may lead to hypoglycaemia in the glycogen-depleted state, the impact of chronic alcohol consumption remains to be elucidated. This is of considerable import given that some alcoholics tend to significantly reduce their food intake (Salasuprano, 1993; Addolorato et al., 1998), or if they do consume food, their diet is low in carbohydrates (Addolorato et al., 1998). Under these circumstances of fasting or inadequate nutritional intake, renal and hepatic glycogen stores would be compromised. For the alcoholic, this may result in greater susceptibility for alcohol-induced hypoglycaemia upon their next consumption of ethanol (Emanuele et al., 1998). Further, in humans, chronic alcohol consumption has been observed to decrease first-pass ethanol metabolism that is exacerbated with fasting (DiPadova et al., 1987). This would elevate the availability of alcohol and could augment its attenuating effect upon glucose production capacity. Moreover, reports in humans (Maly and Sasse, 1991) and in rats (Maly and Sasse, 1985) suggest that there are sex differences in the location of hepatic alcohol dehydrogenase, sex differences in fatty acid accumulation within the liver resulting from chronic alcohol consumption in rats (Shevchuk et al., 1991), and sex differences in the alcohol elimination rate in humans (Van Thiel et al., 1988; Lieber, 2000). Thus, the effect of chronic alcohol consumption upon glucose production capacity between males and females is unknown.

The purpose of the current investigation was to assess whole-body glucose production and glucose carbon recycling after a 48-h fast in male and female rats in the presence and absence of an acute ethanol injection. Specifically, with use of standard tracer techniques to measure in vivo rates of glucose appearance and apparent glucose carbon recycling, we sought to determine if chronic alcohol consumption resulted in:

(1)
any sex difference in glucose production capacity in the absence of acute ethanol and (2) any sex difference in glucose production capacity in the presence of acute ethanol. Based upon previous studies (Souza and Masur, 1981, 1982, 1984), we hypothesized that an acute alcohol injection would lower the gluconeogenic capacity of all animals. In addition, we anticipated a much larger reduction in glucose production from female rats chronically fed ethanol compared to male rats. Finally, we expected the ethanol fed female rats to demonstrate greater susceptibility to alcohol-induced hypoglycaemia in the presence of an acute ethanol load.

MATERIALS AND METHODS

The experimental protocol for this study was pre-approved by the Chapman University Institutional Review Board and complied with the Public Health Service policy on the use of experimental animals for research. Sixty age-matched male and female Wistar rats (initially 100–125 g), obtained from Charles River, were housed individually and maintained on a 12/12 h light/dark cycle. Male animals were randomly assigned to either a control group (MC, n = 15) fed a control liquid diet or an experimental group (ME, n = 15) fed an ethanol liquid diet. In like manner, female animals were randomly assigned to a control group (FC, n = 15) fed the control liquid diet or an experimental group (FE, n = 15) fed the ethanol liquid diet. Male and female animals were pair fed a Lieber DeCarli (Lieber and DeCarli, 1986) liquid diet (Dyets, Bethlehem, PA) containing 36% of the caloric intake as alcohol or a dextrin–maltose substitute for alcohol (to ensure an isocaloric ingestion between the ethanol and control diet). Body weights were monitored weekly and all animals were within 10% of their sex-matched counterpart throughout the feeding period. The feeding period on the liquid diets lasted for 8 weeks. Prior to the experiment, the animals were fasted for 48 h, whereas water continued to be provided ad libitum. The fast was employed to significantly deplete kidney and liver glycogen stores and to minimize the glucose appearance derived from glycogenolysis. In addition, a 48-h fast helped to ensure the clearance of alcohol from the chronically fed ethanol animals in order to distinguish between acute and chronic effects.

All experiments were performed at the same time of day. On the day of the experiment, animals were anesthetized (Ketamine, Rompun, Acepromazine) and catheters (filled with saline) were inserted into the carotid artery (advanced to the aortic arch) and jugular vein (advanced to the right atrium). Once the catheters were secured, the animal was kept warm under a lamp. When blood samples were not collected, the blood pressure was monitored in-line via the carotid artery catheter with use of a blood pressure transducer (Grass-Astro Med, PT-300). For most of the experiments, no additional anaesthesia was needed during the experimental period. In a few instances (a total of three animals), when blood pressures started to elevate, animals were given an additional dose of anaesthesia (Ketamine). For a small minority of animals (n = 1, 2 per group) we collected an initial blood sample to determine the initial pH and blood gas pressures (Radiometer, ABL 5). For all animals, a primed-constant infusion of dual labelled glucose, U-14C at 0.15 μCi/min and 6-3H at 0.45 μCi/min, was initiated via the venous catheter at −75 min, after a priming dose equivalent to 60 times the minute infusion rate was given. Starting at −15 min, sequential arterial blood samples (−150 μl) were collected every 15 min via the carotid catheter. At time 0, a small abdominal incision was made and either water (4 g/kg) or an equivalent volume (4 g/kg) of an ethanol solution (40% w/v) was injected directly into the stomach. The ethanol dose has previously been observed to elicit alcohol-induced hypoglycaemia in rats (Souza and Masur, 1982, 1984). About half the animals (n = 7) from each group (MC, ME, FC, or FE) were injected with water and the other half (n = 8) were injected with ethanol. For the animals given the ethanol injection, a small blood sample (~50 μl) was collected in heparinized tubes 15 min after the injection and every 15 min thereafter. The sample was centrifuged and the plasma used for the determination of alcohol content (Sigma Kit, Catalogue No. 332–5). Prior to the end of the experiment, an additional blood sample was collected from the same small set of animals from each group (n = 1, 2) for the determination of the ending pH and blood gas pressures (PO2 and PCO2). To minimize the amount of blood collected from each animal, we chose to use a representative subset of animals to examine pre- and post- blood pH and blood gas pressures. At 60 min, all animals were killed and the entire liver and right kidney was rapidly removed and freeze-clamped with aluminium tongs precooled in liquid nitrogen. These samples were stored at −85°C for subsequent analyses.

For all animals, the sequential arterial blood samples (~150 μl) collected throughout the experiment were deproteinized in ice-cold perchloric acid (8% w/v), centrifuged, and the supernatant neutralized with 3.5 N KOH. A portion of the supernatant was used for the analysis of glucose (Raabo and Terkildsen, 1960). For the remaining portion, ion-exchange chromatography was used for the separation of radioactive glucose (Donovan and Suniida, 1990) and the subsequent determination of glucose specific activity (GSA). Duplicate aliquots of the glucose eluant derived from the ion-exchange procedure were evaporated to dryness (Organamation, N-Evap dry bath with aluminium beads) and reconstituted in distilled water. The measurement of 14C and H-activities in the remaining samples of the experiment were determined via liquid scintillation counting. Samples of the liver and kidney were pulverized under liquid nitrogen and solubilized in K2HPO4 (Good et al., 1933) for the determination of glycogen content (Dubois et al., 1956).

Glucose rates of appearance (Ra) and disappearance (Rd) were calculated using non-steady-state equations (Steele, 1959), as follows.

\[ Ra = \frac{(R^- - \left[ pVG(t) \times (dSA/dt) \right])}{SA(t)} \]

\[ Rd = Ra - \left[ pV(dG/dt) \right], \]

where \( R^- \) is the rate of tracer infusion (dpm/min), \( pVG \) is the effective glucose distribution pool (150 ml/kg), \( G \) is the blood glucose concentration (μmol/ml), and \( SA \) is the specific activity (dpm/μmol). Derivatives for the specific activity and glucose concentration were determined using the enhanced version of the optimal segments method for smoothing metabolic data (Bradley et al., 1993). The Ra and Rd values were based upon the \([1H]GSA\). Glucose clearance rate was calculated as Rd divided by the glucose concentration. Finally, apparent rates of glucose recycling (an indirect indicator of
By the end of the experimental period (Fig. 1), the glucose Ra concentration (mean ± standard error [SE]) as a function of time prior to and following the injection of water for male rats fed the control diet (MC, n = 7), male rats fed the ethanol diet (ME, n = 7), female rats fed the control diet (FC, n = 7) and female rats fed the ethanol diet (FE, n = 7). *Significant difference between FE and controls, P < 0.05. **Significant difference between the indicated time point and the initial level within FE, P < 0.05.

RESULTS

Body weights between control, 342.7 ± 8.4 g, and ethanol-fed male rats, 353.6 ± 7.2 g, were not significantly different after the 48-h fast. In like manner, the body weights between control, 238.2 ± 3.2 g, and ethanol-fed female rats, 237.7 ± 4.4 g, were not significantly different after the 48-h fast. Further, when the blood pH and blood gas pressures were pooled from all animals (n = 11), there were no significant differences between the initial versus ending experimental pH (7.38 ± 0.01 vs. 7.38 ± 0.01), PO₂ (87.2 ± 2.8 vs. 88.8 ± 2.4 mmHg), and PCO₂ (34.1 ± 0.9 vs. 33.4 ± 1.2 mmHg).

Water injection

Given that each group would be injected with ethanol, we chose to similarly inject all groups with water (our version of a sham operation). We also assumed that there would be no impact upon whole body glucose production between groups in the absence of ethanol (i.e. water injection). However, to substantiate this position, we injected water into the stomach for both male (MC, n = 7 and ME, n = 7) and female (FC, n = 7 and FE, n = 7) animals from each group. As anticipated, whole-body glucose production (Fig. 1) did not significantly vary between control-fed male rats (18.69 ± 1.26 μmol/kg/min) and female rats (19.47 ± 0.79 μmol/kg/min). Further, the glucose Ra did not significantly vary within the control groups throughout the experimental period. In support, the blood glucose concentration did not significantly vary between male and female rats fed the control diet and injected with water at any time point during the experimental period (Fig. 2). While the ME animals demonstrated a lower glucose Ra compared to controls, it was not statistically significant. In contrast, whole-body glucose production was significantly lower from the FE animals compared to the controls for most of the experimental period (Fig. 1). In addition, the glucose Ra was significantly lower for FE toward the end of the experimental time period compared to the initial level. Further, only the FE animals were observed to have a significantly lower blood glucose concentration compared to the control animals (MC and FC) beginning at 15 min and throughout the remaining experimental period. Apparent rates of glucose carbon recycling (Fig. 3) were similar in control groups and did not significantly vary during the experimental period (MC, 13.10 ± 0.72 μmol/kg/min and FC, 13.60 ± 0.77 μmol/kg/min). In contrast, the apparent rate of glucose carbon recycling (10.25 ± 0.72 μmol/kg/min) was significantly lower for the FE animals compared to controls. Consistent with the glucose Ra, glucose carbon recycling from FE animals was significantly lower at the last two points compared to their initial level.

Ethanol injection

For the animals fed the control diet (MC, n = 8 and FC, n = 8) and injected with ethanol, there was a slight decline (13% for
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MC and 10% for FC, respectively) in blood glucose concentration (from 6.53 ± 0.28 μmol/ml to a nadir of 5.65 ± 0.22 μmol/ml for MC and from 6.18 ± 0.39 μmol/ml to a nadir of 5.58 ± 0.54 μmol/ml for FC) after the ethanol injection (Fig. 4). In support, the glucose Ra (Fig. 5) declined for the control animals (nadir of 16.29 ± 1.38 μmol/kg/min for MC and 15.11 ± 1.29 μmol/kg/min for FC). For male animals chronically fed the ethanol diet (n = 8), there was a similar decline (15%) in blood glucose concentration (from 5.91 ± 0.29 μmol/ml to a nadir of 5.03 ± 0.36 μmol/ml) and glucose Ra (nadir of 9.47 ± 1.07 μmol/kg/min) after the ethanol injection. The glucose Ra (Fig. 5) from ME animals was significantly lower for the last experimental time points compared to controls and it was significantly different from their initial level (16.46 ± 2.3 μmol/kg/min). In contrast, the blood glucose concentration for the ME animals did not significantly vary from the control animals at any time point (Fig. 4). The female animals chronically fed the ethanol diet (n = 8) also demonstrated a slight decline (12%) in blood glucose concentration (from 4.16 ± 0.11 μmol/ml to a nadir of 3.65 ± 0.15 μmol/ml) after the ethanol injection (Fig. 4). Moreover, the FE animals started at a blood glucose concentration significantly lower than any other group and sustained the attenuated blood glucose concentration throughout the experimental period. In addition, the FE animals had a significantly lower glucose Ra throughout the experimental period (nadir of 7.67 ± 1.07 μmol/kg/min) compared to controls (Fig. 5). Apparent rates of glucose carbon recycling (Fig. 6) were significantly lower at the final time point compared to the initial level for both MC (17.81 ± 2.15 vs 12.66 ± 1.16 μmol/kg/min) and FC (16.25 ± 2.06 vs 11.02 ± 1.03 μmol/kg/min). Apparent rates of recycling were significantly lower for ME animals compared to controls toward the end of the experimental time period and were significantly lower compared to their initial level at the final

**Fig. 3.** Effects of chronic alcohol consumption on apparent rates of glucose carbon recycling (mean ± standard error [SE]) as a function of time prior to and following the injection of water for male rats fed the control diet (MC, n = 7), male rats fed the ethanol diet (ME, n = 7), female rats fed the control diet (FC, n = 7) and female rats fed the ethanol diet (FE, n = 7). *Significant difference between FE and controls, P < 0.05. #Significant difference between the indicated time point and the initial level within FE, P < 0.05.

**Fig. 4.** Effects of chronic alcohol consumption on blood glucose concentration (mean ± standard error [SE]) as a function of time prior to and following the injection of ethanol for male rats fed the control diet (MC, n = 8), male rats fed the ethanol diet (ME, n = 8), female rats fed the control diet (FC, n = 8) and female rats fed the ethanol diet (FE, n = 8). *Significant difference between FE and controls, P < 0.05.

**Fig. 5.** Effects of chronic alcohol consumption on the rate of glucose appearance (Ra, mean ± standard error [SE]) as a function of time prior to and following the injection of ethanol for male rats fed the control diet (MC, n = 8), male rats fed the ethanol diet (ME, n = 8), female rats fed the control diet (FC, n = 8) and female rats fed the ethanol diet (FE, n = 8). *Significant difference between ME and controls, P < 0.05. #Significant difference between the indicated time point and the initial level within a group, P < 0.05.

All groups demonstrated an initial decline in the Ra–Rd (Fig. 7), which supports the slight decrease in blood glucose concentration after the ethanol injection. While all groups
remained below the line of identity (i.e. where $R_a – R_d = 0$) only the FE animals remained significantly below zero throughout the experimental period following the ethanol injection (Fig. 7). Finally, the glucose clearance rate was not significantly different between groups (Fig. 8). While the glucose clearance rate demonstrated a trend toward a decline after the injection of alcohol in chronically ethanol-fed animals, only the ME animals were significantly lower at the end of the experiment compared to their initial levels.

The plasma alcohol content (Fig. 9) was not significantly different between male animals fed either the control or ethanol diet (for clarity, all male animals were pooled). In contrast, the plasma alcohol content was significantly higher for the female animals chronically fed the ethanol diet at all time points compared to the male animals and was significantly higher for all groups (including FC) at 45 and 60 min (Fig. 9). While all other groups demonstrated a plateau, the female rats fed the ethanol diet appeared to demonstrate a continual rise in plasma alcohol content. Finally, the 48-h fast resulted in significant depletion of liver and kidney glycogen.
demonstrated a significant reduction in glucose ethanol-fed animals. The inability to observe an exacerbated blood glucose concentration from either the control or alcohol ingestion, we failed to observe a significant decline in attenuation in glucose carbon recycling. Following the acute able to lower rates of gluconeogenesis (as indicated by the experimental time period) from the FE animals is attribut-
catecholamine response and a corresponding elevation in blood It is possible that we inadvertently evoked a significant 
we did observe such a decline for a majority of the time points. 
chronically fed the ethanol diet in the absence and presence of 
lower blood glucose concentration in female rats 
in vivo 
observed rates of glucose carbon recycling cannot be equated 
from all groups after the ethanol injection. Although our 
observed significant decreases in glucose carbon recycling 
from all groups after the ethanol injection. Although our 
induced hypoglycaemia for any of the groups. While we 
withholding of food and measurement of blood glucose at 0, 0.5, 
Conversely, blood glucose homeostasis could be attributable 
to alterations in peripheral glucose clearance. Normally, in vivo glucose production and glucose disposal are tightly regulated 
in order to maintain euglycaemia. Xu et al. (1996) observed 
that ethanol caused an acute insulin resistance in skeletal muscle thereby inhibiting whole body glucose utilization.
However, the mechanism for sex differences in glucose production and regulation after chronic ethanol consumption and in the presence of an acute alcohol exposure remains unresolved. Although we failed to observe significant differences in the glucose clearance rate between groups, the ME animals demonstrated clearance rates that were significantly lower than their initial level (i.e. prior to the ethanol injection). Further, the Ra–Rd (Fig. 7) provided an examination of the ability to match rates of glucose production and glucose utilization at a specific time point. Following the alcohol injection, the ME animals were able to match the marked declines in glucose Ra with comparable reductions in glucose clearance. In contrast, the FE animals failed to match the lower glucose production with decreased rates of glucose utilization. Given that the major site of glucose disposal is skeletal muscle, ME animals appear to maintain the ability to regulate glucose production with peripheral glucose utilization. However, because the FE animals demonstrate higher plasma alcohol levels, we cannot rule out the possibility that the amount of alcohol exposure is what caused the more dramatic decline in glucose production capacity and/or the failure to appropriately lower peripheral glucose clearance. We have preliminary evidence to support that chronic ethanol consumption elicits a distinct decline in gluconeogenic capacity from female compared to male rats in the absence of alcohol (Sumida et al., 2000), albeit potential sex differences from alcohol consumption upon peripheral glucose utilization remains to be determined.

Despite an equivalent amount of alcohol injected, the tendency toward higher plasma alcohol contents exhibited by females fed the control diet compared to males is consistent with previous studies in rats (Rivier, 1993; Da-Silva et al., 1996) and humans (Van Thiel et al., 1988; Lieber, 2000). Women have a lower amount of gastric alcohol dehydrogenase compared to men, which decreases first-pass ethanol metabolism (Lieber, 2000). In addition, the distribution space for alcohol is smaller in women than in men (Van Thiel et al., 1988). Further, our observation that FE animals had higher plasma alcohol contents is consistent with human studies which reported that the sex effect is exacerbated by alcoholism (Frezza et al., 1990; Lieber, 2000). In contrast to human studies, we did not observe a higher plasma alcohol content from ME animals compared to male controls. In alcoholic and nonalcoholic men, fasting has been observed to decrease first-pass ethanol metabolism and appears to be dose dependent (DiPadova et al., 1987). As such, it is possible that our 48-h fast and elevated alcohol dose eliminated the normal differences observed between ME and MC animals. For alcoholic women, it has been reported that they lose the gastric protective barrier provided by first-pass ethanol metabolism (Lieber, 2000). Assuming this also occurs in rodents, fasting would have little impact on the FE animals, which supports our observed plasma alcohol levels.

Collectively, the sex difference we report in animals underscores the deleterious effects of chronic alcohol consumption for females. Our female animals chronically fed the ethanol diet had lower resting blood glucose concentrations compared to all other groups. Further, after the ethanol injection FE animals had lower rates of glucose production attributable to lower rates of gluconeogenesis, higher plasma alcohol levels, and lower blood glucose concentrations. Accordingly, this may contribute to a greater vulnerability of alcoholic females to the toxic effects of ethanol. Specifically, the combination of low blood glucose concentration and high plasma alcohol content would enhance the pharmacologic effects of ethanol. Moreover, the higher plasma alcohol levels could generate elevated hepatotoxic products (e.g. acetaldehyde) resulting in greater susceptibility to medical complications.

The lower liver glycogen content in female compared to male rats following the 48-h fast was unexpected, but consistent with a prior report (Teutsch, 1984). Despite these differences, they appear to have no impact upon our observed glucose kinetics. In support, despite the difference in hepatic glycogen content there was no significant difference in glucose Ra and blood glucose concentration between MC and FC after the water or ethanol injection. There was also no significant difference in glycogen content between MC and ME or between FC and FE despite differences in glucose production for the FE animals. Further, the liver was clamped at the end of the experiment after both the water and alcohol injection. Because neither condition yielded a significant difference between a given group injected with water versus a given group injected with ethanol, the total glycogen content was pooled for each group. It was only upon pooling all values (water and ethanol injection) for each group (n = 15) that a significant difference was subsequently attained between groups.

We recognize that a potential limitation might be the use of anesthetized animals. However, the use of conscious animals requires the elimination of any acute stress (i.e. sympathetic activation), which would evoke a counter-regulatory response and the subsequent rise in blood glucose. Naïve control animals (i.e. unaccustomed to drinking alcohol) will not voluntarily drink ethanol. In addition, we wanted to ensure an equivalent dose of alcohol based upon body weight to be given through the gastric route. To accomplish this, the use of conscious animals would have required an intraperitoneal injection or gastric intubation and a corresponding counter-regulatory response. While intravenous infusion was an option, this would have avoided differences attributable to gastrointestinal ethanol oxidation and would have limited the potential for any immediate effects of alcohol upon the liver (a key glucose regulatory organ). The use of anaesthetized animals allowed us to inject the ethanol directly into the stomach. Further, the use of conscious animals requires chronic cannulation and the necessary recovery time where animals do not immediately eat after the surgery. Souza et al. (1981) reported an attenuated effect of ethanol in eliciting a decline in blood glucose concentration from chronically starved rats. As such, we attempted to maximize our ability to elicit alcohol-induced hypoglycaemia by exposing the animals to their first and only bout of fasting prior to the experiment. Further, the only general effect of ketamine/xylazine upon glucose homeostasis in rats we are aware of involves glucose intolerance when given a glucose load (Hindlycke and Jansson, 1992) most likely attributable to insulin resistance. If the anaesthesia were to affect glucose homeostasis in the resting state (an absence of a glucose load), then the insulin resistance should have markedly elevated our blood glucose concentration for all animals. In contrast, we observed no elevation, but we cannot rule out the possibility of an
alcohol/anaesthesia interaction. Even if there was an interaction, this would help to explain our inability to elicit hypoglycaemia suggesting that the effects of chronic alcohol consumption in the conscious (unanesthetized) state might be more detrimental than what we report. Finally, due to the use of anaesthetized animals and the initial time required for the radioactive glucose to distribute into various pools, we only examined the glucose kinetics for 1 h after the ethanol injection. It is possible that more time is required for alcohol to elicit its effect upon blood glucose levels. However, given the prior studies in both rats (Abel, 1996) and humans (Yki-Jarvinen et al., 1988; Siler et al., 1998) it seems unlikely that the glucose kinetics over an additional time period would have altered our conclusion.

In summary, the results indicate that, in female rats, chronic alcohol consumption lowers whole-body glucose production in the absence of alcohol exposure compared to controls. In all groups, following an acute ethanol injection, in vivo glucose production rates decline, attributable to a reduction in gluconeogenesis, but this did not result in significantly lower blood glucose concentrations. Further, after acute alcohol exposure, both ME and FE had lower whole-body glucose production rates compared to corresponding controls. In addition, FE (compared to ME) failed to match the decline in glucose production with a comparable decrease in glucose clearance. Finally, that FE continued to demonstrate reduced rates of glucose production and lower blood glucose levels in both the absence and presence of alcohol suggests a greater vulnerability to the pharmacologic effects of ethanol, enhanced susceptibility to alcohol-induced hypoglycaemia, or elevated risks for medical complications.

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