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Strain-Dependent Differences for Suppression of Insulin-Stimulated Glucose Uptake in Skeletal and Cardiac Muscle by Ethanol

Charles H. Lang¹, Zoltan Derdak², and Jack R. Wands²

¹Department of Cellular and Molecular Physiology, and Surgery, The Pennsylvania State University College of Medicine, Hershey, PA 17033

²Division of Gastroenterology and Liver Research Center, Warren Alpert Medical School of Brown University and Rhode Island Hospital, Providence, RI

Abstract

Background—Chronic ethanol consumption impairs the ability of insulin to suppress hepatic glucose production in a strain-dependent manner, with hepatic insulin resistance being greater in Long-Evans (LE) than Sprague-Dawley (SD) rats. We assessed whether strain differences exist for whole-body and tissue glucose uptake under basal and insulin-stimulated conditions and whether they were associated with coordinate strain-dependent elevations in muscle cytokines.

Methods—Male rats (160 g) were provided the Lieber-DeCarli ethanol-containing (36% total energy) diet or pair-fed a control diet for 8 weeks. Rats were studied in the basal state or during a euglycemic hyperinsulinemic clamp, and whole-body glucose flux assessed using ³H-glucose and in vivo tissue glucose uptake by ¹⁴C-2-deoxyglucose.

Results—Ethanol impaired whole-body insulin-mediated glucose uptake (IMGU) more in SD than LE rats. This difference was due to impaired IMGU by gastrocnemius and heart in ethanol-fed SD vs LE rats. However, decreased IMGU in adipose tissue (epididymal and perirenal) produced by ethanol was comparable between strains. Ethanol-induced insulin resistance in muscle from SD rats was associated with reduced AKT and AS160 phosphorylation and plasma membrane-localized GLUT4 protein as well as enhanced phosphorylation of JNK and IRS-1 (S307), changes which were absent in muscle from LE rats. Ethanol increased TNF α mRNA in gastrocnemius and fat under basal conditions in both SD and LE rats; however, hyperinsulinemia decreased TNF α in skeletal muscle from LE but not SD rats. IL-6 mRNA in gastrocnemius was increased under basal conditions and increased further in response to insulin in SD rats, but no ethanol- or insulin-induced change was detected in muscle IL-6 of LE rats.

Conclusion—These data indicate strain-dependent differences in ethanol-induced IMGU in skeletal and cardiac muscle, but not fat, associated with sustained increases in TNF α and IL-6 mRNA and JNK activation, and decreased plasma membrane GLUT4 in response to insulin.

Keywords

ethanol; glucose uptake; insulin resistance; TNF α , IL-6

INTRODUCTION

Both acute ethanol intoxication and chronic ethanol abuse alter whole-body and tissue carbohydrate metabolism under basal and insulin-stimulated conditions, and chronic ethanol abuse is an independent risk factor for type 2 diabetes (Avogaro and Tiengo, 1993). The associated ethanol-induced abnormalities in glucose metabolism appear dependent on the underlying nutritional state and do not necessarily involve the same cellular mechanisms. Because of the dominant role of the liver in regulating both ethanol metabolism and glucose homeostasis, this organ has been the primary focus of research. However, glucose balance is also influenced by the rate of glucose uptake by numerous peripheral organs mediated by insulin-dependent and -independent mechanisms (Edelman et al., 1990, Lang, 1992). Acute ethanol administration, especially in the fasted state, produces hypoglycemia by reducing hepatic glucose production (HGP), resulting from the combined effects of inhibition of gluconeogenesis (Dittmar and Hetenyi, 1978, Kreisberg et al., 1971, Lochner et al., 1967, Searle et al., 1974) and impaired glycogenolysis (Kubota et al., 1992, Winston and Reitz, 1980). In contrast, the prevailing blood glucose concentration is well-maintained when acute ethanol intoxication is studied either in the fed state or in rats chronically fed an ethanol-containing diet (Dittmar and Hetenyi, 1978, Kreisberg et al., 1971, Molina et al., 1991). However, despite the appearance of normal glucose homeostasis in these latter experimental situations, ethanol has a demonstrable effect on basal whole-body glucose production and disposal (Dittmar and Hetenyi, 1978, Siler et al., 1998, Spolarics et al., 1994, Yki-Jarvinen et al., 1988). Although a decreased basal glucose uptake by select tissues has been reported in response to acute ethanol intoxication (Spolarics et al., 1994), these changes are modest in magnitude and may be transient. However, there are few data pertaining to alterations in tissue-specific glucose disposal produced by chronic ethanol consumption.

Separate from the ethanol-induced alterations in basal glucose metabolism are its effects on insulin action. Ethanol, both the acute infusion and chronic consumption, can impair the ability of insulin to suppress HGP (Derdak et al., 2011, Kang et al., 2007b). Moreover, the severity of ethanol-induced hepatic insulin resistance is strain-dependent, being more pronounced in ethanol-fed Long-Evans (LE) compared to Sprague-Dawley (SD) rats (Derdak et al., 2011). Such differences between strains have the potential to provide mechanistic insight under in vivo conditions by elucidating potential mediators or signaling pathways central to glucose disposal which may be differentially regulated in a strain-specific manner. A similar approach has been used previously in liver to reveal the relative importance of p53 and oxidative stress in the susceptibility of ethanol-induced hepatic insulin resistance and liver damage (Derdak et al., 2011). Long-term ingestion of ethanol impairs insulin stimulated whole-body glucose disposal (Avogaro et al., 1987, Kang et al., 2007b, Yki-Jarvinen et al., 1988), but ethanol-induced changes in insulin-stimulated glucose uptake by individual tissues are more inconsistent and sparse (Qu et al., 2011, Spolarics et al., 1994, Wan et al., 2005, Wilkes and Nagy, 1996, Xu et al., 1996). The presence of

peripheral insulin resistance in other catabolic states has been associated with the overproduction of the proinflammatory cytokines, tumor necrosis factor (TNF)- α or interleukin (IL)-6 (Kim et al., 2004, Lang et al., 1992). Hence, the present study assessed whether strain differences exist for whole-body and tissue glucose uptake under both basal and insulin-stimulated conditions and whether such differences were associated with coordinate elevations in muscle cytokine expression in chronic ethanol-fed rats.

MATERIALS and METHODS

Sprague-Dawley and Long-Evans male rats (~160 g, Harlan, Indianapolis, IN) were fed ad libitum for 8 weeks with a Lieber-DeCarli ethanol-containing liquid diet (ethanol-derived calories were increased stepwise from 12% to 36% of total energy during first two weeks) (Bioserv, Frenchtown, NJ). Control-fed rats received an isonitrogenous isocaloric liquid diet containing maltose dextrin instead of ethanol and the volume provided was the average consumed by ethanol-fed rats of the same strain the previous day. Body fat and fat free mass (e.g., lean body mass [LBM]) were quantitated by ^1H -NMR (Bruker Minispec, LF90, Woodlands, TX) (Lang et al., 2010), immediately prior to surgery. Rats were anesthetized by intramuscular injection of ketamine and xylazine (90 and 9 mg/kg body weight, respectively) and heart function assessed by echocardiography, as described below. Sterile surgery was then performed to implant a single catheter in the carotid artery and two catheters in the jugular vein (Lang et al., 1992). After surgery, rats were housed individually in wire-bottom cages and provided the appropriate ethanol-containing or control diet for 8 weeks. Food was then removed at midnight and the experiment started between 0700-0800 h. This period of food deprivation was imposed to minimize intestinal glucose absorption and glycogenolysis as contributors to HGP but to permit the consumption of ethanol during at least part of the night prior to the insulin clamp. Experimental protocols were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine and adhered to National Institutes of Health (NIH) guidelines.

Basal glucose kinetics and euglycemic hyperinsulinemic clamp

Experiments were performed on catheterized, unrestrained, conscious rats (Crist et al., 1998, Lang, 1992, Lang et al., 1992). In all experiments, control and ethanol-fed rats of both strains were randomized and always studied in the same experiment; all studies were repeated at least 3 times to obtain the desired sample size. A primed, constant intravenous (IV) infusion of [3- ^3H]-glucose (Perkin-Elmer, Waltham, MA) was initiated the morning after surgery to determine glucose kinetics. Rats received a bolus injection of radiolabeled glucose (7- μCi) followed by an IV infusion of tracer (0.83 $\mu\text{Ci}/\text{min}$ at 0.5 ml/hr) for the duration of the protocol. To determine basal glucose flux, blood samples (0.3 ml) were collected from the arterial catheter at 120 and 140 min after starting the ^3H -glucose infusion. Plasma glucose (Analox Instruments; Lunenburg, MA) and insulin (ALPCO; Salem, NH) concentrations were determined, and the plasma ^3H -glucose radioactivity quantitated (Beckman LS6000).

At the conclusion of this basal period, a euglycemic hyperinsulinemic clamp was initiated to determine the ability of insulin to stimulate peripheral glucose uptake and suppress HGP

(Derdak et al., 2011). Insulin (Humulin-R; Eli Lilly, Indianapolis, IN) was administered IV as a primed, constant infusion to rapidly increase the plasma insulin concentration; an exogenous glucose infusion containing 25% D-glucose and sufficient [^3H]-glucose to maintain a constant glucose specific activity (GSA) was initiated after starting the insulin infusion. Insulin was infused (2 mU/min/kg) for 3 h and the glucose infusion rate (GIR) was varied to maintain euglycemia based on the glucose concentration determined every 10-15 min (Lang, 1992, Lang et al., 1992). Blood samples were obtained at 20-min intervals during the last hour of the clamp for quantitating glucose, insulin, free fatty acids (FFAs) and glycerol. The GSA was determined on neutralized supernatants of deproteinized plasma where [^3H]-glucose radioactivity was determined after removal of tritiated water. Rates of glucose appearance (Ra) and disappearance (Rd) were assessed in the basal condition and at 20-min intervals during the last hour of the clamp. The residual HGP rate during the clamp was calculated by subtracting the GIR from the tracer-determined total glucose Ra. The GIR and GSA were statistically unchanged over the final hour of the clamp (data not shown) and the mean was calculated by averaging the three consecutive 20-min interval measurements. The plasma concentration of FFAs and glycerol were determined at selected times (Wako Industrials, Osaka, Japan).

Tissue glucose uptake

In vivo glucose uptake (Rg) by individual tissues was determined using [^{14}C]-labeled 2-deoxyglucose (2-DG) (Lang et al., 1992, Meszaros et al., 1987). Tissue-specific glucose uptake was determined between 140-180 min after starting the euglycemic hyperinsulinemic clamp. Separate rats injected with ^{14}C -2-DG were used to determine tissue Rg under basal (no clamp) conditions. A tracer amount of ^{14}C -2-DG (8 mCi/rat; Amersham, Arlington Heights, IL.) was injected IV and serial arterial blood samples (0.2 ml) collected. Plasma was deproteinized with perchloric acid (PCA) and ^{14}C -radioactivity determined. Rats were then anesthetized with pentobarbital and tissues excised to quantitate the intracellular accumulation of phosphorylated ^{14}C -2-DG. The ^{14}C -GSA was determined on neutralized supernatant of deproteinized plasma. Tissues were homogenized in ice-cold 0.5 N PCA and centrifuged. The concentration of phosphorylated 2-DG in tissues was calculated as the difference between total ^{14}C -radioactivity of the neutral extract and the ^{14}C -radioactivity remaining after Somogyi treatment. In vivo glucose uptake for each tissue was calculated as previously described (Meszaros et al., 1987).

RNA extraction and real-time quantitative PCR

Tissues were homogenized using Tri-reagent (Molecular Research Center, Cincinnati, OH) followed by chloroform extraction and total RNA isolated using the RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturers' protocol. RNA was eluted from the Qiagen mini-spin column with RNase-free water and an aliquot quantitated by the NanoDrop 2000 (Thermo Fisher Scientific; Waltham, MA). RNA quality was analyzed on a 1% agarose gel and total RNA (1 μg) was reverse transcribed using superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) following manufacturer's instruction. Real-time quantitative PCR was performed using cDNA in a StepOnePlus system using TaqMan gene expression assays (Applied Biosystems, Foster City, CA) for tumor necrosis factor (TNF)- α , interleukin (IL)-6 and L32 using primer sequences (Korzick et al., 2013). The comparative

quantitation method 2^{-Ct} was used in presenting gene expression of target genes in reference to the endogenous control.

Western blot analysis

Muscle was homogenized using ice-cold buffer containing (in mM) 20 HEPES (pH 7.4), 2 EGTA, 50 NaF, 100 KCl, 0.2 EDTA, 50 β -glycerolphosphate, 1 DTT, 0.1 PMSF, 1 benzamidine, and 0.5 sodium vanadate (28-30, 40). Equal amounts of protein per sample were subjected to standard SDS-PAGE, using antibodies from Cell Signaling (Beverly, MA) unless otherwise specified. Western analysis was performed for total and phosphorylated AKT (S473), AS160 (T642), insulin-like substrate (IRS)-1 (S307), *c*-Jun N-terminal kinase (JNK) (T183/185), and ribosomal S6 kinase -1 (S6K1) (T389). Blots were washed with TBS-T (1X TBS including 0.1% Tween-20) and incubated with secondary antibody. Blots were incubated with enhanced chemiluminescence (ECL) reagents (Amersham), and dried blots exposed to x-ray film for 1-30 minutes. After development, the film was scanned (Microtek ScanMaker IV) and analyzed using NIH Image 1.6 software

Plasma membrane preparation

For total membrane preparation, muscle was homogenized (1:10 vol) in buffer containing 20 mmol/L HEPES, 5 mmol/L EDTA, 250 mmol/L sucrose, 50 nmol/L okadaic acid, 1 mmol/L Na_3VO_4 , 2 $\mu\text{g/ml}$ pepstatin, 1 mmol/L PMSF, 10 $\mu\text{g/ml}$ aprotinin, and 2 $\mu\text{g/ml}$ leupeptin (pH 7.5) at 4°C. The homogenate was centrifuged at 1200 g at 4 °C for 15 min and the precipitate discarded. The supernatant was then centrifuged at 220,000 g for 90 min at 4°C and the pellet resuspended in the HEPES-EDTA-sucrose buffer for Western analysis using antibodies for GLUT1, GLUT4, $\text{Na}^+\text{-K}^+\text{-ATPase}$ or GAPDH (Abcam, Cambridge, MA).

Ecocardiography

Heart function was assessed by echocardiography (Sequoia C256, Siemens Medical Solutions, Mountain View, CA) in anesthetized rats immediately prior to surgical implantation of catheters (Pruznak et al., 2008). The transducer was placed on the thorax and M-mode recordings were performed by directing the ultrasound beam at the midpapillary muscle level. The operator was blinded to the treatment group. Derived echocardiography parameters included heart rate, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD) and interventricular septal diastolic wall thickness (IVSD). To assess left ventricular systolic function, fractional shortening (FS) and ejection fraction (EF) were calculated as follows: $\text{FS} = [(\text{LVEDD} - \text{LVESD}) / \text{LVEDD}] \times 100\%$, $\text{EF} = [(\text{LVEDD}^3 - \text{LVESD}^3) / \text{LVEDD}^3] \times 100\%$.

Statistical analysis

Data for each condition are summarized as mean $\pm$ SEM where the number of rats per treatment group is indicated in the legend to the figure or table. Data pertaining to body composition, echocardiographic endpoints, insulin-mediated whole-body glucose disposal, insulin-induced suppression of hepatic glucose production and the area under the curve for FFAs were compared by 2-way (ethanol x strain) analysis of variance (ANOVA). All other data were compared using a 3-way ANOVA (ethanol x insulin x strain). The Student-

Neuman-Keuls (SNK) test was used for post hoc comparisons on significant interactive effects (SigmaPlot 11.0 for Windows; San Jose, CA). An α -level of $P < 0.05$ was used for all comparisons and considered statistically different. For all tables and figures, values having the same superscript letter are considered not statistically different ($P > 0.05$); values having different superscript letters (“a” versus “b” versus “c”) were considered statistically significant at $P < 0.05$. The area under the curve (AUC) was calculated using the trapezoidal rule using the basal (time 0) value as zero.

RESULTS

Body composition

The starting weight of all rats regardless of strain or group assignment did not differ (Table 1). The final body weight of both SD (-18%) and LE (-11%) rats consuming ethanol was lower than their respective pair-fed controls (Table 1). As a result, the increment in body weight was significantly less in ethanol-fed SD rats (-27%), compared to ethanol-fed LE rats (-17%). Similarly, ethanol-fed rats had a lower fat free mass (FFM; e.g. lean mass) than control-fed rats, and this decrease averaged -22% in SD rats and -13% in LE rats. Ethanol feeding also tended to reduce the fat mass in both strains of rats, but these differences did not achieve statistical significance. Hence, chronic ethanol feeding slowed the normal increase in body weight gain and this was largely due to the failure to accrete lean body mass in SD compared to LE rats. These changes in body weight and composition between control and ethanol-fed rats and between SD and LE rats did not result from a difference in the volume of liquid diet consumed (Table 1).

Whole-body glucose kinetics

The rates of HGP and whole-body peripheral glucose disposal were determined under basal and insulin-stimulated conditions in control and ethanol-fed rats using ^3H -glucose. The plasma glucose concentration did not differ between SD (Figure 1A) or LE (Figure 1B) rats under either the basal state (time 0) or during the final hour of the euglycemic hyperinsulinemic clamp. There was no strain-dependent change in the plasma insulin concentration in control or ethanol-fed rats under basal conditions or during the insulin clamp (Figure 1C and 1D); although plasma insulin was increased in control and ethanol-fed rats during the insulin clamp, compared to basal values. The rate of whole-body glucose disposal did not differ between control and ethanol-fed rats SD or LE rats under basal conditions (Figure 1E and 1F, respectively). The infusion of insulin increased whole-body glucose disposal to the same extent in control-fed rats regardless of strain. Insulin stimulation of whole-body glucose disposal was decreased to the same extent in ethanol-fed SD and LE rats. Overall, there was no significant strain effect for any variable illustrated in Figure 1.

Calculation of the difference in glucose disposal between basal and insulin-stimulated conditions in the same rat revealed that although ethanol feeding reduced glucose uptake in both LE and SD rats, the attenuation of insulin action was greater in ethanol-fed SD rats (Figure 2A). As rats were in a metabolic steady-state, under basal conditions the rate of whole-body glucose disposal equals the rate of glucose production (i.e., HGP). Hence, basal

HGP did not differ between control and ethanol-fed rats in either group. Chronic ethanol consumption also impaired insulin-induced suppression of HGP and this hepatic insulin resistance was greater in LE compared to SD rats (Figure 2B).

Tissue glucose uptake

Glucose disposal by gastrocnemius, soleus and heart (right and left ventricle) did not differ between control and ethanol-fed rats under basal conditions for SD rats (Figures 3A, 3C, 3E and 3G, respectively) or LE rats (Figures 3B, 3D, 3F and 3H, respectively). Glucose uptake was increased in each tissue during the insulin clamp and the tissue-specific increase was not different between strains. Ethanol blunted the insulin-induced increase in glucose uptake in gastrocnemius, but not soleus, as well as in the right and left ventricle of SD rats. In contrast, this insulin resistance in gastrocnemius and left ventricle was not detected in ethanol-fed LE rats. Apparent strain differences for insulin-mediated glucose uptake by right ventricle did not achieve statistical differences ($P > 0.05$; ethanol x insulin x strain). Glucose uptake by atria did not differ between strains or in response to ethanol feeding and averaged 57 ± 4 nmol/min/g tissue (group data not shown).

As for striated muscle, glucose uptake by epididymal (Figure 4A and 4B) and perirenal fat (Figure 4C and 4D) did not differ under basal conditions and showed no strain differences. Ethanol feeding impaired insulin-stimulated glucose uptake in both fat depots examined and the ethanol-induced insulin resistance in fat did not differ between strains ($P > 0.05$; ethanol x insulin x strain).

Additionally, we determined whether chronic ethanol consumption alters glucose uptake in other peripheral tissues and brain under basal and insulin-stimulated conditions (Table 2). Overall, there was no difference in the basal glucose disposal by liver, ileum, spleen, lung, kidney and brain between control and ethanol-fed rats for either SD or LE rats. There was a significant insulin-induced increase in glucose uptake by liver, spleen, lung and kidney in both rat strains. Insulin did not increase glucose uptake by ileum or brain. Overall, there was no ethanol x insulin x strain interaction for glucose disposal by any individual tissue identified in Table 2.

FFA and glycerol alterations

As insulin inhibits lipolysis and increased circulating FFAs can impair insulin-stimulated glucose uptake (Savage et al., 2007), we also assessed the in vivo anti-lipolytic action of insulin. The basal concentration of FFAs in control and ethanol-fed rats did not differ in either SD or LE rats (Figure 5A and 5B). In response to hyperinsulinemia, the plasma FFA concentration gradually declined in control and ethanol-fed rats ($P < 0.05$ for insulin effect). As assessed by the AUC, the insulin-induced decrease in FFAs was smaller in ethanol-fed compared to control rats, suggesting ethanol impairs the inhibition of lipolysis by insulin (Figure 5C). The magnitude of this insulin resistant state did not differ between SD and LE rats. Ethanol feeding also blunted the insulin-induced decrease the plasma glycerol in both SD and LE rats (data not shown).

Signal transduction in skeletal and cardiac muscle

The ability of insulin to stimulate glucose uptake is dependent on the recruitment of GLUT4 to the plasma membrane, which in turn is dependent upon the up-regulation of AKT and AS160 phosphorylation (Thong et al., 2007). AKT and AS160 phosphorylation in gastrocnemius (Figure 6A and 6B) and heart (Figure 7A and 7B) did not differ between control and ethanol-fed rats under basal conditions, and there was no difference between strains. Ethanol consumption prevented the insulin-induced phosphorylation of both proteins in gastrocnemius and heart of SD, but this suppressive effect was not observed LE rats (ethanol x insulin x strain interaction; $P < 0.01$). In contrast, ethanol did not alter insulin-induced increases in AKT or AS160 phosphorylation in the soleus from SD rats (data not shown). Associated with this insulin resistance in SD rats was an increased S307-phosphorylation of IRS-1 in both the basal and insulin-stimulated state in gastrocnemius (Figure 6C) and heart (Figure 7C). Likewise, the phosphorylation of JNK, one of the stress-activated kinases, was increased in gastrocnemius and heart from ethanol-fed SD rats (Figures 6D and 7D, respectively). No such increase in IRS-1 or JNK phosphorylation was detected in LE rats leading a statistically significant ($P < 0.01$) strain interaction.

S6K1 phosphorylates a number of residues on IRS-1 during conditions producing insulin resistance (Zhang et al., 2008). However, there was no difference in T389-phosphorylated S6K1 in skeletal muscle between control and ethanol-fed rats (either SD or LE) under basal conditions, nor an insulin-induced change in S6K1 phosphorylation (Figure 8). There was also no ethanol- or insulin-induced change in S6K1 phosphorylation in heart (data not shown).

GLUT4 protein in the plasma membrane (PM) fraction of gastrocnemius did not differ between control and ethanol-fed rats under basal conditions for either SD or LE rats (Figures 9B and 9C). However, a significant strain interaction ($P < 0.01$) was observed for the insulin-induced increase in plasma membrane GLUT4 as this increase was blunted in SD but not LE rats. GLUT1 protein did not differ in the total lysate or plasma membrane fraction in response to ethanol and/or insulin stimulation, and did not show a strain difference (Figure 9B).

Tissue cytokines

As chronic ethanol feeding can alter cytokine expression in certain tissues and cause insulin resistance (Olefsky and Glass, 2010), we assessed TNF α and IL-6 mRNA in gastrocnemius, heart and epididymal fat. TNF α mRNA was increased in all three tissues under both basal and insulin-stimulated conditions in ethanol-fed SD rats (Figure 10A, 10C and 10E). While TNF α expression in epididymal fat under control and insulin-stimulated conditions did not differ between strains, a significant strain interaction ($P < 0.01$) was detected in gastrocnemius and heart where LE rats showed no such elevation. A different pattern for IL-6 mRNA was detected in response to ethanol feeding and hyperinsulinemia (Figure 10). Ethanol increased IL-6 mRNA in gastrocnemius from SD but not LE rats under basal conditions (Figure 10B). Hyperinsulinemia further increased IL-6 in skeletal muscle from SD rats. No ethanol- or insulin-induced changes were detected in gastrocnemius from LE rats (strain difference $P < 0.01$). The IL-6 mRNA content in heart did not differ between

control and ethanol-fed SD or LE under basal or hyperinsulinemic conditions (Figure 10D). Finally, IL-6 mRNA was increased in adipose tissue from both SD and LE rats consuming ethanol and this increase was sustained during the glucose clamp (Figure 10F).

Echocardiography

Because of the difference in insulin-stimulated glucose uptake between ethanol-fed SD and LE rats and the potential impact of changes in substrate handling on cardiac function (Abel et al., 2012), we also assessed cardiac function by echocardiography. As presented in Table 3, there was no significant difference between SD and LE rats either in the fed condition or after ethanol feeding.

DISCUSSION

The present study demonstrates *in vivo*-determined whole-body glucose disposal under *basal* conditions does not differ between rats (either SD or LE) fed a nutritionally complete ethanol-containing diet for 8 weeks and pair-fed control animals, a finding in agreement with most reports where the host has not undergone a prolonged fast (Dittmar and Hetenyi, 1978, Molina et al., 1991, Yki-Jarvinen et al., 1988). The lack of an ethanol-induced change in basal glucose uptake in skeletal muscle has also been observed *in vitro* in isolated muscle from ethanol-fed rats (Wilkes and Nagy, 1996). These data are internally consistent with our results showing basal glucose uptake by skeletal muscle (both fast- and slow-twitch), heart (both atria and ventricle), adipose tissue (both epididymal and perirenal), liver, kidney, spleen, lung, gut and brain did not differ between control and ethanol-fed rats. In contrast, a decrease in basal glucose disposal has been reported for red quadriceps, soleus, heart, and ileum in rats following acute ethanol intoxication (Spolarics et al., 1994). The reason for these differences in regional glucose flux between acute and chronic conditions may be related to the higher peak ethanol concentration typically achieved in the former situation (Limin et al., 2009, Wan et al., 2005). Regardless of the exact mechanism, these differences emphasize data obtained using acute ethanol intoxication models may not necessarily accurately reflect the new metabolic steady-state achieved with more prolonged feeding protocols.

Chronic ethanol consumption suppressed the ability of insulin to stimulate whole-body glucose uptake, a response previously reported in rodents (Kang et al., 2007b) and humans (Yki-Jarvinen et al., 1988). The ability of ethanol to produce peripheral insulin resistance appears dose-related with relatively low levels of ethanol consumption often improving insulin action (Ting and Lutt, 2006). Our data extend these observations by demonstrating the magnitude of the ethanol-induced insulin resistance is strain-dependent, with a more severe peripheral resistance observed in SD rats compared to LE rats. In contradistinction, the ability of ethanol to produce insulin resistance in *liver* is more pronounced in LE and SD rats, a difference which in part may be related to strain differences in the formation of reactive oxygen species (Derdak et al., 2011). It is noteworthy that the specific tissues responsible for the development of the peripheral insulin resistance also differed between strains, with suppression of IMGU in fast-twitch muscle in ethanol-fed SD but not LE rats. The lack of an ethanol-induced decrease in IMGU by skeletal muscle has also been reported

in Wistar rats (Kang et al., 2007b, Wilkes and Nagy, 1996). Hence, strain differences in rats may at least partially account for often contradictory findings in the literature regarding the importance of skeletal muscle in mediating the whole-body insulin resistance to ethanol. As our experiments were performed in ~8-hour fasted rats and we have previously reported there was no difference in the blood ethanol concentration between SD and LE rats in the fed state (Derdak et al., 2011), it seems unlikely that a difference in the blood ethanol was causally related to strain differences in glucose metabolism.

Ethanol-fed SD rats also exhibited decreased IMGU in heart and adipose tissue. In this regard, all previous studies have examined the heart as a whole. Our data indicate for both basal and IMGU, the rank order (highest to lowest) for the various parts of the heart was: left ventricle > right ventricle > atria. Moreover, our data indicate the ethanol-induced decrease in myocardial IMGU was restricted to ventricular tissue. In contrast to striated muscle, ethanol only decreased IMGU in adipose tissue from LE rats. IMGU by adipose tissue has been reported in Wistar rats in response to chronic ethanol feeding (Kang et al., 2007b), but not with acute ethanol intoxication (Spolarics et al., 1994). The similar decrement in IMGU by fat in both SD and LE rats is consistent with the comparable efficacy of insulin to decrease the AUC for FFA and glycerol. The ability of ethanol to blunt the inhibitory action of insulin on adipocyte lipolysis is consistent with previous reports (Wilkes and Nagy, 1996, Yki-Jarvinen et al., 1988, Kang et al., 2007a).

Although ethanol could conceivably attenuate insulin action at any number of recognized control points in its metabolism (Wasserman et al., 2011), we initially examined whether ethanol impaired the translocation of GLUT4 to the cell membrane. We confirm previous reports that ethanol does not alter the total amount of GLUT4 in a whole muscle (Wilkes and Nagy, 1996) and demonstrate that ethanol decreases GLUT4 protein in the plasma membrane fraction of gastrocnemius in SD (but not LE) rats. Short-term ethanol exposure in vitro can also acutely decrease insulin-stimulated GLUT4 translocation in myotubes (Yu et al., 2000). The recruitment of GLUT4 in skeletal muscle is dependent upon the phosphorylation of AS160 and its upstream kinase AKT (Thong et al., 2007), and chronic ethanol feeding also prevented insulin-stimulated AKT and AS160 phosphorylation in muscle from SD but not LE rats.

We posit the ethanol-induced increase in TNF α and/or IL-6 in skeletal muscle in the basal state and their continued elevation under hyperinsulinemic conditions increased phosphorylation of JNK and the subsequent phosphorylation of IRS-1 at S307. While these endpoints have been previously reported to be increased in ethanol-fed mice under basal conditions (Li et al., 2009), there are no data on whether such changes persist during a sustained period of hyperinsulinemia. Our current data and those of others (Clary et al., 2011, Korzick et al., 2013) indicate chronic ethanol feeding increases both TNF α and IL-6 in skeletal muscle. Of note, skeletal muscle insulin resistance was only observed in SD rats which exhibited a sustained elevation in both TNF α and IL-6 during basal and hyperinsulinemic conditions. Our hypothesis is supported by the ability of TNF α and other inflammatory cytokines to increased JNK phosphorylation as well as other stress-activated kinases (Hotamisligil, 2005). One downstream target protein of JNK is IRS-1 and elevations in TNF α may impair insulin action, at least in part, by JNK-mediated Ser-phosphorylation

of IRS-I (Aguirre et al., 2000). Our results show ethanol blunts the insulin-induced increase in AKT and AS160 phosphorylation in SD, but not LE, rats and are supportive of a defect in this putative signaling pathway. Collectively, our data are consistent with the ethanol-induced reduction in GLUT4 translocation observed in SD but not LE rats. It is noteworthy, that chronic ethanol consumption also increased TNF α and IL-6 in adipose tissue from both strains of rats, which was associated with impaired IMGU in fat from both SD and LE rats. These data are comparable to those demonstrating ethanol decreases GLUT4 fusion or translocation in adipose tissue (Wilkes et al., 1996, Poirier et al., 2001). In addition, inflammatory and catabolic stimuli can also enhance Ser-phosphorylation of IRS-1 via up-regulation of S6K1 (Zhang et al., 2008). However, this pathway does not appear operational under the present conditions as S6K1 phosphorylation in striated muscle was not altered by ethanol consumption or changed by insulin stimulation in either rat strain. The inability of other anabolic stimuli (i.e., insulin-like growth factor-I) to fully activate S6K1 in muscle and heart has been reported in response to acute ethanol intoxication (Lang et al., 2003, Kumar et al., 2002).

In summary, our data indicate chronic ethanol consumption impairs IMGU in a strain- and tissue-specific manner. While ethanol impairs IMGU by adipose tissue in both SD and LE rats, it decreased insulin action in fast-twitch skeletal and cardiac muscle only in SD rats. As a result, the ethanol-induced whole-body insulin resistance is more severe in SD compared to LE rats. Moreover, strain comparisons suggest the ethanol-induced insulin resistance in muscle may be mediated by TNF α and/or IL-6-induced activation of JNK which inhibits the AKT-AS160-GLUT4 pathway. Finally, these data demonstrate the potential importance of the rat strain in ethanol research and advance our understanding of the cellular mechanism by which chronic ethanol produces peripheral insulin resistance.

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Figure 1.

Effect of ethanol feeding on glucose and insulin concentrations and whole-body glucose disposal in Sprague-Dawley and Long-Evans rats. The plasma glucose or insulin concentrations were determined under basal conditions (i.e., time 0) or during the final hour of the euglycemic hyperinsulinemic clamp for control-fed (C) or ethanol-fed (E) Sprague-Dawley or Long-Evans rats. Quantitation of whole-body glucose disposal under basal conditions and during the last hour of the clamp (+ insulin) in control-fed (C) or ethanol-fed (E) rats. Values are means $\pm$ SEM; $n = 9-12$ per group. Where absent, standard error bars are too small to visualize. For all bar graphs, values having a different superscript letter (a versus b versus c) are statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 2.

Effect of ethanol feeding on whole-body insulin-mediated glucose disposal and the ability of insulin to suppress hepatic glucose production (HGP) determined during the euglycemic hyperinsulinemic clamp. Values are means $\pm$ SEM; $n = 9-12$ per group. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 3.

Rate of in vivo-determined glucose uptake (Rg) by skeletal and cardiac muscle in Sprague-Dawley and Long-Evans rats. Glucose Rg was determined in vivo using ^{14}C -2-deoxyglucose (DG) during either the basal period or during the last 40 min of the euglycemic hyperinsulinemic clamp. Values are means $\pm$ SEM; $n = 9-12$ per group. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 4.

Rate of in vivo-determined glucose uptake (Rg) by adipose tissue in Sprague-Dawley and Long-Evans rats. Glucose Rg was determined in vivo using ^{14}C -2DG during either the basal period or during the last 40 min of the euglycemic hyperinsulinemic clamp. Values are means $\pm$ SEM; $n = 9$ -12 per group. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 5.

Insulin-induced decrease in plasma free fatty acids (FFA) in control and ethanol-fed Sprague-Dawley and Long-Evans rats. Time 0, prior to the start of the euglycemic clamp. Values are means $\pm$ SEM; $n = 9-12$ per group. * $P < 0.05$, compared to time-matched value from control-fed rats. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 6.

Effect of ethanol feeding on insulin signaling in gastrocnemius from Sprague-Dawley and Long-Evans rats under basal and hyperinsulinemic conditions. Values are means $\pm$ SEM; $n = 9-12$ per group. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 7. Effect of ethanol feeding on insulin signaling in heart from Sprague-Dawley and Long-Evans rats under basal and hyperinsulinemic conditions. Values are means $\pm$ SEM; $n = 9-12$ per group. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 8.

Effect of ethanol feeding on S6K1 phosphorylation (T389) in gastrocnemius from Sprague-Dawley and Long-Evans rats under basal and hyperinsulinemic conditions. Values are means $\pm$ SEM; $n = 9-12$ per group. There were no significant differences between values in either rat strain.

Figure 9.

Effect of ethanol feeding on GLUT4 translocation in gastrocnemius in Sprague-Dawley and Long-Evans rats. *Panel A*, markers for fractionation of muscle where Na^+/K^+ -ATPase and GAPDH were used as plasma membrane (PM) and cytosolic markers, respectively. *Panel B*, representative Western blots of GLUT4 and GLUT1 protein in PM and total cell lysate under basal conditions and following the euglycemic hyperinsulinemic clamp (+insulin) in control-fed (C) and ethanol-fed (E) rats. *Panel C*, quantitation of Western blots for GLUT4 protein in PM where the value from the control-fed basal condition was set at 1.0 arbitrary units (AU). There were no significant differences detected for GLUT1 protein between groups (data not shown). Values are means $\pm$ SEM; $n = 5-7$ per group. Values with different superscript letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Figure 10.

Tissue TNF α and IL-6 mRNA content in control and ethanol-fed Sprague-Dawley and Long-Evans rats under basal conditions and at the conclusion of the euglycemic hyperinsulinemic clamp. Values are means $\pm$ SEM; $n = 9-12$ per group. Values with different superscripted letters (a,b,c) were statistically different ($P < 0.05$); values with the same letter are not significantly different.

Table 1Body weight and composition by ^1H -NMR and food consumption in control and ethanol-fed rats

	Sprague-Dawley		Long-Evans	
	Control	Ethanol	Control	Ethanol
Body Weight, g				
Starting	161 $\pm$ 5	163 $\pm$ 6	159 $\pm$ 6	160 $\pm$ 6
Final	476 $\pm$ 12 ^a	393 $\pm$ 7 ^b	464 $\pm$ 15 ^a	412 $\pm$ 17 ^b
Change	315 $\pm$ 7 ^a	229 $\pm$ 8 ^b	305 $\pm$ 9 ^a	252 $\pm$ 7 ^c
FFM, g	288 $\pm$ 7 ^a	226 $\pm$ 7 ^b	271 $\pm$ 10 ^a	236 $\pm$ 10 ^b
Fat, g	109 $\pm$ 2	99 $\pm$ 2	113 $\pm$ 7	103 $\pm$ 4
Food Consumption, ml/day/100 g BW	22.0 $\pm$ 0.8	22.9 $\pm$ 1.1	20.4 $\pm$ 0.5	21.3 $\pm$ 1.2

Values are means $\pm$ SEM; n = 9-12 rats per group. FFM = fat free mass (e.g., lean body mass). Values with different superscripts for each row are statistically different ($P < 0.05$). Food consumption was calculated as an average during the final 4 weeks of the ethanol feeding protocol. Food consumption during the first 4 weeks of ethanol feeding were higher for all groups compared to the final 4 weeks, but did not differ between strains (data not shown). Values which share a common letter are not statistically different. Data analyzed by 2-way ANOVA with SNK post hoc analysis.

Table 2

Tissue glucose uptake assessed under basal and insulin-stimulated conditions in control and ethanol-fed rats

	Sprague-Dawley				Long-Evans			
	Basal		+ Insulin		Basal		+ Insulin	
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
Liver	36 ± 5 ^a	32 ± 3 ^a	95 ± 11 ^b	82 ± 13 ^b	41 ± 5 ^a	38 ± 5 ^a	83 ± 10 ^b	72 ± 9 ^b
Ileum	246 ± 22	237 ± 38	260 ± 24	245 ± 39	211 ± 31	245 ± 27	241 ± 10	259 ± 26
Spleen	230 ± 18 ^a	218 ± 20 ^a	337 ± 24 ^b	312 ± 33 ^b	251 ± 31 ^a	232 ± 18 ^a	447 ± 44 ^b	429 ± 68 ^b
Lung	145 ± 18 ^a	129 ± 24 ^a	276 ± 23 ^b	256 ± 34 ^b	161 ± 21 ^a	157 ± 14 ^a	301 ± 38 ^b	287 ± 34 ^b
Kidney	98 ± 7 ^a	102 ± 11 ^a	174 ± 20 ^b	169 ± 23 ^b	69 ± 7 ^a	76 ± 11 ^a	189 ± 23 ^b	164 ± 27 ^b
Brain	543 ± 32	555 ± 50	526 ± 41	558 ± 61	601 ± 59	574 ± 26	573 ± 38	579 ± 47

Values are means ± SEM; n = 9-12 rats per group. Tissue glucose uptake was determined in vivo using ¹⁴C-2-deoxyglucose in separate groups of rats either under basal conditions or during the last 40 min of the euglycemic hyperinsulinemic clamp. Units = nmoles glucose/min/g wet weight tissue. Data were analyzed by 3-way (ethanol x insulin x strain) ANOVA and values with different superscripts for each row are statistically different ($P < 0.05$).

Table 3

Echocardiographic endpoints in control and ethanol-fed rats

	Sprague-Dawley		Long-Evans	
	Control	Ethanol	Control	Ethanol
MABP, <i>mmHg</i>	118 ± 3	117 ± 4	121 ± 5	115 ± 4
HR, <i>bpm</i>	365 ± 21	330 ± 19	381 ± 23	374 ± 28
LVEDD, <i>mm</i>	7.2 ± 0.4	6.8 ± 0.3	7.5 ± 0.3	7.1 ± 0.4
LVEDS, <i>mm</i>	3.8 ± 0.2	3.6 ± 0.3	3.9 ± 0.2	3.5 ± 0.2
FS, %	46 ± 3	48 ± 5	48 ± 4	50 ± 4
EF, %	85 ± 7	85 ± 9	87 ± 6	88 ± 6

Values are means ± SEM; n = 12-16 rats per group. MABP, mean arterial blood pressure; HR, heart rate; LVEDD, left ventricular (LV) end diastolic diameter; LVEDS, LV end systolic diameter; FS, fractional shortening; EF, ejection fraction. Data were analyzed by 2-way (ethanol x strain) ANOVA which showed no ethanol and/or strain interaction.