Alcohol-Related Diols Cause Acute Insulin Resistance In Vivo

Dan Xu, Amardeep S. Dhillon, Anke Abelmann, Kevin Croft, Timothy J. Peters, and T. Norman Palmer

Epidemiological studies suggest that alcohol consumption is an independent risk factor for the development of non–insulin-dependent diabetes mellitus (NIDDM). Alcoholism is known to be associated with increased plasma levels of two novel diols, 2,3-butanediol and 1,2-propanediol, metabolites known to impair insulin action in isolated adipocytes. This study examines whether 2,3-butanediol and 1,2-propanediol have the capacity to impair insulin action acutely in vivo in the rat. Using the euglycemic-hyperinsulinemic clamp, it is shown that the two diols reduce whole-body glucose utilization (by ~30%), with the onset of insulin resistance in vivo occurring at plasma concentrations of 2,3-butanediol (33 μmol/L) at least one order of magnitude ($P < .001$) lower than 1,2-propanediol (432 μmol/L). Tracer methodologies using [U-^{14}C]glucose and 2-deoxy[1-^{3}H]glucose indicate that the reduction in whole-body glucose utilization is accompanied by a reduction in glucose uptake and glycogen synthesis in the skeletal muscle and heart. The association between elevated plasma diol levels and insulin resistance demonstrated in this report raises the question of whether there is a link between the high plasma diol levels in alcohol abusers and their increased susceptibility to NIDDM.

ETHANOL (ALCOHOL) use is associated with a range of abnormalities in carbohydrate metabolism. These effects of ethanol invariably depend on the nutritional state of the individual and on the duration of ethanol consumption. In normal fasted individuals,[1-2] elderly men,[3] and rats,[4-5] ethanol causes acute and profound insulin resistance. This insulin resistance, at least in the rat, has been shown to involve reductions in glucose utilization and glycogen synthesis, primarily in skeletal muscle.[4-5] In contrast to the acute effects of ethanol, the impact of chronic ethanol consumption on carbohydrate metabolism is complex, partly due to the complicating effects of alcohol-related diseases such as pancreatitis, fatty liver, cirrhosis, and myopathy in alcoholics.[6]

2,3-Butanediol and 1,2-propanediol are two novel metabolites for which serum levels have been reported to be elevated in 70% of alcoholics with measurable ethanol levels[7-10] and in 30% of abstinent patients with alcoholic cirrhosis,[11-12] with reported serum levels of 5 to 154 μmol/L and 5 to 2,200 μmol/L for 2,3-butanediol and 1,2-propanediol, respectively. An elevation in serum diols in alcoholics is observed in acute response to consumption of distilled spirits, which, unlike beer[7] and wine,[13-14] do not contain measurable levels of the diols.[7] This would suggest that in alcoholics, at least, the diols are synthesized from endogenous precursors via pathways that have yet to be fully elucidated.[12,15] Elevated levels of 1,2-propanediol have also been reported in humans during starvation and diabetic ketoacidosis, most probably as a metabolite of acetone.[16-17]

Insulin resistance is widely recognized to be associated with a broad spectrum of common metabolic disorders, including non–insulin-dependent diabetes mellitus (NIDDM), obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Epidemiological studies suggest that alcohol consumption is an independent risk factor for the development of NIDDM,[18-19] alcoholics being at higher risk for NIDDM compared with nonalcoholics.[19-20] Moreover, it is known that long-term ethanol feeding in the rat is associated with insulin resistance in isolated adipocytes.[21] Lomeo et al[22] have reported that 2,3-butanediol and 1,2-propanediol inhibit both basal and insulin-stimulated lipogenesis and glucose oxidation in isolated rat adipocytes, with the inhibition occurring at diol concentrations (0.5 to 50 μmol/L) comparable to the plasma levels reported in alcoholics with measurable ethanol levels[7-10] and in abstinent patients with alcoholic cirrhosis. Others[23] have failed to observe impaired insulin action in adipocytes in vitro from rats administered 1,2-propanediol long-term, but it remains to be established whether insulin action in vivo is affected. Thus, the aim of the present study was to establish whether the acute inhibitory effects on isolated adipocytes are indicative of the capacity of 2,3-butanediol and 1,2-propanediol to impair insulin action in vivo and lead to insulin resistance. Long-term ethanol feeding in the rat leads not only to insulin resistance in isolated adipocytes[21] but also to increases in plasma 1,2-propanediol concentrations,[24] suggesting a possible link between abnormal diol levels in alcoholism and the genesis of insulin resistance. In this study, acute effects of the diols on whole-body glucose utilization were assessed using the euglycemic-hyperinsulinemic clamp technique, whereas effects on insulin-stimulated glucose uptake and glycogen synthesis in individual tissues were examined using tracer methodologies.[5]

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MATERIALS AND METHODS

Treatment of Animals

Adult male Wistar rats (body weight [BW], 300 to 350 g) maintained on a 12-hour light-dark cycle (light from 7:30 AM) at 22°C and with free access to water and a standard laboratory chow diet supplied by Glen Forest Stockfeeders (Glen Forest, Western, Australia: 55% digestible carbohydrate, 19% protein, 5% lipid, and 21% nondigestible residue by weight) were starved for 24 hours before commencement of the experiments.

In preparation for the euglycemic-hyperinsulinemic insulin clamp, Silastic catheters (Dow Corning, Midland, MI) were implanted into the left carotid artery and right jugular vein and exteriorized through the

back of the neck. Surgical anesthesia was attained using a combination of ketamine hydrochloride (90 mg/kg intraperitoneally [IP]) and xylazine (60 mg/kg IP) in conjunction with atropine sulfate (0.6 mg/kg intramuscularly [IM]) to prevent airway obstruction during surgery. Catheter patency was ensured by daily flushing with 0.9% (wt/vol) saline and by filling the catheters with heparin (50 IU heparin/mL in 0.9% saline). To reduce the possibility of infection, each animal was administered gentamycin (40 mg/kg intravenously [IV]) and Duplocillin (10 mg/kg IM; Intervet, Lane Cove, New South Wales, Australia) immediately following surgery. All animals were allowed to recover for at least 3 days before study.[25]

Euglycemic-Hyperinsulinemic Clamp and Experimental Design

Whole-body insulin sensitivity was assessed using the euglycemic-hyperinsulinemic clamp technique.[25] Briefly, before commencement of the clamp, animals were fitted with long sampling cannulae and placed in specially designed metabolic cages, which they were then allowed to acclimatize to for at least 30 minutes before experimentation.[25] Thereafter, human neutral insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused IV at a rate of 5 mU/kg/min using a syringe pump. Glucose (25% wt/vol) was delivered IV at a variable rate during insulin infusion to maintain euglycemia; blood glucose was determined in 35-μL samples at 5 to 10-minute intervals using a YSI Model 2700 Select Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH). A steady-state glucose infusion rate was achieved in most cases within 45 minutes of commencement of the insulin and glucose infusion. The glucose infusion rate (GIR) required to maintain euglycemia is a recognized measure of in vivo insulin sensitivity.

In our initial study (Fig 1), at 60 minutes after initiation of insulin infusion, rats received a bolus injection of either 2,3-butanediol (0.1 μmol/100 g BW IV over 2 minutes or 1,2-propanediol (10 μmol/100 g BW, IV over 2 minutes; both diols obtained from Sigma Chemical, St Louis, MO). Plasma samples were taken immediately before and at intervals after injection of the diol. Preliminary experiments showed that bolus injection of the two diols rapidly increased plasma butanediol and propanediol concentrations to steady-state values of approximately 55 and 700 μmol/L, respectively. Control animals received an isovolumic injection of normal saline instead of the diol. At 105 minutes after initiation of the insulin infusion, animals were administered an IV bolus of [U-^{14}C]glucose (30 μCi) and 2-deoxy[1-^{3}H]glucose (50 μCi) (Amersham, Poole, UK). The plasma tracer radioactivity (dpm/100 μL) was estimated from plasma samples (150 to 200 μL) obtained at intervals (2, 5, 10, 15, 20, 30, and 45 minutes) thereafter. At the end of the study (150 minutes), animals received IV sodium pentobarbitone (25 to 30 mg), which resulted in rapid surgical anesthesia during which the individual leg muscles (extensor digitorum longus and red and white gastrocnemius) and other tissues (liver, heart, diaphragm, brain, and brown adipose tissue) were removed and rapidly frozen in aluminium clamps precooled in liquid nitrogen. Arterial blood samples were also taken for determination of plasma radioactivity and insulin levels.

In a separate study, the dose-dependency of the effects of butanediol and propanediol on the GIR was investigated. In this protocol, at 60 minutes after initiation of the insulin infusion, animals received increasing doses of either butanediol (0.0001, 0.01, 0.1, 1, and 10 μmol/100 g BW, IV over 2 minutes) or propanediol (1, 5, 10, and 30 μmol/100 g BW IV over 2 minutes). Plasma samples were taken both at 10 minutes after administration of each dose and immediately before administration of the next dose. The interval between the two doses was approximately 30 minutes and was dependent on achievement of a steady-state GIR over 20 minutes.

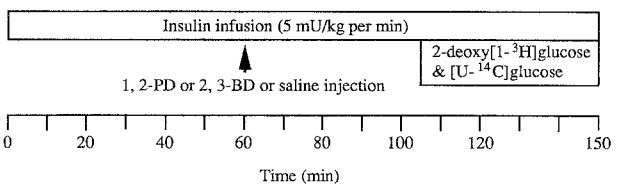

Fig 1. Protocol used to study the acute effects of 1,2-propanediol or 2,3-butanediol on insulin-stimulated glucose uptake and glycogen synthesis in individual tissues of the rat.

Analytical Procedures

The glucose metabolic index (Rg′), used as an estimate of the glucose metabolic rate, was calculated by the equation,

$$R_g' = \frac{C_p \cdot C_{m^*}}{\int_{45}^{0} C_{p^*}(t)dt},$$

where C_p is the steady-state plasma glucose concentration in millimolars, C_m* is the accumulation of 2-deoxy[1-^{3}H]glucose 6-phosphate in the tissue at 45 minutes in dpm per milligram wet weight, C_p*(t) is the plasma 2-deoxy[1-^{3}H]glucose concentration in dpm per milliliter, and t = 0 represents the time when the radiolabel bolus is administered. The derivation of the equation and assumptions involved in the calculation have been described previously.[26]

The calculation for the incorporation of [U-^{14}C]glucose into glycogen is essentially as described for R_g', with the following modifications: C_m* represents tissue [U-^{14}C]glucose content in dpm per milligram wet weight at 45 minutes after radiolabel administration, and C_p*(t) represents plasma [U-^{14}C]glucose concentrations in dpm per milliliter. Although the method used for determination of glycogen synthesis[27] is well established in the literature, it does have the disadvantage of using dpm per milliliter as a specific measure of plasma [U-^{14}C]glucose radioactivity, whereas in fact much of the radioactivity will be associated with other metabolites.

Plasma samples used for determination of plasma tracer radioactivity were deproteinized in 2.5% (wt/vol) $ZnSO_4$ and saturated $Ba(OH)_2$, and an aliquot of the supernatant was taken for measurement of radioactivity by scintillation counting. Tissue uptake of 2-deoxy[1-^{3}H]glucose and its conversion to 2-deoxy[1-^{3}H]glucose 6-phosphate was determined as described by Ferré et al,[28] whereas [U-^{14}C]glucose incorporation into glycogen, a measure of glycogen synthesis, was measured according to the method of Chan and Exton.[29] Plasma insulin levels were determined by radioimmunoassay.[30]

Plasma levels of 2,3-butanediol and 1,2-propanediol were determined by a modification of the method of Needham et al,[31] with the analysis performed on a Hewlett-Packard (HP; San Francisco, CA) 5890 gas chromatograph coupled with a HP 5970 series mass-selective detector and a HP ChemStation using HP G1034C MS ChemStation software. Samples were injected on a DB-Wax column (15 m × 0.25 mm, 0.25 film thickness; J&W Scientific, Folsom, CA) using helium as the carrier gas with an inlet pressure of 30 kPa. Injections were made in a splitless mode for 0.75 minutes, followed by the split mode with a split flow of 30 mL/min and a septum purge of 4 mL/min. The injection temperature was 250°C, and the transfer line temperature was 250°C. The initial column temperature of 80°C was maintained for 3 minutes and then programed to increase at a rate of 10°C/min until 150°C, where it was kept for 2 minutes, for a total run time of 12 minutes. The mass spectrometer was operated in the electron-impact mode (70 eV). Mass chromatograms in the scan mode were recorded in the mass range 50 to 650 atomic mass units (AMU) at a scan rate of 1.1 to 1.3 scans/s. Selected ion monitoring was performed according to the protocols of the HP MS ChemStation software program. The detector was turned on 4 minutes into the program, and four characteristic ions (mass 45, 57, 58, and 71) were used for both diols. Peak identification was based on a relative retention time and total ion mass comparison with external

standards. In addition, a comparison to spectra installed in the HP MS ChemStation NBS Mass Spectral Data Library of compounds was possible, yielding in most cases a greater than 98% match. For quantitation of the diols in samples, calibration curves were established by measuring peak areas versus response. 1,3-Propanediol was used as the internal standard.

Statistics

Results are presented as the mean ± SEM. Statistical significance was assessed using either the Student's unpaired *t* test or one-factor ANOVA for repeated measures followed by the Scheffe F test.

RESULTS

Plasma Insulin, Blood Glucose, and GIR

Euglycemic-hyperinsulinemic clamp studies (Fig 1) were undertaken to establish the extent, if any, to which 2,3-butanediol and 1,2-propanediol affect whole-body insulin sensitivity. The rate of insulin infusion in the clamp studies was 5 mU/kg/min, which, in agreement with others,[5,31] resulted in plasma insulin concentrations at the termination of the clamp (~150 minutes) that did not differ significantly between controls (553 ± 76 pmol/L) and animals infused with either 2,3-butanediol (537 ± 69 pmol/L) or 1,2-propanediol (737 ± 85 pmol/L). Administration of 2,3-butanediol (0.1 μmol/100 g BW IV over 2 minutes) resulted in a rapid increase in plasma diol to levels (54.6 ± 4.5 μmol/L) that remained relatively constant for the duration of the study (Fig 2). 2,3-Butanediol administration significantly decreased the GIR required to maintain euglycemia by 14% ($P < .05$) at 30 minutes and by 22% ($P < .001$) at termination of the clamp (Fig 2), consistent with diminished whole-body insulin sensitivity. The blood glucose level remained relatively constant throughout the experiment (3.82 mmol/L; coefficient of variation [CV], 3.9%). Administration of 1,2-propanediol (10 μmol/100 g BW IV over 2 minutes), which resulted in mean plasma diol levels of 708 ± 40 μmol/L, produced a reduction (of 30%) in the GIR (from a mean value at 40 to 60 minutes of 21.8 ± 1.9 to 15.3 ± 1.3 mg/kg/min at termination of the clamp) that assumed statistical significance ($P < .05$) after 120 minutes (Fig 3). It is important to recognize that plasma insulin levels were only determined before and at termination of the clamp. Thus, it cannot be precluded that insulin levels changed initially in response to diol infusion but thereafter returned to levels similar to those in control (saline-infused) rats. However, this seems unlikely, given that the GIR decreased in response to diol administration to levels that remained essentially constant throughout the last phase (80 minutes) of the clamp.

Dose-Dependency of the Effects of Diols on GIR

Increasing the plasma level of 2,3-butanediol under the conditions of the euglycemic-hyperinsulinemic clamp by bolus administration of increasing amounts of the diol led to a pronounced decrease in GIR (Fig 4). At a plasma 2,3-butanediol concentration of 13 μmol/L, the GIR was unaltered, while at plasma levels more than 33 μmol/L, the GIR was reduced by an approximately constant amount (30%, $P < .001$). In 1,2-propanediol–treated animals, the GIR remained at control levels even at plasma concentrations up to 432 μmol/L. At higher concentrations (>771 μmol/L), the GIR was decreased by an approximately constant amount (by 36%, $P < .001$). Blood glucose levels were relatively constant irrespective of plasma 2,3-butanediol (3.9 ± 0.1 mmol/L, CV 9.5%) or 1,2-propanediol (3.6 ± 0.1 mmol/L, CV 12.1%) concentrations, whereas plasma insulin increased from basal values of 13 ± 1 to levels at the end of the clamp that did not differ significantly between animals administered 2,3-butanediol (720 ± 46 pmol/L, n = 4) or 1,2-propanediol (510 ± 69 pmol/L, n = 4).

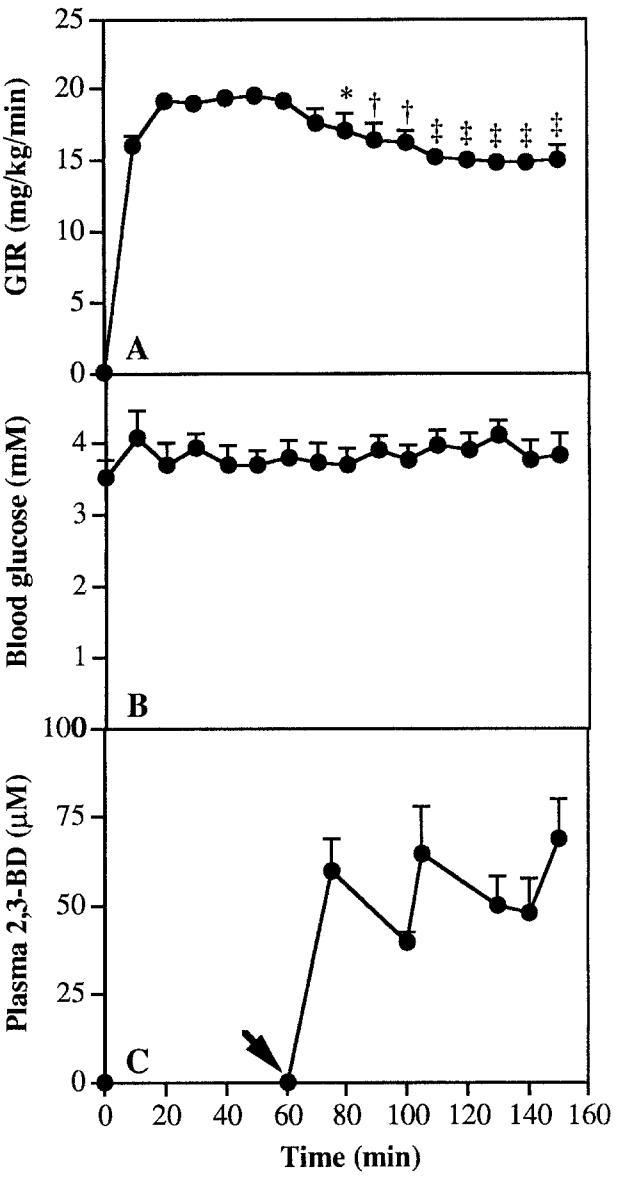

Fig 2. Acute effects of 2,3-butanediol on (A) GIR, (B) blood glucose level, and (C) plasma 2,3-butanediol level during the euglycemic-hyperinsulinemic clamp. Arrow indicates the time at which the diol was administered. Values for plasma 2,3-butanediol are derived from 2 separate clamp studies. Data are the mean ± SEM for 5 rats. Statistically significant effects of the diol on the GIR relative to the mean GIR over the 30-minute period before diol administration: *$P <$ .05, †$P <$ .01, and ‡$P <$ .001.

Glucose Disposal in Individual Tissues

The finding that the diols reduce whole-body insulin sensitivity poses the question of whether the diol-mediated decrease in

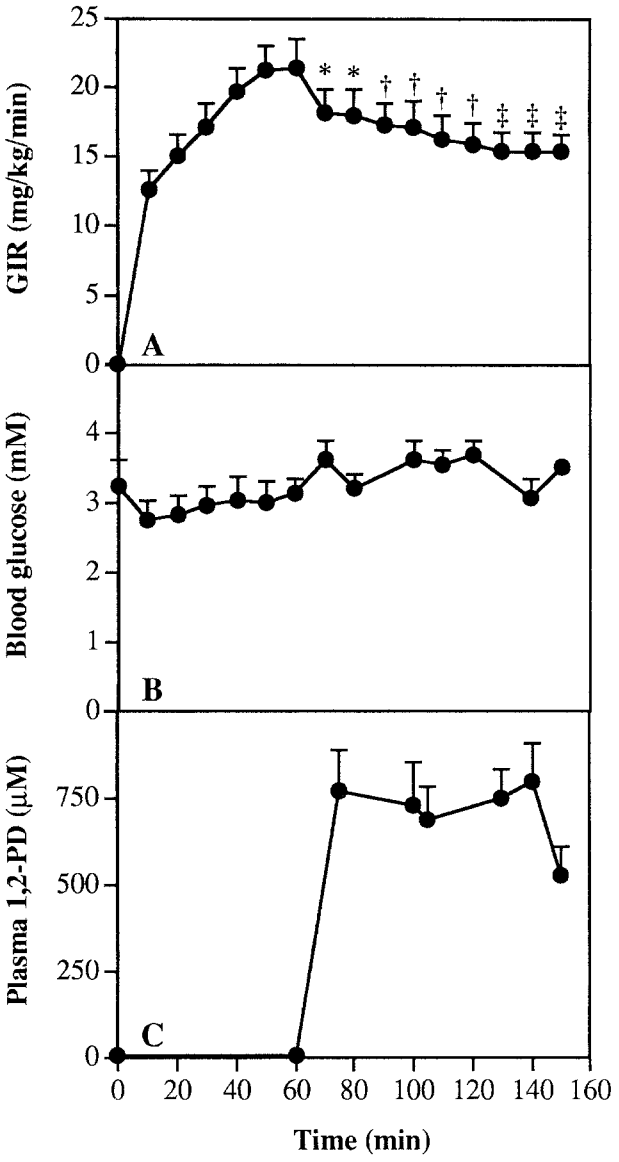

Fig 3. Acute effects of 1,2-propanediol on (A) GIR, (B) blood glucose level, and (C) plasma 1,2-propanediol level during the euglycemic-hyperinsulinemic clamp. Arrow indicates the time at which the diol was administered. Values for plasma 1,2-propanediol are derived from 2 separate clamp studies. Data are the mean ± SEM for 5 rats. Statistically significant effects of the diol relative to the mean GIR over the 30-minute period before diol administration: *$P < .05$, †$P < .01$, and ‡$P < .001$.

insulin sensitivity is accompanied by reduced glucose disposal in tissues, particularly skeletal muscle. The glucose metabolic index (R_g'), a measure of glucose uptake and phosphorylation in tissues, is calculated from tissue 2-deoxy[1-^{3}H]glucose 6-phosphate levels adjusted for plasma glucose and the rate of plasma 2-deoxy[1-^{3}H]glucose disappearance.[26] R_g' values obtained for the different tissues in the euglycemic-hyperinsulinemic state are in close agreement with published values[5,32,33] (Fig 5). The rates of glucose metabolism were higher ($P < .01$) in heart, oxidative skeletal muscle rich in slow-twitch oxidative type I muscle fibers (namely diaphragm and red gastrocnemius), and brown adipose tissue than in nonoxidative skeletal muscle containing a high proportion of fast-twitch glycolytic type II muscle fibers (namely extensor digitorum longus and white gastrocnemius) and brain. Administration of 2,3-butanediol (0.1 μmol/100 g BW IV over 2 minutes to yield plasma diol concentrations of 54.6 ± 4.5 μmol/L) resulted in a highly significant decrease in R_g' in diaphragm (by 60.7%), heart (by 76.1%), red gastrocnemius muscle (by 63.7%), and brown adipose tissue (by 79.5%). In contrast, R_g' values were unaffected by 2,3-butanediol in white gastrocnemius and extensor digitorum longus muscle and brain. Administration of 1,2-propanediol (10 μmol/100 g BW IV over 2 minutes to yield plasma levels of 708 ± 40 μmol/L) led to a significant reduction in R_g' in diaphragm (by 60.7%), heart (by 55.0%), red gastrocnemius muscle (by 55.9%), and brown adipose tissue (by 63.2%), but not in white gastrocnemius and extensor digitorum longus muscle and brain (Fig 5).

Rates of Glycogen Synthesis

A major fate of glucose under euglycemic-hyperinsulinemic conditions is storage as glycogen, posing the question of whether this process is affected by 2,3-butanediol and 1,2-propanediol. Glycogen synthesis, measured as the rate of incorporation of [U-^{14}C]glucose into glycogen, was highest in the diaphragm and red gastrocnemius muscles (Fig 6). 2,3-Butanediol administration (0.1 μmol/100 g BW IV over 2 minutes; plasma diol concentration, 54.6 ± 4.5 μmol/L) resulted in a significant impairment in glycogen synthesis in the diaphragm (by 67.4%) and red gastrocnemius (by 49.1%) muscles, whereas glycogen synthesis in the liver, heart, brain, and extensor digitorum longus and white gastrocnemius muscles was unaffected. Similarly, 1,2-propanediol administration (10 μmol/100 g BW IV over 2 minutes; plasma level, 708 ± 40 μmol/L) significantly reduced the rate of glycogen synthesis in

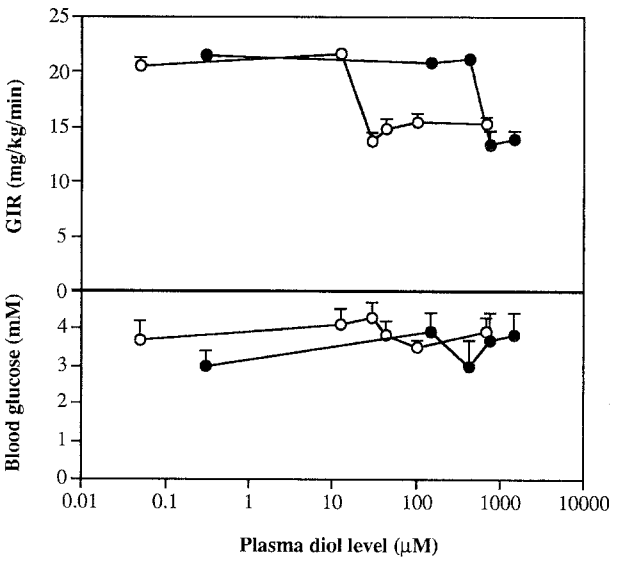

Fig 4. Relationship between plasma 2,3-butanediol (○) and 1,2-propanediol (●) concentration and the GIR under conditions of the euglycemic-hyperinsulinemic clamp. Mean blood glucose concentrations at each diol level are also shown. Data are the ± SEM for at least 4 rats.

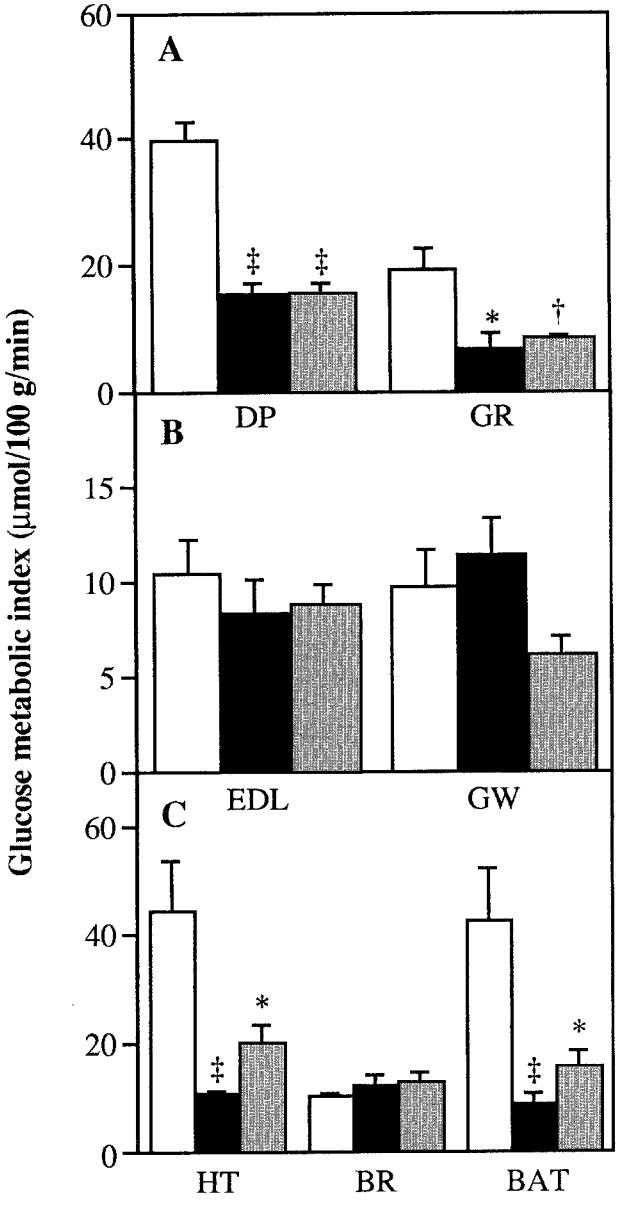

Fig 5. Acute effects of 2,3-butanediol (■) and 1,2-propanediol (▨) on glucose metabolic index (Rg') relative to controls (saline infusion, □) in (A) oxidative skeletal muscle (diaphragm, DP; red gastrocnemius, GR), (B) nonoxidative skeletal muscle (extensor digitorum longus, EDL; white gastrocnemius, GW), and (C) other tissues (heart, HT; brain, BR; brown adipose tissue, BAT). Data are the mean ± SEM for 5 rats. Statistically significant effects of diols compared with saline treatment: *$P < .05$, †$P < .01$, and ‡$P < .001$.

the red gastrocnemius (by 76.2%), diaphragm (by 52.5%), extensor digitorum longus (by 78.4%), and white gastrocnemius (by 74.5%) muscles, whereas glycogen synthesis in the liver, heart, and brain was unaffected (Fig 6).

DISCUSSION

Plasma levels of 2,3-butanediol and 1,2-propanediol are reported to be elevated in 70% of alcoholics with measurable ethanol levels[7-10] and in 30% of abstinent patients with alcoholic cirrhosis.[11-12] This study shows that these two diols cause an acute and profound insulin resistance in vivo in the rat, decreasing whole-body glucose utilization as indicated by a reduction in the GIR. Since glucose turnover was not measured in this study, the reduction in the GIR could result from either peripheral or hepatic insulin resistance or a combination of both. However, previous studies have shown that insulin resistance caused by other alcohols is primarily due to a reduction in peripheral glucose utilization.[5]

Skeletal muscle is recognized as the major site of insulin-

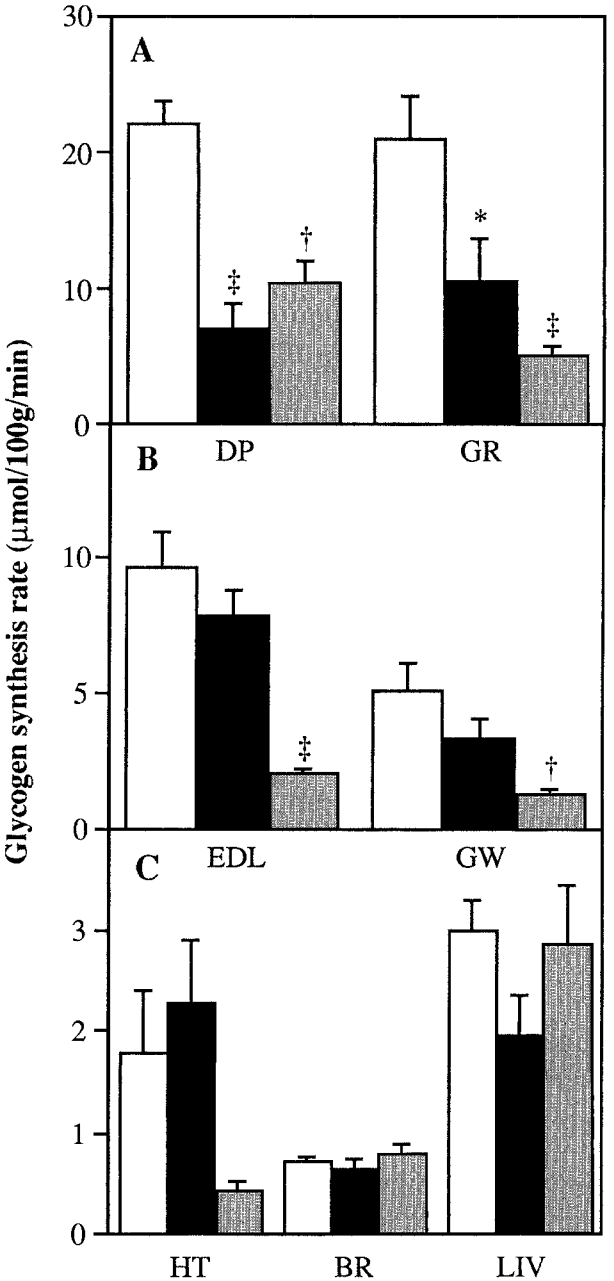

Fig 6. Acute effects of 2,3-butanediol (■) and 1,2-propanediol (▨) on the rate of glycogen synthesis relative to controls (saline infusion, □) in (A) oxidative skeletal muscle (diaphragm, DP; red gastrocnemius, GR), (B) nonoxidative skeletal muscle (extensor digitorum longus, EDL; white gastrocnemius, GW), and (C) other tissues (heart, HT; brain, BR; liver, LIV). Data are the mean ± SEM for 5 rats. Statistically significant effects of diols compared with saline treatment: *$P < .05$, †$P < .01$, and ‡$P < .001$.

stimulated glucose disposal in vivo,[34] and defects in skeletal muscle glucose utilization are found in a number of insulin-resistant states.[35] The present study identifies skeletal muscle as a major site of the diol-mediated defect in glucose disposal, with R_g' being significantly decreased by the two diols in red gastrocnemius and diaphragm muscles and heart (Fig 3). The extent of the decrease in R_g' in these insulin-sensitive tissues was so substantial (55% to 76%) that it alone could be the major factor responsible for the diol-mediated reduction in whole-body glucose disposal. R_g' was unaltered in nonoxidative extensor digitorum longus and white gastrocnemius muscles, consistent with nonoxidative muscles being less insulin-sensitive than oxidative muscles.[25]

In addition to muscle, the effects of 2,3-butanediol and 1,2-propanediol on glucose utilization by other tissues were also assessed. In the insulin-sensitive brown adipose tissue, both diols reduced R_g' significantly, possibly consistent with previous studies showing diol-mediated inhibition of glucose oxidation in isolated adipocytes.[22] The two diols had no effect on glucose uptake by the brain, and this may reflect its insulin-independent glucose transport system.[36]

During hyperinsulinemia, the storage of glucose as glycogen is the major route of nonoxidative glucose disposal in skeletal muscle. Although the rats used in our studies were fasted, glycogen synthesis accounted for up to 100% of glucose metabolism in certain tissues (extensor digitorum longus and red gastrocnemius muscles of saline-treated rats), due presumably to the effects of hyperinsulinemia coupled with those of exogenous glucose infusion. In the present study, 2,3-butanediol administration substantially decreased the rate of insulin-stimulated glycogen synthesis in red gastrocnemius and diaphragm muscles (49% to 67%), while 1,2-propanediol reduced glycogen synthesis in all skeletal muscles examined (by 50% to 79%). These findings would suggest that a defect(s) in the nonoxidative pathway of glucose disposal may be an important contributory factor to the diol-mediated reduction in R_g' in skeletal muscle. In contrast, both diols had no inhibitory effect on the rate of glycogen synthesis in the liver, brain, or heart. The low rates of hepatic glycogen synthesis probably reflect the fact that the liver takes up very little glucose in the euglycemic state.[37]

In isolated adipocytes,[22] 2,3-butanediol and 1,2-propanediol are reported to be equipotent in inhibiting (by 43% to 52% at a diol concentration of 50 μmol/L) insulin-stimulated triglyceride synthesis and glucose oxidation, although others[23] have been unable to confirm this inhibition of glucose metabolism by 1,2-propanediol. However, in vivo, the onset of insulin resistance occurs at plasma concentrations of 2,3-butanediol (33 μmol/L) at least one order of magnitude ($P < .001$) lower than with 1,2-propanediol (432 μmol/L), implying that 2,3-butanediol is a more potent agent. However, despite these differences, the two diols cause approximately the same degree of insulin resistance (28% to 36% reduction in GIR; Fig 4). This is roughly comparable to the inhibition (43% to 46%) of glucose oxidation by 2,3-butanediol and 1,2-propanediol in isolated adipocytes.[22] The metabolism rates of the two diols are low. For example, the reported plasma elimination half-life of 2,3-butanediol is 3.9 days in alcoholic subjects.[38] Consistent with this low rate of metabolism, plasma levels of 2,3-butanediol (Fig 3) and 1,2-propanediol (results not shown) remained constant over the 90-minute study period, despite being administered via a single injection. The observation that the two diols are equipotent in inhibiting metabolism in isolated adipocytes[22] but show marked differences in potency in vivo may reflect the fact that adipose tissue, unlike skeletal muscle, plays a relatively minor role in insulin-stimulated glucose disposal in vivo.

An important question relates to the mechanism of diol-mediated insulin resistance. In this context, it is of interest that ethanol has similar inhibitory effects as the diols on whole-body and individual tissue glucose metabolism,[4-5] albeit at higher blood levels (>2 mmol/L).[39] The ability of the diols and ethanol to impair insulin-stimulated glucose utilization in vivo may be related to their properties as an alcohol. Previous studies in this laboratory have shown that *tert*-butanol, an alcohol that is not a substrate for hepatic alcohol dehydrogenase, causes insulin resistance in vivo.[39] The reason(s) for differences in the potency of the different alcohol molecules is presently unknown. There are studies showing that ethanol can modify the function of membrane-associated proteins, as well as the components of intracellular signal transduction pathways.[40-41] Thus, it is possible that ethanol and other alcohols interfere with the microenvironment in the cell membrane and thereby impair important membrane-related processes such as glucose transport and insulin receptor function and signaling. Several recent studies suggest that alcohol consumption is an independent risk factor for development of NIDDM,[18-19] posing the question of how alcohol abuse can alter insulin sensitivity in vivo.

This study shows that 2,3-butanediol and 1,2-propanediol, two novel diol metabolites with increased serum levels in alcoholism, cause acute and profound insulin resistance in the rat. It remains to be established whether there is a link between an elevation in the plasma levels of these diols in human alcohol abusers and their susceptibility to NIDDM.

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