The inhibition of gluconeogenesis following alcohol in humans

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Siler, Scott Q., Richard A. Neese, Mark P. Christiansen, and Marc K. Hellerstein. The inhibition of gluconeogenesis following alcohol in humans. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E897–E907, 1998.—Accurate quantification of gluconeogenic flux following alcohol ingestion in overnight-fasted humans has yet to be reported. [2-13C1]glycerol, [U-13C6]glucose, [1-2H1]galactose, and acetaminophen were infused in normal men before and after the consumption of 48 g alcohol or a placebo to quantify gluconeogenesis, glycogenolysis, hepatic glucose production, and intrahepatic gluconeogenic precursor availability. Gluconeogenesis decreased 45% vs. the placebo (0.56 ± 0.05 to 0.44 ± 0.04 mg·kg−1·min−1) vs. placebo (0.44 ± 0.05 to 0.63 ± 0.09 mg·kg−1·min−1), respectively, P < 0.05) in the 5 h after alcohol ingestion, and total gluconeogenic flux was lower after alcohol compared with placebo. Glycogenolysis fell over time after both the alcohol and placebo cocktails, from 1.46–1.47 mg·kg−1·min−1 to 1.35 ± 0.17 mg·kg−1·min−1 (alcohol) and 1.26 ± 0.20 mg·kg−1·min−1, respectively (placebo, P < 0.05 vs. baseline). Hepatic glucose output decreased 12% after alcohol consumption, from 2.03 ± 0.21 to 1.79 ± 0.21 mg·kg−1·min−1 (P < 0.05 vs. baseline), but did not change following the placebo. Estimated intrahepatic gluconeogenic precursor availability decreased 61% following alcohol consumption (P < 0.05 vs. baseline) but was unchanged after the placebo (P < 0.05 between treatments). We conclude from these results that gluconeogenesis is inhibited after alcohol consumption in overnight-fasted men, with a somewhat larger decrease in availability of gluconeogenic precursors but a smaller effect on glucose production and no effect on plasma glucose concentrations. Thus inhibition of flux into the gluconeogenic precursor pool is compensated by changes in glycogenolysis, the fate of triose-phosphates, and peripheral tissue utilization of plasma glucose.

Inhibition of gluconeogenesis has also been implicated in the etiology of alcoholic hypoglycemia in the clinical setting. Freinkel et al. (9) presented indirect evidence for the inhibition of gluconeogenesis by EtOH when they were unable to counteract EtOH-induced hypoglycemia with infusion of glucagon in normal subjects who had fasted 24–48 h. Other groups have measured a decrease in hepatic glucose production as hypoglycemia developed during the infusion of EtOH after a 2- to 3-day fast (43, 53, 54). Because hepatic glycogen levels after a long fast are already substantially depleted (35), these findings suggest that the decrease in hepatic glucose production after EtOH infusion likely reflects a reduction in gluconeogenesis.

Direct quantitative evidence for the inhibition of gluconeogenesis in humans by EtOH has yet to be presented, however, with reliable techniques. New techniques for the measurement of total gluconeogenesis (i.e., from all precursors) with stable isotope tracers have recently been developed (15, 24, 25, 33, 48, 49), including mass isotopomer distribution analysis (MIDA; Refs. 15 and 33). Our goal in this study was to directly measure the effects of EtOH on gluconeogenesis, glycogenolysis, and other hepatic metabolic fluxes in normal, overnight-fasted humans by combining MIDA with probes of intrahepatic metabolism (11, 12, 29) and other isotopic techniques (13, 16, 15). Portions of the material presented here have been previously reported in abstract form (45).

METHODS

Materials. [2-13C1]glycerol, [U-13C6]glucose, and [1-2H1]galactose were purchased from CIL (Andover, MA) and/or Isotec (Miamisburg, OH). Isotopic purity was >98% for all tracers used. Acetaminophen was purchased from Mallinkrodt (Phillipsburg, NJ).

Subject characteristics. Volunteers were recruited by advertisement and gave written informed consent before enrolling in the study. All protocols were approved by the University of California at San Francisco Committee on Human Research and the University of California at Berkeley Committee for the Protection of Human Subjects. All five subjects were males and moderate consumers of EtOH (<120 g/wk). Subject characteristics are listed in Table 1. None had any history of alcoholism, and all the subjects had normal liver enzyme levels in blood. All subjects but one had normal serum lipid levels; fasting serum triglyceride levels were elevated in one individual (409 mg/dl). The data generated from this subject were similar to the data from the other subjects and, thus, are included here. None of the subjects had history of any medical diseases, metabolic disorders, diabetes mellitus, or a family history of diabetes mellitus, and none were using medications.
with known metabolic effects. Body composition was measured with bioelectrical impedance analysis (model no. 1990B, Valhalla Scientific, San Diego, CA). Body fat and total body water were calculated according to the equations of the manufacturer.

Study design. Subjects were admitted twice to the General Clinical Research Center at San Francisco General Hospital for separate studies. During one admission, four alcoholic beverages containing 12 g EtOH (40% vodka, Absolut, Ahus, Sweden) mixed with sugar-free lemonade (Kraft General Foods, White Plains, NY) were administered, whereas during the other admission the sugar-free lemonade was given as a placebo. Each EtOH cocktail contained 84 kcal from EtOH and 4 kcal from the sugar-free lemonade, whereas the placebo cocktails contained 4 total kcal (as measured by bomb calorimetry). The order of the treatment was randomized, with the second admission following 1 wk after the first.

The infusion protocol is shown in Fig. 1. After an evening meal (40% total daily caloric requirement, 55:30:15, carbohydrate:fat:protein) at 1700, intravenous infusion of isotopes was begun at 0400. [U-13C6]glucose (0.02 mg · kg$^{-1}$ · min$^{-1}$) and [1-2H1]galactose (0.05 mg · kg$^{-1}$ · min$^{-1}$) were infused at a constant rate after a 1-h priming bolus at 0400. Acetaminophen was also infused at a constant rate (3.7 mg/min), starting at 0400. Acetaminophen was prepared for infusion by Dr. Lou Tomimatsu (University of California San Francisco School of Pharmacy). Acetaminophen (dense powder; 5 g; USP) was mixed into 100 ml final volume containing propylene glycol (40 ml), ethanol (10 ml), and 5% dextrose (as 100 ml USP). An aliquot of this solution was added to 0.45% saline and neutralized with NaOH. After centrifugation, the supernatant was injected onto a Waters 0.2 × 10 cm reverse-phase C18 resolve high-performance liquid chromatography column. The mobile phase was 2% acetonitrile, aldonitrile pentaacetate, and glycerol triacetate.

Glucose and glycerol were isolated from plasma by ion-exchange chromatography as described previously (16, 15, 33). Plasma was deproteinized with perchloric acid (1:2), desalted with 6 N KOH, and loaded with a water wash onto gravity-flow columns. One set of columns contained an anion-exchange resin (AG 1-X8, Bio-Rad, Hercules, CA) and the other contained a cation-exchange resin (AG 5OW-X8, Bio-Rad). The two columns were used in sequence, with the eluent collected completely and lyophilized. The samples were then divided and derivatized three ways: glucose pentaacetate, aldonitrile pentaacetate, and glycerol triacetate. Glucose pentaacetate and glycerol triacetate were prepared by combining 100 µl of 2:1 acetic anhydride:pyridine with the lyophilized sample at room temperature for 15 min. After drying under nitrogen gas, the samples were reconstituted in ethyl acetate for gas chromatography-mass spectrometry (GC-MS) analysis. The aldonitrile derivative was formed by combining the lyophilized sample with hydroxylamine in pyridine (2%) for 30 min at 100°C. After cooling, 100 µl of 2:1 acetic anhydride:pyridine was added to the solution and kept at room temperature for 15 min. The solution was then reconstituted in ethyl acetate after drying under nitrogen gas.

Acetaminophen-glucuronide (GlcUA) was isolated from urine and derivatized to either the saccharic acid or the methyl-tetraacetate derivative, as previously described (13, 15, 29, 31). Briefly, the urine was acidified to pH 1.0 with HCl and neutralized with NaOH. After centrifugation, the supernatant was injected onto a Waters 0.2 × 10 cm reverse-phase C18 resolve high-performance liquid chromatography column in a radial compression module system (Waters, Milford, MA) with a C18 precolumn. The mobile phase was 2% acetonitrile in water with 1 ml glacial acetic acid per liter. Absorbance was measured at 254 nm with a variable wavelength ultraviolet detector (160 Absorbance Detector, Beckman, San Ramon, California). See METHODS for details.

Table 1. Subject characteristics

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<th>Age, yr</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Body Fat, %</th>
<th>Total Body Water, liters</th>
<th>Triglycerides, mg/dl</th>
<th>Total Cholesterol, mg/dl</th>
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<td>25.5 ± 1.9</td>
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<td>56.3 ± 13.6</td>
<td>179 ± 134</td>
<td>177 ± 21</td>
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</table>

Values are means ± SD of 5 subjects. One subject had triglycerides >400 mg/dl but was included in the data set. BMI, body mass index. See METHODS for details.
were determined by comparison with a standard curve. The [1-2H1]galactose and [13C]glycerol) must be corrected to the galactose and GlcUA derivatives (which contain label from both acid derivatives were used for the measurement of gluconeogenesis (33); the methyl-GlcUA derivative was analyzed using an initial temperature of 100°C and rising to 180°C at a rate of 40°C/min then rising 4°C/min until 252°C, and rising to 280°C at 45°C/min. The methyl-GlcUA derivative was separated by using a starting temperature of 150°C and rising at a rate of 40°C/min to a final temperature of 270°C. The ions monitored were the 328 (M1-aldonitrile tetraacetate), 329 (M1), and 330 (M2). The methyl-GlcUA derivative was analyzed using an initial temperature of 75°C and rising 45°C/min to 160°C, 5°C/min to 171°C, and 40°C/min to a final temperature of 220°C. The M0 and M1 of 159 and 160, respectively, were collected. Calculations. The rates of appearance (Rf) of plasma glucose and hepatic UDP-glucose (via acetaminophen-GlcUA) were calculated by the dilution technique (13, Table 2), Rf UDP-glucose was calculated based on the assumption of a constant and complete entry of galactose into the UDP-glucose pool in the liver (13). Fractional gluconeogenesis in both plasma glucose and hepatic UDP-glucose (as measured in secreted acetaminophen-GlcUA) was calculated by MIDA (Table 2) as described in detail elsewhere (14, 33). With the use of combinatorial probabilities, the enrichment of the true gluconeogenic precursor (triose-phosphate) can be inferred from the ratio of the excess double-labeled to single-labeled species (EM2/EM1) of glucose. The precursor-product relationship can then be used to calculate the fractional contribution of gluconeogenesis to plasma glucose or hepatic UDP-glucose production.

The fractional contribution of plasma glyceraldehyde to plasma gluconeogenesis was calculated as the ratio of the calculated (by MIDA) triose-phosphate enrichment to the measured plasma glyceraldehyde enrichment. The absolute rate of contribution of plasma glyceraldehyde to plasma gluconeogenesis was calculated by multiplying the fractional contribution of plasma glyceraldehyde to plasma gluconeogenesis by the absolute rate of plasma gluconeogenesis (Table 2).

The fractional release of labeled hepatic UDP-glucose in plasma glucose (R) was calculated from the recovery of infused [1-2H1]galactose in plasma glucose (15), on the assumption that all galactose passes through hepatic UDP-glucose (Fig. 2, Ref. 13). The Mf enrichment of glucose was multiplied by Rf glucose and divided by the galactose infusion rate to calculate R (Table 2). The deuterium-influenced Mf enrich-
Table 2. Equations for calculated parameters

1. \( R_g \) glucose (mg·kg\(^{-1} \)·min\(^{-1} \)) = \( l_{glc}/M_6 \) glucose enrich - \( l_{glc} \)
2. \( R_g \) UDP-glucose (mg·kg\(^{-1} \)·min\(^{-1} \)) = \( l_{gal}/(1-2H_3)GUA \) enrich - \( l_{glc} \)
3. Fractional GNG = \( EM_1 \) glucose enrich/A\( \ddagger \) glucose enrich
4. Fractional UDP-GNG = \( EM_1 \) GUA enrich/A\( \ddagger \) GUA enrich
5. Fractional contribution of plasma glycerol to GNG = triose-P enrich/plasma glycerol enrich
6. Fraction of plasma glucose from UDP-glucose = \( R = \frac{1-3H_2}{1} \) glucose enrich/(1-3H\(_2\))GUA enrich
7. Fractional direct pathway = \( D = M_6 \) GUA enrich/M\(_6 \) glucose enrich
8. Absolute GNG (mg·kg\(^{-1} \)·min\(^{-1} \)) = \( R_g \) glucose × fractional GNG
9. Absolute glycogen to glucose flux (mg·kg\(^{-1} \)·min\(^{-1} \)) = \( 1 - \) fractional GNG × \( R_g \) glucose
10. Absolute UDP-GNG (mg·kg\(^{-1} \)·min\(^{-1} \)) = \( R_g \) UDP-glucose × fractional UDP-GNG
11. Corrected \( R_d(H) \) = \( D \) × \( R_g \) UDP-glucose × \( (1-R) \)
12. Total GNG pathway flux (mg·kg\(^{-1} \)·min\(^{-1} \)) = (absolute GNG) + [absolute UDP-GNG × \( (1-R) \)] - [fractional GNG × corrected \( R_d(H) \)]
13. GNG partitioning coefficient into plasma glucose = absolute GNG/(absolute GNG + absolute UDP-GNG)
14. Absolute contribution from plasma glycerol to GNG (mg·kg\(^{-1} \)·min\(^{-1} \)) = absolute GNG × fractional contribution of plasma glycerol to GNG

\( R_g \), rate of appearance or turnover/flux; \( R_d(H) \), rate of direct pathway hepatic glucose disposal; \( l_{glc} \) and \( l_{gal} \), infusion rates of glucose and galactose, respectively; \( M_6 \), M\(_6\) isotope of glucose; GUA, glucuronate; GNG, gluconeogenesis; EM\(_1\), excess M\(_1\) isotope; A\( \ddagger \), asymptotic value for EM\(_1\) as calculated by mass isotope distribution analysis (MIDA); enrich, enrichment; triose-P, triose-phosphates.

RESULTS

Plasma metabolite and hormone concentrations. Blood alcohol concentrations rose significantly from undetectable levels to peak values of \( 14.7 \pm 3.2 \) mM and decayed linearly thereafter (Fig. 3) in the EtOH protocol. Plasma glucose concentrations did not change significantly throughout either the EtOH or placebo studies. At 0800, 1000, 1200, and 1300, plasma glucose concentrations were \( 83.6 \pm 7.6 \), \( 80.7 \pm 4.9 \), \( 79.7 \pm 6.6 \), and \( 83.0 \pm 4.9 \) mM, respectively. The glycogenolytic contribution to plasma glucose was calculated as the ratio of the M\(_6\) enrichment in acetaminophen-GlcUA to the M\(_6\) enrichment in plasma glucose (Ref. 15, Table 2).

Absolute gluconeogenesis was calculated as the product of \( R_g \) glucose and fractional gluconeogenesis (Table 2). The glycogenolytic contribution to plasma glucose was calculated as the difference between \( R_g \) glucose and absolute gluconeogenesis (Table 2). Absolute UDP-glucose-gluconeogenesis was calculated as the product of fractional UDP-glucose-gluconeogenesis and \( R_g \) UDP-glucose. Total gluconeogenesis was calculated as the sum of absolute plasma gluconeogenesis and UDP-glucose-gluconeogenesis, after correcting for duplicate fluxes (i.e., gluconeogenic flux that passed through UDP-glucose, then cycled into plasma glucose or entered plasma glucose then UDP-glucose by the direct pathway; Table 2, Ref. 15). The partitioning coefficient for glucose 6-phosphate was calculated as the ratio of absolute plasma gluconeogenesis to the sum of plasma gluconeogenesis and UDP-glucose-gluconeogenesis (Table 2). A more detailed explanation of the above calculations is presented in Ref. 15.

Statistics. Multifactorial ANOVA with time and treatment as variables was performed for all parameters except blood alcohol concentration, for which time was the only variable. Tukey’s post hoc test was used to determine statistical significance (\( P < 0.05 \)).

Fig. 2. Key pathways of gluconeogenesis after EtOH. gly, glucose; gal, galactose; glyc-6P, glucose 6-phosphate; glyc-1P, glucose 1-phosphate; GUA, glucuronate; aceta, acetaminophen; AcHO, acetaldehyde; OAA, oxaloacetate; AcoA, acetyl-coenzyme A; pyr, pyruvate; PEP, phosphoenolpyruvate; triose-P, triose-phosphates; glyc-3P, glycerol 3-phosphate; glyc, glyceral. *Metabolic reaction that includes interconversion between NAD and NADH.
3.9 mg/dl, respectively, in the placebo phase and 84.2 ± 7.2, 82.3 ± 10.0, 78.7 ± 6.3, and 76.0 ± 5.5 mg/dl, respectively, in the EtOH phase of the study. Plasma glucagon and insulin values over time were similar (and not statistically different) in both the EtOH and placebo studies (Fig. 4).

Plasma glucose flux, gluconeogenesis, and glycogenolysis. In the EtOH treatment protocol (Fig. 5, Table 3), the $R_a$ of plasma glucose ($R_a$ glucose) fell over time from a value of 2.03 ± 0.21 mg·kg$^{-1}$·min$^{-1}$ to 1.79 ± 0.21 mg·kg$^{-1}$·min$^{-1}$ (statistically significant vs. pre-EtOH, $P < 0.001$). $R_a$ glucose did not change significantly over time in the placebo treatment protocol (1.91 ± 0.20 mg·kg$^{-1}$·min$^{-1}$ initially to 1.92 ± 0.22 mg·kg$^{-1}$·min$^{-1}$ at the end of the infusion). There were no statistically significant differences between the EtOH and placebo treatments for $R_a$ glucose.

The fractional gluconeogenic contribution to plasma glucose (Fig. 6A, Table 4) dropped significantly ($P < 0.001$) from an initial value of 28.0 ± 4.7% to 23.5 ± 1.7% after EtOH, and it rose from 23.2 ± 1.3% to 32.3 ± 4.3% after consumption of placebo. Significant differences were observed between treatments for several time points. Fractional gluconeogenesis reached relatively stable values after both EtOH and placebo treatments. Absolute plasma gluconeogenic fluxes changed in the same manner as fractional gluconeogenesis (Fig. 6B, Table 3). Initial rates of absolute gluconeogenesis were 0.56 ± 0.08 mg·kg$^{-1}$·min$^{-1}$ and 0.44 ± 0.05 mg·kg$^{-1}$·min$^{-1}$ in the EtOH and placebo treatments, respectively. After consumption of EtOH, the steady-state value was 0.44 ± 0.04 mg·kg$^{-1}$·min$^{-1}$, representing a statistically significant decrease from baseline of 21% ($P < 0.05$). In contrast, the absolute rate of plasma gluconeogenesis rose 42% ($P < 0.05$ vs. pre-EtOH) to 0.63 ± 0.09 mg·kg$^{-1}$·min$^{-1}$ in the placebo treatment. The response of absolute gluconeogenesis after the placebo and EtOH treatments was significantly different ($P < 0.001$).

The contribution from glycogen to plasma glucose flux dropped in both treatments over time from similar initial values of 1.47 ± 0.22 (EtOH) and 1.46 ± 0.16 (placebo) mg·kg$^{-1}$·min$^{-1}$ (Fig. 6C, Table 3). Glycogenolysis fell 8% after EtOH and 14% after placebo, to 1.35 ± 0.17 mg·kg$^{-1}$·min$^{-1}$ and 1.26 ± 0.20 at the end of each infusion, respectively. There were no statistically significant differences between treatments, although the placebo 1230 and 1300 time points were significantly lower ($P < 0.05$) than the pretreatment values.

Plasma glycerol enrichments. Plasma glycerol enrichments rose after EtOH and decreased after consumption of the placebo, reflecting a change in adipose
lipolysis (17). The pre-ETOH plasma glycerol enrichment was 49.4 ± 7.2% and rose to a steady-state value of 58.6 ± 10.1% after ETOH; these values corresponded to endogenous Ra glycerol values of 2.53 ± 0.73 µmol·kg⁻¹·min⁻¹ before ETOH and 1.85 ± 0.77 µmol·kg⁻¹·min⁻¹ after ETOH. The preplacebo plasma glycerol enrichment was 47.0 ± 8.3% and decreased to 38.2 ± 7.0% at the end of the infusion (endogenous Ra glycerol there was equal to 2.38 ± 1.27 µmol·kg⁻¹·min⁻¹ before placebo and 3.78 ± 1.95 µmol·kg⁻¹·min⁻¹ after placebo). These changes were not statistically significant. The contribution of plasma glycerol to gluconeogenesis was calculated to be 24 ± 2% before ETOH, 35 ± 5% after ETOH, 28 ± 5% before placebo, and 32 ± 7% after placebo. The absolute contribution of glycerol to gluconeogenesis was 0.14 ± 0.01 mg·kg⁻¹·min⁻¹ before ETOH, 0.15 ± 0.02 mg·kg⁻¹·min⁻¹ after ETOH, 0.12 ± 0.02 mg·kg⁻¹·min⁻¹ before placebo, and 0.20 ± 0.04 mg·kg⁻¹·min⁻¹ after placebo. These differences were not statistically significant.

Intrahepatic metabolite enrichments and fluxes. Intrahepatic triose-phosphate enrichments (p) responded differently to the two treatments. Values of p increased by 61% after ETOH, from a before ETOH enrichment of 0.121 ± 0.012 to 0.195 ± 0.012 at the end of the infusion (Fig. 6D, Table 3). In contrast, p did not change in the placebo treatment, maintaining a steady value of 0.127 ± 0.011. The differences between the two treatments were statistically significant (P < 0.001) for the time points from 900 through 1300, and the post-ETOH values were significantly different from the pre-ETOH values.

The fractional gluconeogenic contribution to UDP-glucose was significantly lower after ETOH (16.1 ± 1.6%) compared with after placebo (24.2 ± 7.1%). Ra UDP-glucose, representing the intrahepatic turnover of UDP-glucose, did not change significantly in either the ETOH or placebo studies (Table 3). Ra UDP-glucose was 0.99 ± 0.04 mg·kg⁻¹·min⁻¹ before and 1.09 ± 0.16 mg·kg⁻¹·min⁻¹ after ETOH and 0.95 ± 0.15 mg·kg⁻¹·min⁻¹ before and 1.04 ± 0.14 mg·kg⁻¹·min⁻¹ after placebo. The absolute rate of UDP gluconeogenesis (calculated as the fractional contribution from gluconeogenesis to UDP-glucose multiplied by Ra UDP-glucose) after ETOH was 0.17 ± 0.03 mg·kg⁻¹·min⁻¹ and after placebo was 0.25 ± 0.09 mg·kg⁻¹·min⁻¹ (Table 3).

The fractional recovery of labeled hepatic UDP-glucose in plasma glucose (Ra) did not change after ETOH (from 25 ± 3% to 27 ± 4%) or placebo (from 27 ± 1% to 25 ± 6%). Incorporation of [1-14C]galactose into plasma glucose was stable over time and reproducibly measured (Fig. 7). The direct plasma glucose contribution to UDP-glucose decreased significantly (P < 0.05) after ETOH but not after the placebo; the pre-ETOH value was 29 ± 5%, and the post-ETOH value was 18 ± 6%, whereas the pre- and postplacebo values were 22 ± 8% and 20 ± 9%, respectively. Total gluconeogenic flux (plasma gluconeogenesis + UDP-glucogenesis) was significantly higher after the placebo (0.75 ± 0.10 mg·kg⁻¹·min⁻¹) compared with after ETOH (0.45 ± 0.05 mg·kg⁻¹·min⁻¹, Table 4). The glucose-6-phosphate partitioning coefficient for gluconeogenic flux (10) was not different (75–80% of flux into plasma glucose) after either ETOH or placebo ingestion.

**DISCUSSION**

The consumption of ETOH (48 g) inhibited gluconeogenesis in normal, healthy, overnight-fasted men. Plasma gluconeogenic flux fell by 21% after ETOH instead of rising by 43% after placebo (a significantly different response between the two treatments); total gluconeogenic flux (plasma gluconeogenesis + UDP-glucogenesis) was also lower. The effect of ETOH on plasma gluconeogenesis (Fig. 6B, Table 3) can therefore be calculated to be a 45% inhibition (79%/143% of baseline values for ETOH/placebo treatments). The increase in triose-phosphate enrichment (61%, Fig. 6D and Table 4) was of a similar magnitude as that in the inhibition of gluconeogenesis. The borderline significant increase in glycogenolysis compared with placebo prevented a greater reduction in hepatic glucose output after ETOH.

In previous studies (5, 22, 23), gluconeogenesis was assessed by the infusion of radioactive precursors ([14C]lactate or [14C]alanine) with and without ETOH. In each of these studies, the specific activity of plasma lactate or alanine was compared with the specific activity of plasma glucose to estimate gluconeogenic
flux. Kreisberg et al. (22) observed a 66% reduction in the incorporation of labeled lactate into glucose. Similarly, the incorporation of [14C]alanine into glucose was inhibited 75% after EtOH (23). Clore and Blackard (5) also measured a 50% reduction in the incorporation of labeled alanine into glucose with administration of EtOH. Labeled lactate and glycerol have been similarly infused into non-insulin-dependent diabetics with and without EtOH (5, 40); a 71% and a 65% reduction in the incorporation of each into glucose was observed. There appears to be a disparity between the 45–50% reduction in gluconeogenesis after EtOH observed in our study and the 65–75% changes reported in several of these previous studies.

Measurement of label incorporation from a plasma precursor into plasma glucose is not a quantitative index of gluconeogenesis, however. Dilution occurs as the labeled metabolite moves from the plasma into the hepatocyte, particularly as the carbon skeleton enters the TCA cycle (Fig. 2; Refs. 18 and 21). The extent of this dilution is not constant, however, and changes with different physiological conditions (18, 21). Use of MIDA avoids this problem by measuring the enrichment of the intrahepatic triose-phosphates. Quantification of the enrichment of the triose-phosphate pool with MIDA not only enabled us to calculate fractional gluconeogenesis but also allowed us to observe changes in the availability of the intracellular precursors for gluconeogenesis (i.e., the metabolic flux into the precursor triose-phosphates). Whereas there was no change in triose-phosphate enrichment over time after the placebo, triose-phosphate enrichments increased consider-

![Figure 6](image_url)

**Table 4. Calculation of fractional GNG by MIDA:** comparison of EtOH vs. placebo

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<th>Subject</th>
<th>M1</th>
<th>M2</th>
<th>EM1</th>
<th>EM2</th>
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<td>0.0148</td>
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<tr>
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Values for posttreatment glycogenolysis are final values measured just before termination of infusion. Conditions and calculations are as described in text. P, precursor pool enrichment for GNG; MPE, molar percent excess. * Significant difference vs. pretreatment value; † significant difference between treatments.
of gluconeogenesis. The reduction in gluconeogenesis are presumed to reflect exclusively the rates reduced after a 2- to 3-day fast (35), rates of glucose 53). As hepatic glycogen levels are substantially re-

et al. (25, 22, 23, and 40). Previous observations may therefore gest inhibition of labeled metabolites entry into the triose-phosphate pool. Even so, if ethanol inhibits hepatic uptake of glycerol (27), the effect should be to reduce triose-phosphate enrichment; in-

Fig. 7. Incorporation of [1-2H]galactose into plasma glucose in individual subjects. Stability of calculated plasma M1 enrichments during placebo (A) and EtOH (B).}

ably after EtOH. The 61% increase in precursor enrichment likely represents at least a comparable decrease in triose-phosphate flux, that is, dilution by input from unlabeled precursors. There is some evidence to sug-

glucose after a 2- to 3-day fast in these previous studies (43, 53).

One potential concern is whether the administration of acetaminophen, with propylene glycol-ethanol for solubilization, might have affected the baseline results. The infusion rate of the propylene glycol-ethanol solution was 4 ml/h or 2 g/h. We have previously compared fractional gluconeogenesis in overnight-fasted normal humans, measured by MIDA with [2-13C]glycerol, in 21 subjects with and 17 subjects without intravenous acetaminophen-propylene glycol-ethanol (Neese, Siler, and Hellerstein, unpublished observations). The gluco-

clearly does not affect baseline measurements of gluconeogenesis.

It should be noted that the dilution of glucose tracer in the plasma glucose pool reflects whole body glucose production. Recent work (46) has supported the view that the kidney contributes to glucose production. Direct measurement of renal glucose output and gluco-

neogenesis after EtOH has not been reported. Because our measurement of gluconeogenesis, in principle, includes renal gluconeogenesis that may be present (33), it is therefore likely that both renal gluconeogenesis and glucose production are included in our results. The stability of the plasma glucose concentrations despite significantly lower $R_a$ glucose after EtOH consumption signifies a reduced metabolic clearance rate for blood glucose. This observation is consistent with previous reports (2, 44, 56) of impaired peripheral glucose re-

moval during euglycemic hyperinsulinemic clamp con-

ditions.

The fraction of plasma glucose from gluconeogenesis in these subjects as measured by MIDA was lower than some previous estimates with different techniques (25, 42), although consistent with some others. Previs et al. (38) reported that measurement of gluconeogenesis with [13C]glycerol and MIDA systematically underesti-

mated fractional gluconeogenesis. These authors did not isolate or measure triose-phosphate pools in the liver but inferred that their lower-than-expected values of gluconeogenesis would be compatible with an isotopic gradient of labeled glycerol across the hepatic lobule. It is unlikely that our values of gluconeogenesis are systematically underestimated, however, for se-

eral reasons.

First, several laboratories (not only our own) have reported appropriately high values of gluconeogenesis when MIDA with [2-13C]glycerol is used in the manner and at the infusion rates that we initially described (15, 33) and use here. Fractional gluconeogenesis reaches 90–95% in 48-h-fasted rats (16, 33, 37) and 85–90% in mice (51). In perfused livers from fasted rats, it is >90% (37); in Kenyan children with malaria it is up to 96% with a mean value of 75% (7); in neonatal humans, it is up to 88–100% of glucose production, with a mean value of 72% (47); in fasted adults it reaches 92% (15);
and in overnight-fasted humans (50) or fasted rats (32) it is not affected by adding $^{13}$C$_3$alanine to $^{13}$C$_1$glycerol, contrary to the prediction of the labeled-glycerol gradient model (38).

Second, the reported values of gluconeogenesis in the literature are extremely variable among individuals. Landau et al (25) reported values ranging between 33 and 69% based on $^2$H$_2$O incorporation; Rothman et al. (42) reported a range between 46 and 81% with NMR spectroscopy. Prolongation of fasting from 14 to 22 h increased the gluconeogenic contribution from 47 to 67% (25) by the $^2$H$_2$O method. Thus the composition or energy sufficiency of recent or chronic dietary intake, duration of fasting, and exercise habits could clearly have a substantial impact on gluconeogenesis. Comparison between studies should therefore be made with caution, and it is premature to assume what the fractional gluconeogenic values should have been.

Finally, some variations in the estimates of fractional gluconeogenesis were obtained with the use of [U-$^{13}$C$_3$]- instead of [2-$^{13}$C$_1$]glycerol (38). It is possible that very low enrichments of (M$_x$4) species of glucose could lead to some technical difficulties in their accurate measurement. Indeed it appears that the lowest estimates of fractional gluconeogenesis were obtained with uniformly labeled substrates. In perfused livers, however, estimates with either [U-$^{13}$C$_3$]glycerol (38) or [2-$^{13}$C$_1$]glycerol (37, 38) yielded similar estimates for gluconeogenesis.

Accordingly, it seems unlikely that gluconeogenesis is systematically underestimated by MIDA from [2-$^{13}$C$_1$]glycerol. In any event, our directional comparisons within subjects (before vs. after interventions) would not be affected even if systematic underestimation were present here.

It appears from the results of this study that the partial preservation of gluconeogenesis is achieved at the expense of other fates of triose-phosphate flux. Alternative metabolic fates of intrahepatic triose-phosphates include entry into the pentose-phosphate shunt, passing down the glycolytic pathway back to pyruvate, conversion to glycerol 3-phosphate, and UDP-gluconeogenesis. There is some evidence from work in hepatocytes for a decrease in pentose-phosphate shunt flux in the presence of EtOH (41). A decrease in the flux from triose-phosphate to pyruvate has not been directly measured by us or others. The pathway does contain a redox-sensitive metabolic reaction (conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate at glyceraldehyde-3-phosphate dehydrogenase) (Fig. 2). The decreased availability of NAD due to the oxidation of EtOH by alcohol dehydrogenase could reduce metabolite flux through this reaction as well as the rest of the pathway. There is also evidence for a directional flux from triose-phosphate to glyceraldehyde 3-phosphate under the influence of EtOH (41). Yu and Cronholm (57) have provided evidence for a conversion from the triose-phosphates to glyceraldehyde 3-phosphate in studies with rats, and elevated hepatic glyceraldehyde 3-phosphate concentrations with EtOH have been observed in other studies (4, 30, 34). Quantification of the flux from the triose-phosphates to glyceraldehyde 3-phosphate under the influence of EtOH has not yet been determined, however.

One of the possible fates of triose-phosphate flux is glycogen synthesis (the indirect pathway, Ref. 19). We were able to quantify the partitioning of the flow of newly synthesized glucose 6-phosphate (via gluconeogenesis) into either plasma glucose (plasma gluconeogenesis) or into glycogen (UDP-gluconeogenesis). The glucose 6-phosphate partitioning coefficient did not change over time in either the EtOH or placebo treatments in this study, and neither did the UDP-glucose flux; if there were a diversion of glucose 6-phosphate flow away from glycogen synthesis and toward plasma glucose, UDP-glucose flux would be expected to decrease with EtOH. These results suggest that there is no decrease in the flux of triose-phosphates toward UDP-gluconeogenesis.

It is unlikely that changes in catecholamines could explain our observation of stable values for $R_a$ glucose in these subjects after EtOH ingestion. When given in larger doses than were administered in this study, EtOH has been shown to initiate a response from the sympathetic nervous system (3). The decrease in lipolysis, by diversion of triose-phosphates from other kinetic fates and into gluconeogenic pathways, and by reduced peripheral tissue clearance of plasma glucose.

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REFERENCES


E906 GLUCONEOGENESIS AFTER ETHANOL


