De novo lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption[1–3]

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ABSTRACT

Background: Acute alcohol intake is associated with changes in plasma lipid concentrations and whole-body lipid balances in humans. The quantitative roles of hepatic de novo lipogenesis (DNL) and plasma acetate production in these changes have not been established, however.

Objective: We used stable-isotope mass spectrometric methods with indirect calorimetry to establish the metabolic basis of changes in whole-body lipid balances in healthy men after consumption of 24 g alcohol.

Design: Eight healthy subjects were studied and DNL (by mass-isotopomer distribution analysis), lipolysis (by dilution of $[1,2,3,4-^{13}C_4]$palmitate and $[^2H_5]$glycerol), conversion of alcohol to plasma acetate (by incorporation from $[1-^{13}C_1]$ethanol), and plasma acetate flux (by dilution of $[1-^{13}C_1]$acetate) were measured.

Results: The fractional contribution from DNL to VLDL-triacylglycerol palmitate rose after alcohol consumption from $2 \pm 1\%$ to $30 \pm 8\%$; nevertheless, the absolute rate of DNL (0.8 g/6 h) represented $<5\%$ of the ingested alcohol dose; $77 \pm 13\%$ of the alcohol cleared from plasma was converted directly to acetate entering plasma. Acetate flux increased 2.5-fold after alcohol consumption. Adipose release of nonesterified fatty acids into plasma decreased by 53% and whole-body lipid oxidation decreased by 73%.

Conclusions: We conclude that the consumption of 24 g alcohol activates the hepatic DNL pathway modestly, but acetate produced in the liver and released into plasma inhibits lipolysis, alters tissue fuel selection, and represents the major quantitative fate of ingested ethanol.   *Am J Clin Nutr* 1999;70:928–36.

KEY WORDS   Fatty acid synthesis, mass-isotopomer distribution analysis, lipolysis, hypertriglyceridemia, acetate, ethanol, stable isotopes, lipogenesis, men

INTRODUCTION

Alcohol (or ethanol) intake results in many changes in lipid metabolism. Alterations in whole-body fuel utilization (1, 2), lipolysis (3–6), plasma triacylglycerol concentrations (7–11), and hepatic triacylglycerol content (12–14) have all been reported in humans after the acute or chronic consumption of ethanol. The effects of ethanol on macronutrient balances and, ultimately, on body composition are of particular interest but are complex. Ethanol intake acutely reduces net whole-body lipid oxidation as measured by indirect calorimetry (1, 2), possibly because of the reduced availability of nonesterified fatty acids in the circulation associated with inhibition of lipolysis by ethanol (3–6) or because of altered fuel selection by tissues. It is also possible that the reduction in net lipid oxidation reflects stimulation of hepatic de novo lipogenesis (DNL) by ethanol, without a reduction in lipid oxidation by peripheral tissues, ie, ethanol might balance rather than reduce ongoing lipid oxidation by tissues. Although ethanol was shown to induce hepatic DNL in some animal studies (15, 16), other studies indicated no effect (17–20). Few studies have attempted to quantify DNL after ethanol consumption in humans (21–23). Moreover, these reports are confounded by methodologic constraints, such as inadequate access to the hepatic lipogenic intracellular precursor pool.

There also appears to be a disproportionate increase in energy expenditure associated with the consumption of ethanol compared with other macronutrients (24). Several possible explanations for this increase in energy expenditure have been offered, including induction and utilization of the microsomal ethanol oxidizing system (25, 26), compromised mitochondrial membrane integrity (27, 28), and substrate cycling (29). Another possible explanation is that ethanol fuels energetically costly biosynthetic pathways such as DNL (30).

The goal of the present study was to establish the metabolic basis for these effects of acute ethanol intake on whole-body lipid balances. In particular, we assessed the quantitative roles of DNL, lipolysis, changes in tissue utilization of lipids, and plasma acetate released from the liver after the consumption of

TABLE 1
Subject characteristics[1]

	Value
Age (y)	26.8 ± 0.5
Weight (kg)	74.0 ± 2.1
BMI (kg/m^2)	23.6 ± 0.5
Body fat (%)	13.4 ± 1.6
Total body water (L)	48.3 ± 1.6
Serum triacylglycerols (mmol/L)	1.20 ± 0.05
Serum total cholesterol (mmol/L)	3.55 ± 0.18

[1] $\bar{x} \pm$ SEM.

24 g ethanol. Portions of this work were presented previously in abstract form (31, 32).

SUBJECTS AND METHODS

Subject characteristics

Volunteers were recruited by advertisement and gave 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. Because of sex differences in ethanol metabolism (33), all sub-

jects recruited ($n = 8$) were men. All subjects were moderate consumers of ethanol (< 120 g/wk) and had no history of alcoholism. All subjects had normal liver enzyme and blood lipid concentrations and were seronegative for HIV (34). None of the subjects had a history of medical diseases, had metabolic disorders, or were using any medications with known metabolic effects. Subject characteristics are listed in **Table 1**. Body composition was measured by bioelectrical impedance analysis (model 1990B; Valhalla Scientific Inc, San Diego). Body fat and total body water were calculated by using the manufacturer's equations.

Study design

Subjects were admitted to the General Clinical Research Center at San Francisco General Hospital twice, once for the intravenous infusion of stable-isotope tracers (protocol 1) and once for oral administration of the stable-isotope tracers (protocol 2). The design for protocol 1 is shown in **Figure 1**A. After an evening meal providing 40% of total daily energy requirements (55% as carbohydrate, 30% as fat, and 15% as protein), the intravenous infusion of isotopes began at 0400. Sodium [1-^{13}C$_1$]acetate (1 g/h), [1,2,3,4-^{13}C$_4$]palmitic acid (7 μg·kg^{-1}·min^{-1}), and [^{2}H$_5$]glycerol (9.7 μg·kg^{-1}·min^{-1}) were infused at a constant rate until 1400. At 0800 and again at 0830, a cocktail containing 12 g ethanol (40% vodka; Absolut, Ahus, Sweden) mixed with sugar-free lemonade (Kraft General Foods, White Plains, NY) was administered orally. Each ethanol cocktail contained 351 kJ (84 kcal) from
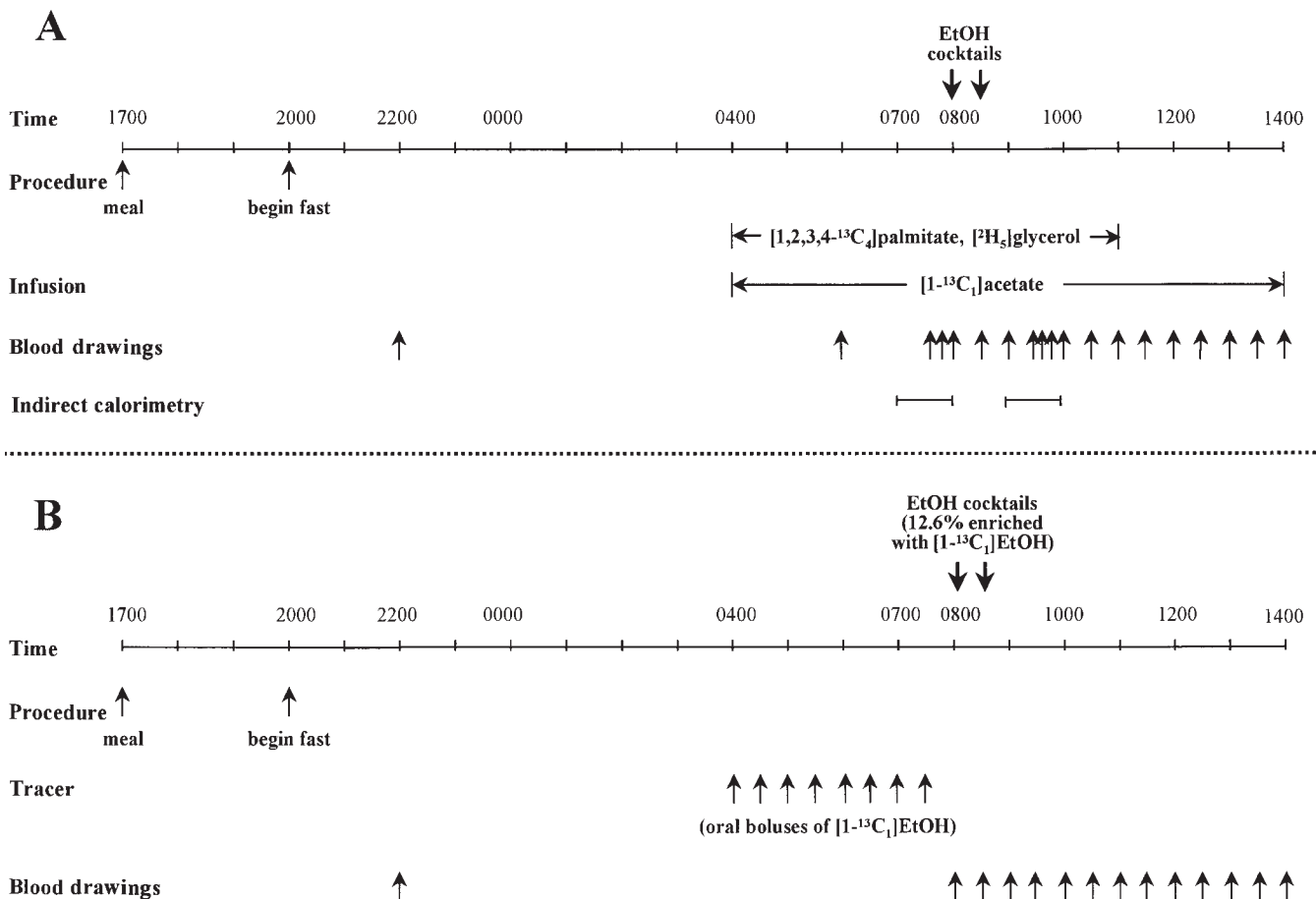

FIGURE 1. Study design of protocols 1 (A) and 2 (B). EtOH, ethanol.

ethanol and 17 kJ (4 kcal) from the sugar-free lemonade. Values obtained at 0740, 0750, and 0800 were averaged to represent values before ethanol consumption, whereas the values obtained at 0930, 0940, 0950, and 1000 were averaged to represent values after ethanol consumption. Indirect calorimetry was performed before (0700–0800; baseline) and after (0900–1000) ethanol consumption. Three 20-min measurements were made during each of these periods with a Deltatrac Metabolic Cart (SensorMedics, Yorba Linda, CA) in the hooded mode. The urinary nitrogen content was quantified with an automated nitrogen analyzer (Leco Corporation, St Joseph, MI) to estimate protein oxidation. No food was consumed until the isotopic tracer infusions ended.

A second study (protocol 2) was performed in 7 of the 8 subjects 1 wk after the first study ended. The protocol was similar to the study described above except for the isotopic tracer used and its route of delivery (Figure 1B). Tracer quantities of [1-$^{13}C_1$]ethanol were administered orally (0.075 mg·kg^{-1}·min^{-1}) every 0.5 h between 0400 and 0800. At 0800 and 0830, cocktails containing 12 g of a mixture of unlabeled ethanol and [1-$^{13}C_1$]ethanol (final enrichment = 12.6%) were administered orally. Blood was drawn at 0.5-h intervals from 0800 to 1400, separated, and stored as plasma at 20°C until analyzed.

Materials

Sodium [1-$^{13}C_1$]acetate, [1,2,3,4-$^{13}C_4$]palmitic acid, [1-$^{13}C_1$]ethanol, and [$^2H_5$]glycerol were purchased from CIL (Andover, MA) or Isotec (Miamisburg, OH). Isotopic purity was >98% for all tracers used.

Metabolite isolation and measurement

VLDLs, nonesterified fatty acids, and glycerol were isolated from plasma and derivatized as described previously (10, 35). Plasma acetate was derivatized and isolated according to the method described by Powers et al (36) with the following minor modifications. Plasma was initially deproteinized by adding *sec*-butanol (1:2 by vol) and the supernate was used for the remaining procedures. Analysis of acetate is extremely sensitive to contamination of unlabeled acetate from reagents and other environmental factors (36, 37); therefore, care was taken to avoid contamination (eg, separate reagents were used and all reagents were tested for contamination). To verify the absence of contamination, water blanks were converted regularly in conjunction with sample analyses and tested for the presence of acetate via gas chromatography–mass spectrometry. If a blank contained acetate, the samples that were converted concomitantly were not analyzed; instead, a new preparation from those samples was made.

Blood alcohol concentration was determined by the alcohol dehydrogenase method (Sigma, St Louis). Plasma glucose concentrations were determined with a glucose analyzer (YSI, Yellow Springs, OH). Serum insulin concentrations were measured by radioimmunoassay (Diagnostic Products, Los Angeles). Triacylglycerol concentrations were determined by colorimetric assay (Sigma). Nonesterified fatty acid concentrations were measured by including an internal standard as described previously (35). The relative proportions of fatty acids from plasma and VLDLs were quantified by gas chromatography with flame ionization detection as described previously (35).

Mass spectrometry

Gas chromatography–mass spectrometry was performed with an HP 5971 instrument (Hewlett-Packard, Palo Alto, CA) to deter-

mine isotopic enrichment of fatty acid methyl esters from VLDLs and plasma, glycerol from plasma, and acetate from plasma. For fatty acid methyl esters, analysis was performed in the electron-impact ionization mode. A DB-1 column (J&W Scientific, Folsom, CA) was used for chromatographic separation. The initial temperature was 80°C and rose to 310°C at a rate of 40°C/min, followed by a rise to 325°C at a rate of 3°C/min. The molecular ions observed by selected ion monitoring (M_0, M_1, M_2, and M_4) had mass-to-charge ratios (*m/z*) of 270, 271, 272, and 274, respectively. For glycerol, the chemical ionization mode was used. A DB225 column (J&W Scientific) was used to separate glycerol-triacetate at an initial temperature of 75°C, rising to 160°C at 45°C/min, to 171°C at 5°C/min, and to a final temperature of 220°C at 40°C/min. M_0 (*m/z:* 159), M_1 (*m/z:* 160), and M_5 (*m/z*:164) were collected in the selective ion monitoring mode. Acetate was analyzed as described by Powers et al (36) by monitoring M_0 (*m/z*: 171) and M_1 (*m/z*: 172). Isotope enrichments were quantified by comparisons with standard curves of [1,2,3,4-$^{13}C_4$]palmitate, [$^2H_5$]glycerol, or [1-$^{13}C_1$]acetate.

Calculations

The fraction of plasma acetate derived from ethanol was calculated by using the precursor-product relation; the plateau enrichment of plasma acetate was divided by the enrichment of administered ethanol (12.6%) during protocol 2. The absolute rate of conversion of ethanol into plasma acetate was then calculated by multiplying the fraction of acetate derived from ethanol by the plasma acetate flux (from protocol 1) and comparing that with the ethanol clearance rate. The clearance rate was calculated from the plasma ethanol die-away curve (33, 38). The appearance rates (R_a) of plasma nonesterified fatty acids, glycerol, and acetate were calculated by isotope dilution (10, 39). The fraction of VLDL palmitate synthesized through the DNL pathway (fractional DNL) was calculated by using mass-isotopomer distribution analysis (40), described elsewhere in detail (41, 42). Briefly, the ratio of excess double-labeled to excess single-labeled species ($\Delta A_2/\Delta A_1$) in VLDL palmitate shows the isotopic enrichment of the true precursor for lipogenesis (hepatic cytosolic acetyl-CoA) by applying a probability model based on the binomial expansion. The fractional contribution from DNL to the VLDL fatty acids is then calculated based on the precursor-product relation (40–42). Specifically, the equation for the calculation of *p* was as follows:

$$p = R^3(0.01578508) - R^2(0.08572153) + R(0.2561532) - 0.04748384 \qquad (1)$$

where *R* is the ratio of ΔA_2 to ΔA_1 and ΔA_1 and ΔA_2 are changes in the fractional abundance of M_{+1} and M_{+2} isotopomers, respectively, from natural abundance values.

Fractional DNL was calculated as follows:

$$f = \frac{[R_{(B)} \times M_{1(meas)}] - [M_{1(B)}]}{[M_{1(E)} - M_{1(B)}] + [(R_{(B)} - R_{(E)}) \times M_{1(meas)}]} \qquad (2)$$

where $R_{(B)}$ is the theoretical proportion of ions monitored in the total ion envelope at baseline, $R_{(E)}$ is the theoretical proportion of ions monitored in the total ion envelope for enriched molecules at the calculated *p*, $M_{1(meas)}$ is the measured M_1 fractional abundance, $M_{1(B)}$ is the theoretical proportion of M_1 ions at baseline, and $M_{1(E)}$ is the theoretical proportion of M_1 ions for enriched molecules at the calculated *p*. *See* reference 43 for a more detailed explanation.

Mass-isotopomer distribution analysis has been used to measure DNL in humans under a variety of conditions (34, 39, 40, 44,
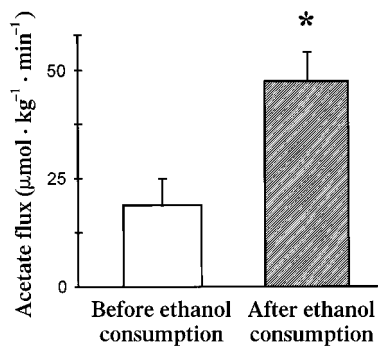
FIGURE 2. Mean (±SEM) plasma acetate flux over the 1 h before and 1–2 h after consumption of 24 g ethanol ($n = 8$). *Significantly different from before ethanol consumption, $P < 0.0001$.

45). VLDL-triacylglycerol production and half-life ($t_{1/2}$) were calculated by kinetic modeling of the rate of rise toward the plateau of fractional DNL during labeling from [1-$^{13}C_1$]acetate (39, 41, 45). Fractional DNL values from half-hourly blood drawings between 0800 and 1400 were plotted and the rate constant for VLDL-triacylglycerol synthesis was determined by best fit for the curve of DNL versus time. The SIGMAPLOT computer program (Jandel Scientific, San Rafael, CA) was used. As discussed elsewhere, the rate constant is determined by the replacement rate of VLDL triacylglycerol from all sources, so the rate constant for VLDL-triacylglycerol turnover is in principle the same whether labeled via DNL, glycerol, fatty acids, etc (10). Indeed, we showed previously that incorporation of [^{13}C]glycerol and [^{13}C]acetate into VLDL triacylglycerol gives identical rate constants after ethanol intake (10). The data were fit to the equation

$$y = A_0 \times (1 - e^{-k_s (t - c)}) \tag{3}$$

where y is fractional DNL, A_0 is the plateau value of fractional DNL, k_s (h) is the rate constant of the rise to plateau in VLDL triacylglycerol, t is time in hours, and c is the lag period before isotope incorporation into secreted VLDL triacylglycerol. Data from 2 subjects could not be modeled and were not included in these analyses. VLDL-triacylglycerol production was then calculated as

$$k_s \times \text{pool size} \tag{4}$$

where pool size is the triacylglycerol concentration (mmol/L) × estimated plasma volume (L). The plasma volume was estimated to be 5% of total body weight (46, 47). Absolute DNL was then calculated by combining fractional DNL measurements with VLDL-triacylglycerol production (39); absolute DNL (g) is the product of total VLDL-triacylglycerol production (μmol/h), time (h), and fraction of fatty acids in VLDL triacylglycerols that were derived from DNL.

Two values were calculated for absolute DNL. The first was for VLDL-triacylglycerol palmitate; the second was for all nonessential fatty acids (stearate, oleate, palmitoleate, palmitate, and myristate) in VLDL triacylglycerols. For the latter calculation it was assumed that the fractional DNL value for the other nonessential fatty acids (which we did not measure) was the same as that determined for palmitate. This represents a maximal (upper bound) estimate of DNL in that the fractional synthesis of these fatty acids is generally less than that of palmitate (39). Non-DNL VLDL-triacylglycerol production was then calculated as the

difference between the total VLDL-triacylglycerol production rate and the absolute DNL rate for all nonessential fatty acids.

Nonprotein respiratory quotients (NPRQs) before ethanol consumption and whole-body oxidation of lipid and carbohydrate were calculated by indirect calorimetry, according to standard equations (48). NPRQs after ethanol consumption were adjusted for the oxidation rate of ethanol (ie, to calculate a nonethanol NPRQ). It was assumed that all the ethanol cleared from plasma that was not lost in the urine or converted to lipid via DNL was completely oxidized to carbon dioxide (1, 2). The ethanol clearance rate during a period of saturated plasma ethanol clearance was corrected for DNL, urinary losses, or both to generate an ethanol oxidation rate, which was used to calculate nonethanol NPRQs.

Statistics

All data were expressed as means ± SEMs. The statistical significance of differences between values before and after ethanol consumption was determined by using one-way analysis of variance with Scheffe's multiple-comparison procedures. P values <0.05 represented statistical significance. STATVIEW for WINDOWS (version 4.53, 1996; Abacus Concepts, Inc, Berkeley, CA) was used for the analyses.

RESULTS

Plasma acetate flux and recovery of ethanol as acetate

Plasma acetate flux after consumption of 24 g ethanol was 47.5 ± 2.5 μmol·kg^{-1}·min^{-1}, significantly higher than the value measured before ethanol consumption (19.0 ± 2.3 μmol·kg^{-1}·min^{-1}) (**Figure 2**). The proportion of labeled ethanol accounted for by entry into plasma as acetate after consumption of 24 g ethanol was $77 \pm 13\%$, whereas $68 \pm 2\%$ of plasma acetate was derived directly from ingested ethanol (ie, plasma acetate enrichment was $68 \pm 2\%$ of administered ethanol enrichment).

Hepatic DNL and VLDL-triacylglycerol production

Fractional DNL (the fraction of VLDL palmitate derived from the DNL pathway) exhibited a robust and reproducible increase in response to ethanol consumption (**Figure 3**). The rise in VLDL-
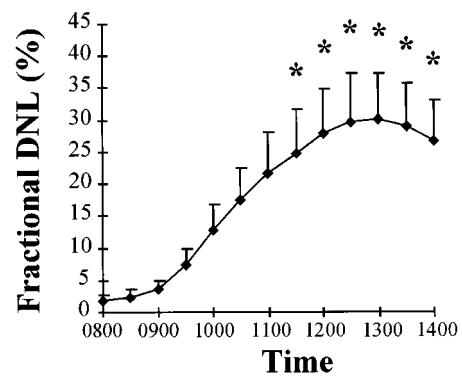

FIGURE 3. Mean (±SEM) fractional contribution from de novo lipogenesis (DNL) to palmitate isolated from circulating VLDL fatty acids over time after consumption of 24 g ethanol ($n = 8$). Values were determined by using mass-isotopomer distribution analysis. *Significantly different from before ethanol consumption, $P < 0.005$.

TABLE 2
Fractional and absolute de novo lipogenesis (DNL) and VLDL-triacylglycerol (TG) production rates after consumption of 24 g ethanol[1]

Parameter	Value
Fractional DNL (%)	33 ± 2 (24–40)
k_s (h^{-1})	0.23 ± 0.02 (0.14–0.27)
A_0 (%)	57 ± 3 (46–67)
$t_{1/2}$ (h)	3.18 ± 0.36 (2.73–5.10)
VLDL-TG production rate (μmol · kg^{-1} · h^{-1})	11.66 ± 1.52 (7.04 – 16.69)
Palmitate synthesis rate via DNL (μmol · kg^{-1} · h^{-1})	1.13 ± 0.20 (0.47 – 1.96)
Total palmitate synthesis via DNL over 6 h (g)	0.32 ± 0.06 (0.13–0.57)
Nonessential fatty acid synthesis rate via DNL (μmol · kg^{-1} · h^{-1})	2.84 ± 0.39 (1.40 – 4.10)
Total nonessential fatty acid synthesis via DNL over 6 h (g)	0.80 ± 0.10 (0.41–1.21)
Non-DNL VLDL-TG production rate (μmol · kg^{-1} · h^{-1})	8.82 ± 1.29 (5.19 – 13.25)
Total non-DNL VLDL-TG production (g)	2.47 ± 0.31 (1.66–3.42)

[1] $\bar{x}$ ± SEM; range in parentheses. k_s, rate constant; A_0, plateau value; $t_{1/2}$, half-life.

palmitate DNL reached a plateau value of 30 ± 3% from baseline values of 1 ± 1%. Lipogenic precursor pool enrichment values fell after ethanol consumption ($P < 0.03$); the initial value at 0800 was 0.106 ± 0.008 MPE (mole percent excess) and the steady state postethanol value was 0.078 ± 0.002 MPE (data not shown).

The increase in fractional DNL permitted kinetic modeling to calculate k_s. Many parameters then were calculated (**Table 2**). The k_s value after 24 g ethanol was 0.23 ± 0.02 h. The corresponding VLDL-triacylglycerol production rate and $t_{1/2}$ were 11.66 ± 1.52 μmol·kg^{-1}·min^{-1} and 3.18 ± 0.36 h, respectively. Total palmitate synthesis via DNL over the 6 h after acute consumption of 24 g ethanol was 0.32 ± 0.06 g for VLDL-triacylglycerol palmitate. The estimated value for all nonessential fatty acid synthesis in VLDL triacylglycerol (*see* calculations) was only 0.80 ± 0.10 g. Non-DNL VLDL-triacylglycerol production was therefore 2.47 ± 0.31 g.

Lipolysis

The R_a of nonesterified fatty acids decreased by 53% (**Figure 4**), from 4.12 ± 0.39 μmol·kg^{-1}·min^{-1} before ethanol consumption to 1.94 ± 0.14 μmol·kg^{-1}·min^{-1} after ethanol consumption. Similarly, plasma nonesterified fatty acid concentrations decreased by 47% ($P < 0.0001$), from 429 ± 93 μmol/L at baseline to 229 ± 68 μmol/L after ethanol consumption. Changes in the R_a of glycerol were less than those observed for the R_a of nonesterified fatty acids. The R_a of glycerol decreased by 25% (NS) after ethanol consumption, falling from 3.04 ± 0.44 to 2.28 ± 0.33 μmol·kg^{-1}·min^{-1}.

Substrate oxidation

Whole-body carbohydrate oxidation did not change significantly after ethanol consumption (before: 8.00 ± 0.67 μmol·kg^{-1}·min^{-1}; after: 7.89 ± 0.44 μmol·kg^{-1}·min^{-1}), but whole-body lipid oxidation fell substantially (**Figure 5**). The baseline value was 4.08 ± 0.32 μmol·kg^{-1}·min^{-1} and dropped to 1.09 ± 0.18 μmol·kg^{-1}·min^{-1} after ethanol consumption, representing a decrease of 73%. The nonethanol NPRQ was 0.78 ± 0.01 after ethanol consumption. Total energy expenditure was 7778 ± 289 kJ/d (1859 ± 69 kcal/d) before and 8134 ± 356 kJ/d (1944 ± 85 kcal/d) after ethanol consumption. The decrease in whole-body fat oxidation nearly balanced, but was slightly less than the calculated ethanol oxidation rate.

Plasma concentrations of metabolites

The blood alcohol concentration rose significantly, from 0 mmol/L before to a peak value of 9.5 ± 0.8 mmol/L after ethanol

consumption (**Figure 6**), returning to baseline values in all subjects by 1300. The ethanol clearance rate was 0.032 ± 0.005 (mmol/L)·kg^{-1}·min^{-1}. Plasma triacylglycerol concentrations were 0.99 ± 0.18 mmol/L before ethanol consumption and rose by 25% to 1.23 ± 0.10 mmol/L by 1400 (NS), after ethanol consumption. Plasma glucose concentrations did not change significantly after ethanol consumption (before: 4.94 ± 0.22 mmol/L; after: 4.83 ± 0.17 mmol/L). Similarly, serum insulin concentrations did not change significantly after ethanol consumption (before: 34.73 ± 7.64 pmol/L; after: 22.85 ± 4.87 pmol/L).

DISCUSSION

The consumption of 24 g ethanol elicited dramatic changes in lipid metabolism in the healthy men in this study. On the basis of these findings, several questions concerning whole-body lipid balances were answered and a model of the metabolic fate and consequences of ethanol consumption may be proposed.
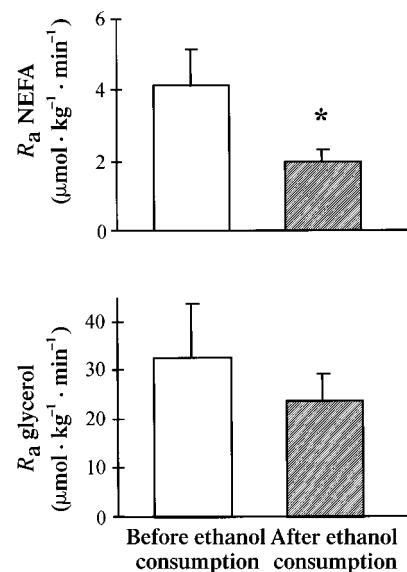

FIGURE 4. Mean (±SEM) appearance rates (R_a) of plasma nonesterified fatty acids (NEFA) and glycerol over the 1 h before and 1–2 h after consumption of 24 g ethanol ($n = 8$). *Significantly different from before ethanol consumption, $P < 0.005$.
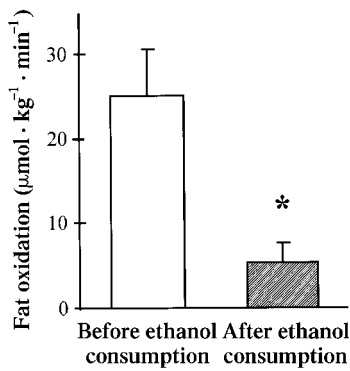
FIGURE 5. Mean (±SEM) whole-body lipid oxidation over the 1 h before and 1–2 h after ethanol consumption of 24 g ethanol (n = 8). *Significantly different from before ethanol consumption, $P < 0.0001$.

The first issue addressed was the quantitative role of DNL after ethanol consumption. Attempts to measure DNL in response to ethanol consumption (21–23) in humans have been flawed by the lack of access to the lipogenic precursor pool, hepatic acetyl-CoA. Use of mass-isotopomer distribution analysis in the current study allowed true precursor enrichments to be calculated (40–42). A sizable increase in the contribution of newly made fatty acid molecules to circulating VLDL-triacylglycerol was observed after consumption of 24 g ethanol. Also, we previously observed fractional DNL values of 37% on average in men who ingested 48 g ethanol (10). It is clear that the hepatic lipogenic pathway is activated after ethanol consumption, in agreement with studies that measured the enzymatic activity of fatty-acyl-CoA synthase in animals not exposed previously to ethanol (49).

Nevertheless, conversion to fatty acids in the liver was not the primary fate of ingested ethanol. The maximum amount of newly synthesized fatty acids released into the circulation was 0.8 g, representing <5% of the ingested ethanol load. This result is roughly comparable with the estimate offered by Koziet et al (10%; 23), who compared the enrichment of ^{13}C in circulating fatty acids with that of ^{13}C in administered ethanol without measuring the dilution in the lipogenic acetyl-CoA pool. There are 2 potential explanations for the relatively minor contribution from the DNL pathway. The first is that little hepatic conversion of ethanol to fatty acids occurred and the lipoprotein particles released into the plasma accurately represent the molecules synthesized in the liver. If this is true, then the contribution of DNL to the increased energy expenditure (24, 50) seen after ethanol intake is minimal: the estimated energy costs for the conversion of ethanol to 0.8 g triacylglycerol fatty acids (before their ultimate oxidation) is <12.6 kJ (3 kcal) (30). The other potential explanation for low rates of DNL is that not all of the newly synthesized fatty acids in the liver were released to the circulation over the 6 h of the study. Studies with cultured HepG2 cells (51), however, showed that de novo synthesized fatty acids are preferentially and rapidly secreted, rather than entering the cytosolic storage pathway. Moreover, the source of the lipid that accumulates in the liver after regular consumption of ethanol (12–14) has been investigated (17, 52, 53) and newly synthesized fatty acids contribute only a small percentage. Substantial hepatic retention of fatty acids derived from DNL in the 6 h after ethanol consumption seems unlikely.

If most of the ethanol metabolized to acetate in the liver is not converted to lipid (54–56), what is the primary fate of ethanol cleared by the liver? Our results provide the first direct isotopic evidence that the primary fate of ethanol is its release as acetate into the circulation. Of the administered [^{13}C]ethanol, 77% was recovered as [^{13}C]acetate in plasma. Our results are similar to the estimate of Lundquist et al (54), who used arteriovenous differences across the splanchnic bed to calculate that 70% of administered ethanol was released as acetate by the liver. This concordance supports the validity of the noninvasive labeling approach.

After ethanol consumption, plasma acetate flux reached a steady state value that was 2.5 times its preconsumption value. This dramatic increase in acetate availability to the peripheral tissues has important metabolic consequences because acetate inhibits lipolysis in vitro (57) and in vivo (58, 59). We observed a 53% decrease in the entry of nonesterified fatty acids into plasma and a 47% reduction in nonesterified fatty acid concentrations after ethanol consumption, despite unchanged circulating insulin concentrations. Others have reported a decrease in nonesterified fatty acid turnover (4–6) and concentrations (58–61) after ethanol consumption. In addition to a reduction in the availability of circulating lipid fuels for tissues, the increased plasma acetate concentration may initiate changes in tissue fuel selection. The primary fate of circulating acetate is oxidation (1, 37), and acetate may have priority over other substrates for oxidation (62). Pouteau et al (37) recovered 69% of infused [^{13}C]acetate as $^{13}CO_2$ in humans and Shelmet et al (1) reported that 101% of [^{14}C]ethanol was recovered as $^{14}CO_2$. In extrahepatic tissues, acetyl-CoA synthetase is located primarily in the mitochondria (63); studies in the perfused heart suggest that acetate has priority for oxidation relative to a variety of other substrates (62). In the present study, oxidation of acetate after ethanol administration replaced lipid, not carbohydrate. Although net lipid oxidation, as measured by indirect calorimetry, has been shown to decrease after ethanol consumption (1, 2), previous studies (2) could not discount the possibility that increased synthesis of lipid merely balanced the ongoing oxidation of fat. Our results exclude hepatic DNL as a quantitatively significant disposal route for ethanol in the liver, and adipose DNL is unlikely to occur in this situation because adipose acetyl-CoA synthetase is primarily mitochondrial (63). Thus, ongoing lipid synthesis did not mask lipid oxidation and lipid oxidation was indeed reduced by ethanol consumption, supporting the priority of plasma acetate in the hierarchy of tissue fuels (62).
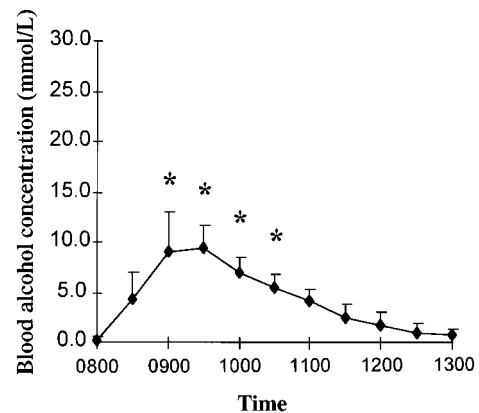

FIGURE 6. Mean (±SEM) blood alcohol concentrations after consumption of 24 g ethanol over 1 h (n = 8). *Significantly different from before ethanol consumption, $P < 0.0001$.
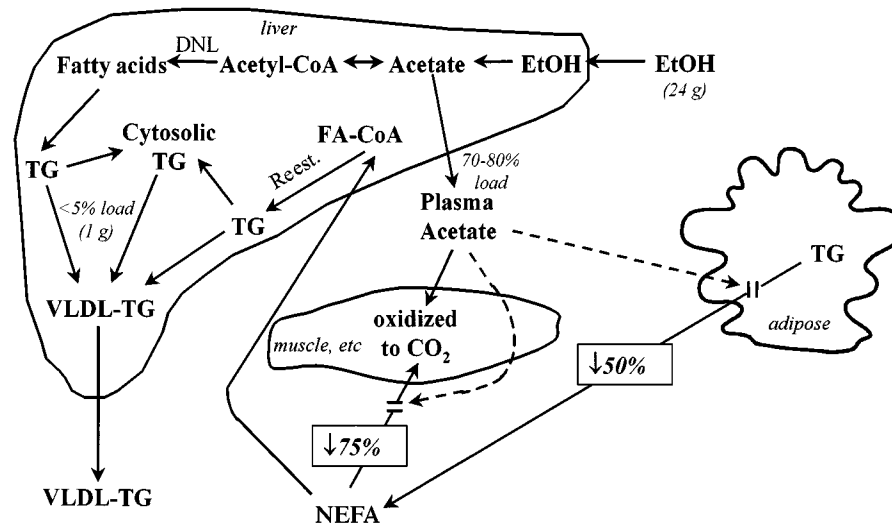
FIGURE 7. Model of whole-body lipid metabolism after consumption of moderate quantities of ethanol. *See* Discussion for details. EtOH, ethanol; DNL, de novo lipogenesis; TG, triacylglycerol; FA-CoA, fatty-acyl-CoA synthase; Reest, reesterification of fatty-acyl-CoA synthase to triacylglycerol; NEFA, nonesterified fatty acids.

These observations form the basis of a model of the integrated lipid response to ethanol consumption (**Figure 7**). Ethanol is converted in the liver to acetate; an unknown portion is then activated to acetyl-CoA, but only a small portion is converted to fatty acids. Most of the acetate is released into the circulation, where it affects peripheral tissue metabolism; adipocyte release of nonesterified fatty acids is decreased and acetate replaces lipid in the fuel mixture.

The response of the liver to the availability of surplus 2-carbon units after ethanol consumption is in many ways analogous to the hepatic response to carbohydrate overfeeding (39). Addition of surplus dietary carbohydrate energy expands hepatic glycogen stores, but does not result in a quantitatively important stimulation of DNL. Instead, hepatic release of glucose in the postabsorptive state is greatly increased (39, 64), leading to increased serum insulin concentrations, reduced lipolysis, and replacement of lipid by carbohydrate in the whole-body fuel mixture. In this manner, the liver alters whole-body lipid balances not by synthesizing lipid but by changing fuel selection of peripheral tissues. Similarly, the liver does not convert a great deal of ethanol to lipid but alters whole-body lipid balances by releasing an oxidizable fuel (acetate). The major difference from carbohydrate overfeeding is that acetate directly reduces lipolysis and replaces fatty acids in the tissue fuel mixture, without mediation by insulin. In both cases, the inability of hepatic DNL to provide a quantitative disposal route for the surplus substrates plays a permissive role in the system, allowing the liver to export the surplus to peripheral tissues for oxidation.

In summary, the consumption of 24 g ethanol activates the lipogenic pathway in humans, but de novo synthesis of fatty acids represents a quantitatively minor fate (<5%) of ingested ethanol. The primary fate (70–80%) of ethanol is conversion to acetate by the liver, release into the circulation, and oxidation by tissues. Inhibition of lipolysis and whole-body lipid oxidation are secondary consequences of the greatly increased availability of acetate. The liver, therefore, mediates the positive whole-body lipid balance induced by ethanol by providing acetate systemically, not through the direct conversion of ethanol to lipids.

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