Ethanol Exerts Acute Protein-Sparing Effects During Postabsorptive But Not During Anabolic Conditions in Man

Kaspar Berneis, Ronald Ninnis, and Ulrich Keller

Ethanol abuse is frequently associated with protein malnutrition. To assess the acute effects of ethanol on whole-body protein metabolism, [1-^{13}C]leucine kinetics were measured in eight postabsorptive normal male subjects three times, ie, during administration of two doses of ethanol (dose 1, 0.52 g/kg during 2 hours and 0.3 g/kg during 3 hours; dose 2, 0.69 g/kg during 2 hours and 0.3 g/kg during 3 hours) and during saline (controls). During the last 2 hours of the studies, glucose, insulin, and amino acids were infused to assess the effects of ethanol on protein kinetics under anabolic conditions (euglycemic clamp). The decreases in leucine flux (reflecting whole-body protein breakdown) and nonoxidative leucine disappearance (a parameter of protein synthesis) during saline infusion were abolished in both ethanol protocols ($P < .05$ or less *v* saline). The rate of leucine oxidation decreased during the higher dose of ethanol compared with saline ($P < .005$), indicating an anticatabolic effect. During anabolic conditions (clamp), leucine flux and nonoxidative leucine disappearance were significantly higher in both ethanol studies compared with saline ($P < .05$). Resting energy expenditure (REE) and oxygen consumption (Vo_2) during the euglycemic clamp increased to a greater degree during both ethanol studies than during saline ($P < .05$ or less). Thus, an elevation of blood ethanol concentrations to the levels observed in social drinking results in a net anticatabolic effect (diminished leucine oxidation) when ethanol is administered alone. However, during administration of other nutritional substrates, the anticatabolic effect was not detectable, possibly because ethanol enhanced nutrient-induced thermogenesis.

APPROXIMATELY 6% of the total caloric intake of adults in the United States[1] and 9% in Switzerland[2] is consumed in the form of ethanol. Alcoholic liver disease in its ultimate stage is a major cause of morbidity (with a prevalence of 3.6 in 1,000)[3] and mortality in the United States.[4]

Malnutrition is typical for chronic ethanol abuse.[5,6] Although insufficient nutrient intake is probably the main cause, the question as to whether ethanol favors or prevents protein malnutrition is not clearly answered.

Ethanol administration decreased protein oxidation in humans assessed by indirect calorimetry,[7,8] whereas it diminished albumin synthesis in man[9] and in isolated perfused rat liver.[10] Isocaloric replacement of a dietary regimen in humans with ethanol during an 8-day period resulted in negative nitrogen balance.[11] A bottle of wine together with a mixed meal did not affect whole-body protein breakdown and synthesis,[9] whereas ethanol-fed rats demonstrated an increase in leucine flux and oxidation.[12] However, the acute effect of graded doses of ethanol on whole-body protein turnover and its interaction with other nutrients (glucose and amino acids) have not been explored previously in humans. The present studies were designed to assess the acute effects of elevated blood ethanol concentrations in man. Ethanol was administered intravenously to achieve steady and reproducible blood ethanol concentrations; its influence on whole-body protein metabolism was determined using a tracer technique ([1-^{13}C]leucine infusion).

From the Departments of Research and Internal Medicine, University Hospital Basel, Basel, Switzerland.

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Address reprint requests to Ulrich Keller, MD, Departments of Research and Internal Medicine, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland.

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SUBJECTS AND METHODS

Subjects

Written informed consent was obtained from eight healthy young volunteers aged 26 ± 1.5 years with a body mass index of 23.5 ± 0.9 kg/m^2. A medical history, physical examination, and routine laboratory tests before the studies provided no evidence of cardiopulmonary, renal, hepatic, or metabolic diseases. The subjects were on no medication, consumed less than 20 g ethanol per day, and did not perform vigorous exercise during the study period. The study protocol was reviewed and approved by the ethics committee of Basel University Hospital.

Procedures

Each subject was studied during three conditions (ethanol dose 1, ethanol dose 2, and saline (control)) on 3 different days in randomized order with intervals of at least 1 week (Fig 1).

At 7 AM after a 12-hour fast, a teflon cannula was placed into the right antecubital vein for infusions. A superficial dorsal vein of the right hand was cannulated in a retrograde manner using a 21-gauge butterfly needle for blood sampling. The hand was kept in a thermostat-controlled warming chamber at a temperature of approximately 55°C to allow arterialization of hand venous blood.

After obtaining blood and breath samples to determine background isotopic enrichment of plasma [1-^{13}C]leucine and [1-^{13}C]-α-ketoisocaproate (α-KIC) and of breath $^{13}CO_2$, priming doses of 3 μmol/kg [1-^{13}C]leucine (99% enriched, sterile, and pyrogen-free; Mass Trace, Woburn, MA) and 3.5 μmol/kg $NaH^{13}CO_3$ (99% enriched, sterile, and pyrogen-free; Mass Trace) were injected; thereafter, a continuous infusion of [1-^{13}C]leucine (0.06 μmol/kg · min $^{-1}$) was administered during the following 390 minutes. After 120 minutes of tracer equilibration, blood and breath samples were obtained at 15-minute intervals during a 30-minute basal period and during the infusion period. A loading dose of ethanol (10%) (dose 1, 0.52 g/kg; dose 2, 0.69 g/kg) was administered during 2 hours, and a holding dose (0.3 g/kg in both dose groups) during the last 3 hours. The volume of saline administered was 200 mL/h during the ethanol loading dose and 70 mL/h during the last 3 hours. During the last 120 minutes of the infusion period, insulin was infused continuously at 60 mU · m^{-2} · min^{-1} during 3 minutes, and then at 15 mU · m^{-2} · min^{-1} during 117 minutes.[13] Glucose 20% (wt/vol) was infused at variable rates and adjusted every 5 to 10 minutes according to rapidly measured plasma glucose concentrations to maintain euglycemia. In addition, a standard mixed amino acid solution

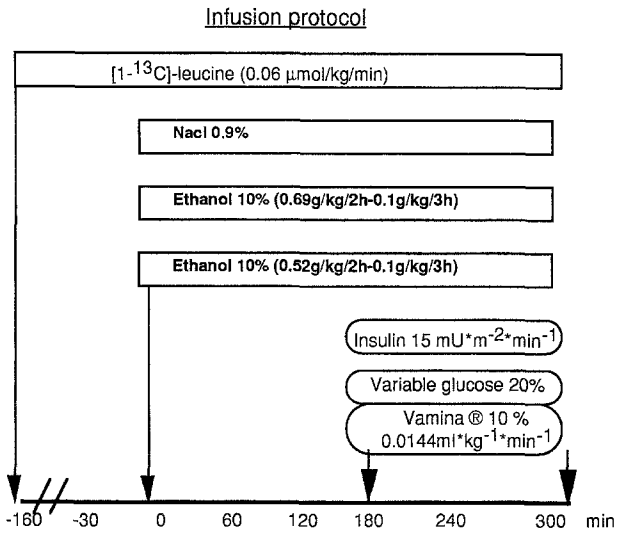

Fig 1. Infusion protocol.

(Vamina 10%) was administered intravenously at a rate of 0.0144 mL/kg, corresponding to a leucine infusion rate of 0.65 μmol/kg/min. The composition of Vamina (Pharmacia, Glattbrugg, Switzerland) (in grams per liter) was aspartate 2.5, glutamate 4.2, glycine 5.9, alanine 12.0, arginine 8.4, cysteine 0.42, histidine 5.1, isoleucine 4.2, leucine 5.9, lysine 6.8, methionine 4.2, phenylalanine 5.9, proline 5.1, serine 3.2, threonine 4.2, tryptophan 1.4, tyrosine 0.17, and valine 5.5. The amino acid solution was enriched with 0.295 g [1-^{13}C]leucine/1,000 mL solution to approximately 5% TTR (tracer to tracee ratio) to maintain the plasma α-KIC TTR during infusion. Plasma was rapidly obtained by refrigerated centrifugation (4°C) and stored at −70°C until later assay. Expired air was collected into gas-tight 20-mL glass tubes (Vacutainer; Becton-Dickinson, Meylan, France) for later $^{13}CO_2$ analysis. To assess the effects of the various infusions (ethanol, glucose, and amino acids) on background $^{13}CO_2$ atom percent excess (APE), three separate sets of studies with ethanol dose 2 and three studies with saline were performed without [1-^{13}C]leucine infusions. $^{13}CO_2$ APE after 3 hours of infusion was not different between ethanol and saline protocols. However, during glucose clamping, APE increased from basal values in all studies similarly by 6.4%, indicating that leucine oxidation was overestimated by 6.4% during clamping; this fact was not considered in the calculation of results.

Analytical Methods

All tracer infusates were ultrafiltered (0.1 μm) and analyzed by gas chromatography–mass spectrometry (GC-MS) (model 5890/5790; Hewlett-Packard, Palo Alto, CA) for tracer concentration, isotopic enrichment, and chemical purity. The plasma TTRs of [1-^{13}C]leucine and α-KIC were measured by GC-MS selected ion monitoring.[14] Plasma concentrations of leucine and α-KIC were determined by the same methodology using (D_7) leucine and (D_3) α-KIC as internal standards, respectively. Isotopic enrichment of $^{13}CO_2$ in expired air was measured by isotope ratio mass spectrometry (SIRA Series II; Isotech, Cheshire, UK). CO_2 production ($\dot{V}CO_2$) was determined by indirect calorimetry using a ventilated-hood metabolic monitor (Deltatrac II MBM-200; Datex, Helsinki, Finland). Plasma glucose concentrations were measured using glucose oxidase and a hydrogen peroxide sensor (Glucose Analyzer 2300 STAT Plus; YSI, Yellow Springs, OH).

Plasma concentrations of C-peptide,[15] glucagon, and insulin were measured using radioimmunoassays (CIS BIO International, Gif-Sur-Yvette, France; Diagnostic Products, Los Angeles, CA; and Insik-5 (P2796) kit, Sorin Biomedica, Italy, respectively). Plasma concentrations of nonesterified fatty acids (NEFAs) were determined using enzymatic methods.[16]

Calculations

Estimates of whole-body leucine were made at near–steady-state conditions during the baseline period (−15 to 0 minutes), during ethanol/saline infusion (150 to 180 minutes), and during the end of the clamp period (280 to 300 minutes). Total leucine flux was calculated by dividing the infusion rate of [1-^{13}C]leucine by the [1-^{13}C]α-KIC TTR according to the reciprocal pool model.[17,18] Leucine oxidation rate (representing irreversible leucine catabolism) was calculated by dividing the product of $^{13}CO_2$ APE and $\dot{V}CO_2$ in expired air by plasma [1-^{13}C]α-KIC TTR. A CO_2 retention factor of 0.81 was used.[19] Nonoxidative leucine disappearance (representing whole-body protein synthesis) was calculated by subtracting the rate of leucine oxidation from total leucine flux. Endogenous leucine flux (a parameter of protein breakdown) was calculated by subtracting the infusion rate of unlabeled leucine from total leucine flux. The net balance of leucine metabolism was calculated as the difference between the rate of nonoxidative disappearance and endogenous flux of leucine.

Respiratory quotients (RQ) were calculated by dividing VCO2 (ml/min) by VO2(ml/min). Resting energy expenditures (REE) (kcal/24h) were calculated using indirect calorimetry formulas.[20] Since ethanol was used as a fourth substrate oxygen consumption and carbon dioxide production rates excluding ethanol oxidation during ethanol infusion were calculated by using energy equivalents of 7.09 kcal/g ethanol and of 4.859 kcal per liter O_2 consumed during ethanol oxidation.[21] We assumed an oxidation rate for ethanol for 0.1g/kg/h according to the results of the present study and to those by Shelmet et al,[8] which observed steady blood ethanol concentrations during this infusion rate.

Statistical Analysis

Repeated-measures ANOVA of Statview, and Students paired *t* tests (Abacus Concepts, Berkeley, CA) on Power Macintosh 7100/80 were used to detect differences between and within the three protocols. Bonferroni/Dunn and Scheffé's F procedures were performed for correction of multiple comparisons.

RESULTS

Ethanol Plasma Concentrations

Plasma ethanol increased rapidly after starting the infusions, reaching steady-state concentrations of 13.2 ± 0.6 mmol/L after 150 minutes during dose 1 and 18.8 ± 0.7 mmol/L during dose 2. Between 150 and 300 minutes, blood ethanol concentrations changed by less than 2.5% in both ethanol studies (Fig 2).

α-KIC Plasma Concentrations and TTR and $^{13}CO_2$ APE

Plasma concentrations of α-KIC until 150 to 180 minutes remained unchanged for saline and ethanol dose 2 and increased for ethanol dose 1 ($P < .02$ *v* basal and $P < .03$ *v* saline). During clamping, α-KIC decreased in the saline group ($P < .0005$) and remained unchanged in both ethanol groups ($P < .005$ or less *v* saline). α-KIC TTR until 150 to 180 minutes increased in the saline group ($P < .005$) and remained unchanged in both ethanol groups (comparison of change over time with saline, $P < .01$ or less). During clamping, α-KIC TTR increased in all groups ($P < .005$ or less), and the increase was less pronounced in both ethanol groups compared with saline ($P < .01$ or less *v* saline). $^{13}CO_2$ APE increased in the

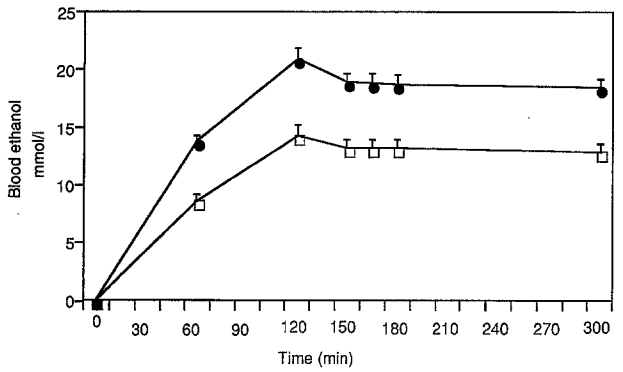

Fig 2. Ethanol blood concentrations during ethanol dose 1 (□) and ethanol dose 2 (●). Data are the mean ± SEM (n = 8).

saline group ($P < .02$ *v* basal) and decreased in both ethanol groups ($P < .02$ or less *v* basal and $P < .005$ or less *v* saline) until 150 to 180 minutes. During clamping, $^{13}CO_2$ APE increased in all three protocols ($P < .0001$ *v* saline/ethanol), and the increase was less pronounced in both ethanol groups compared with the saline group ($P < .05$ or less) (Fig 3).

Leucine Kinetics

The decreases in leucine flux (reflecting whole-body protein breakdown) and nonoxidative leucine disappearance (a parameter of protein synthesis) during saline infusion were abolished in both ethanol protocols ($P < .05$ or less *v* saline). The leucine oxidation rate decreased slightly during saline until 150 to 180 minutes. Administration of ethanol dose 2 resulted in a more pronounced decrease ($P = .005$) versus saline. During anabolic conditions (clamp period), leucine flux and nonoxidative disappearance were higher for both ethanol studies than for saline (dose 1, $P < .05$; dose 2, $P < .05$). At the same time, leucine oxidation increased in all three protocols (saline, $P < .005$; ethanol dose 1, $P < .01$; ethanol dose 2, $P < .01$). Net leucine balance reflected (inversely) leucine oxidation. During anabolic conditions (clamp period), net leucine balance was positive in all three protocols, without a significant difference between the three protocols. Plasma leucine concentrations were 157 ± 19, 120 ± 8, and 166 ± 4 μmol/L during the basal period for saline, ethanol dose 1, and ethanol dose 2 studies, respectively. The plasma leucine concentration increased after 150 to 180 minutes by 30 ± 3 μmol/L during ethanol dose 1 and by 32 ± 5 μmol/L during dose 2, whereas it remained unchanged during saline ($P < .05$ or less *v* dose 1 and 2). During clamping, plasma leucine increased further to 173 ± 12 and 235 ± 15 μmol/L during dose 1 and 2, respectively, whereas it remained unchanged during saline (135 ± 10 μmol/L, $P < .01$ *v* ethanol dose 1 and 2) (Fig 4).

Indirect Calorimetry

Resting energy expenditure (REE) after 120 to 150 minutes of saline/ethanol increased in both ethanol protocols, but remained unchanged in the saline protocol ($P < .05$ or less *v* saline); during the clamp period, energy expenditure and $\dot{V}O_2$ increased to a greater extent during both ethanol studies than during saline ($P < .005$ or less) (Table 1). The respiratory quotient (RQ) was lower after 150 to 180 minutes of ethanol (dose 2) than after saline ($P < .038$). The RQ increased in the saline group during anabolic conditions (clamp period) ($P < .05$) and was higher during saline compared with both ethanol groups ($P < .005$ or less).

Plasma NEFAs, Glucose, Insulin, C-Peptide, and Glucagon

Plasma NEFA concentrations decreased until 150 to 180 minutes during both ethanol protocols compared with saline (dose 1, $P = .01$; dose 2, $P = .005$). During glucose clamping, plasma NEFAs decreased in all three protocols, without signifi-

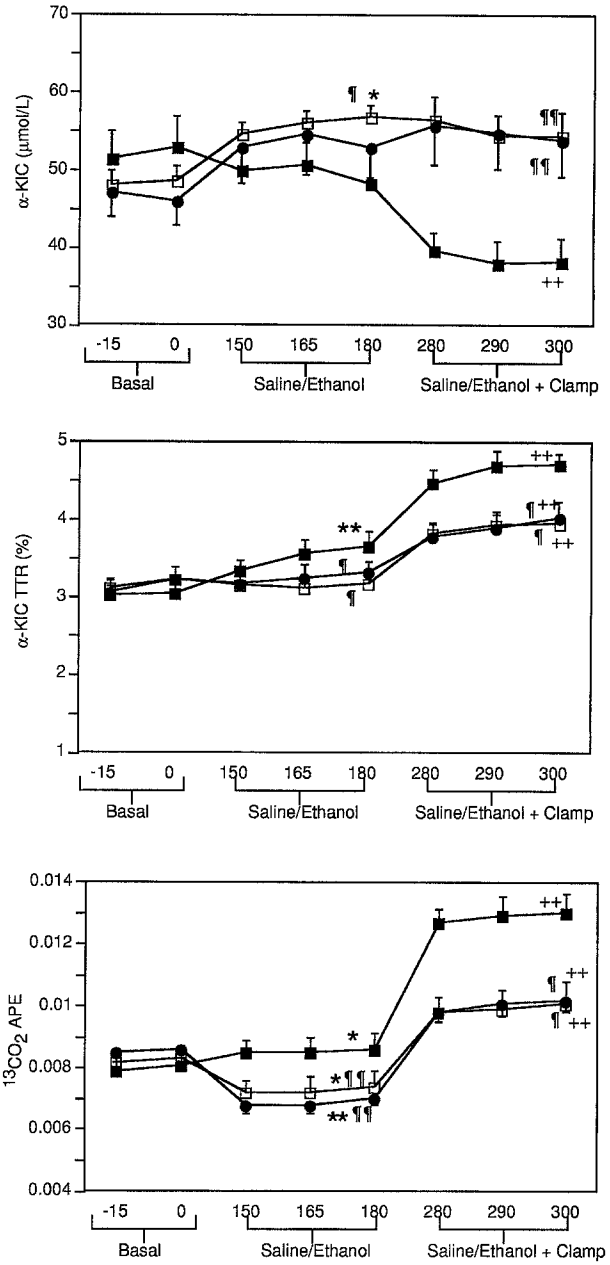

Fig 3. α-KIC plasma concentration and TTR and $^{13}CO_2$ APE during the saline control study (■), ethanol dose 1 (□), and ethanol dose 2 (●). Data are the mean ± SEM (n = 8). ¶$P < .05$, ¶¶$P < .005$ *v* saline; *$P < .05$, **$P < .005$ *v* basal; †$P < .05$, ‡$P < .005$ *v* saline/ethanol (ANOVA).

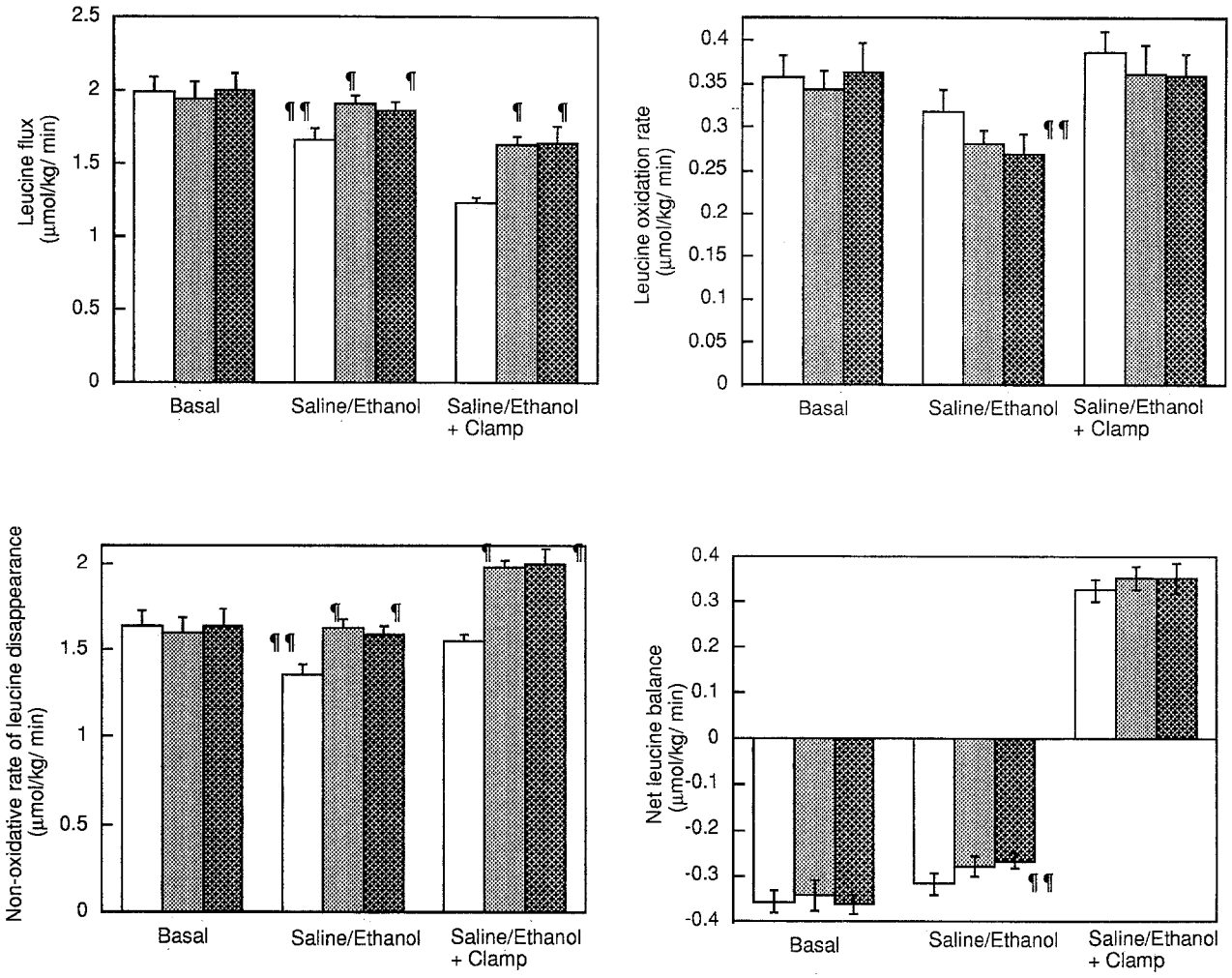

Fig 4. Leucine flux, leucine oxidation rate, nonoxidative rate of leucine disappearance, and net leucine balance during the basal period (−15 to 0 minutes), after saline/ethanol infusion alone (150 to 165 minutes), and after ethanol/saline infusion combined with insulin, glucose and amino acids (euglycemic clamping, 290 to 300 minutes). (□) Saline control study; (▨) ethanol dose 1; (▩) ethanol dose 2. Data are the mean ± SEM. ¶$P < .05$, ¶¶$P < .005$ *v* saline (ANOVA).

cant differences between groups. Plasma glucose concentrations decreased until 150 to 180 minutes in all groups ($P < .02$ or less, NS between groups). Plasma glucose concentrations remained unchanged during clamping in all three groups. Glucose infusion rates during the last 30 minutes of clamping were 3.0 ± 0.3, 2.9 ± 0.3, and 2.7 ± 0.2 mg/kg/min in the saline, ethanol dose 1, and ethanol dose 2 studies, respectively (NS between groups). The decreases of insulin and C-peptide plasma levels during saline infusion were abolished by both ethanol infusions ($P < .05$ or less *v* saline). Plasma insulin levels increased similarly during glucose clamping in all three studies to approximately fourfold above baseline, whereas C-peptide concentrations remained unchanged. Plasma glucagon increased during the clamp period in all groups ($P < .05$ or less) (Table 2).

DISCUSSION

The present study is the first to assess the effect of ethanol administration on leucine kinetics both during postabsorptive conditions and during supply of other nutrients in the same subjects. The results demonstrated that elevation of blood ethanol to concentrations observed during social drinking results in increased leucine turnover with an increase in both endogenous appearance of leucine (whole-body protein breakdown) and nonoxidative disappearance into body proteins. The rate of nonoxidative leucine disappearance has been demonstrated to reflect whole-body protein synthesis.[22,23] The decrease of leucine flux observed in the saline group during postabsorptive conditions may be due to the ongoing fast; however, it cannot be ruled out that it was partly caused by tracer recycling.[24] Ethanol administered alone (without other substrates) decreased leucine oxidation significantly; since this indicated an increase in net leucine balance, it can be concluded that ethanol exerted a net anticatabolic effect in the postabsorptive state. This effect was not observed during administration of glucose, amino acids, and insulin (clamp); nevertheless, these nutritional substrates exerted a positive effect on net leucine balance in all three protocols. The data indicate that only the

Table 1. REE, Respiratory Gas Exchange, and RQ (n = 8, mean ± SEM)

Parameter	Basal	Saline/ Ethanol	Saline/Ethanol + Clamp
Saline			
REE (kcal/24 h)	1,681 ± 86	1,644 ± 73	1,687 ± 87‡
$\dot{V}O_2$ (mL/min)	242 ± 11	237 ± 10	239 ± 12
$\dot{V}CO_2$ (mL/min)	205 ± 13	202 ± 11	218 ± 11
RQ	0.844 ± 0.023	0.852 ± 0.016	0.912 ± 0.012‡
Ethanol dose 1			
REE (kcal/24 h)	1,537 ± 55	1,666 ± 75*‖	1,922 ± 76§¶
$\dot{V}O_2$ (mL/min)	221 ± 8	234 ± 11	270 ± 10§¶
$\dot{V}CO_2$ (mL/min)	191 ± 9	182 ± 7	215 ± 10
RQ	0.863 ± 0.025	0.782 ± 0.023	0.795 ± 0.012¶
Ethanol dose 2			
REE (kcal/24 h)	1,606 ± 77	1,745 ± 85†¶	1,915 ± 90§¶
$\dot{V}O_2$ (mL/min)	233 ± 12	245 ± 14	270 ± 15§¶
$\dot{V}CO_2$ (mL/min)	194 ± 10	192 ± 10	208 ± 13
RQ	0.912 ± 0.035	0.795 ± 0.016‖	0.770 ± 0.016¶

*$P < .05$, †$P < .005$: *v* basal.
‡$P < .05$, §$P < .01$: *v* saline/ethanol.
‖$P < .05$, ¶$P < .005$: *v* saline.

higher dose of ethanol resulted in net protein-sparing, whereas an increase in leucine flux was detectable during both doses.

The inhibitory effect of ethanol on leucine oxidation during postabsorptive conditions was probably due to the fact that ethanol is a substrate that is oxidized preferentially,[8] leaving fewer amino acids for oxidation. This finding obtained after intravenous administration of ethanol in the postabsorptive state agrees with data previously reported by De Feo et al[9] after oral ethanol administration together with a mixed meal.[9] It is similar to that observed with another metabolic substrate that is oxidized preferentially, NEFAs.[25] Therefore, the present data suggest that ethanol per se does not contribute to the protein malnutrition observed in chronic ethanol abusers, but instead prevents it, at least when ingested alone. The finding that coadministration of ethanol with nutritional substrates was not associated with a significant effect on leucine balance was in agreement with De Feo et al,[9] who compared the acute effects of oral ethanol during administration of a mixed meal: they found no effect of ethanol on whole-body protein synthesis and breakdown. They even observed a decrease in the synthesis of the visceral protein albumin. Measurements of whole-body leucine kinetics as performed in the present study do not exclude the possibility that ethanol affected the synthesis of specific proteins. Therefore, these data indicate contrasting effects of ethanol on protein metabolism depending on the nutritional state of the subject—protein may be spared when it is administered alone, but the synthesis of specific proteins (eg, albumin) may be diminished during postprandial conditions.

Table 2. Plasma Concentrations of NEFAs, Glucose, Insulin, C-Peptide, and Glucagon (n = 8, mean ± SEM)

Parameter	Basal	Saline/ Ethanol	Saline/ Ethanol + Clamp
Saline			
NEFA (μmol/L)	408 ± 38	496 ± 61	271 ± 35§
Glucose (mmol/L)	5.0 ± 0.1	4.8 ± 0.1*	5.0 ± 0.1
Insulin (pmol/L)	65 ± 7	50 ± 7*	203 ± 14§
C-peptide (pmol/L)	459 ± 39	332 ± 33†	295 ± 42
Glucagon (pmol/L)	14.1 ± 1.7	13.2 ± 1.7	19.3 ± 2.6
Ethanol dose 1			
NEFA (μmol/L)	405 ± 55	347 ± 72¶	215 ± 26‡
Glucose (mmol/L)	4.9 ± 0.1	4.6 ± 0.1†	4.6 ± 0.1
Insulin (pmol/L)	58 ± 7	58 ± 7‖	210 ± 14§
C-peptide (pmol/L)	428 ± 39	395 ± 41‖	309 ± 74
Glucagon (pmol/L)	13.8 ± 2	16.6 ± 2.6	24.1 ± 2.9
Ethanol dose 2			
NEFA (μmol/L)	422 ± 77	394 ± 96¶	257 ± 48‡
Glucose (mmol/L)	5.0 ± 0.1	4.7 ± 0.1†	4.7 ± 0.1
Insulin (pmol/L)	65 ± 7	65 ± 7‖	217 ± 14§
C-peptide (pmol/L)	477 ± 39	448 ± 46‖	386 ± 53
Glucagon (pmol/L)	14.3 ± 2.6	16.6 ± 2.3	26.7 ± 2.3

*$P < .05$, †$P < .005$: *v* basal.
‡$P < .05$, §$P < .01$: *v* saline/ethanol.
‖$P < .05$, ¶$P < .005$: *v* saline (ANOVA).

REE increased during infusion of ethanol alone in the present study, in agreement with previous data[26-29] but at variance with others.[30-32] REE increased to a greater extent during nutrient administration in the ethanol protocols compared with saline, suggesting that diet-enhanced thermogenesis was increased by ethanol, in agreement with previous data.[28,32,33] This effect may explain the failure of ethanol to inhibit leucine oxidation during nutrient administration.

Regarding the mechanism of ethanol-induced thermogenesis, the evidence that increased sympathetic nervous system activity is responsible for this phenomenon is conflicting. Ingestion of ethanol in humans resulted in no significant change in the urinary excretion of norepinephrine and epinephrine,[26] or in only a transient increase in plasma norepinephrine concentrations.[8] However, Randin et al[34] recently demonstrated a sympathoexcitatory and pressor effect of bolus infusions of high-dose ethanol. Alternatively, the thermic effect of ethanol may have been due to insulin-induced ethanol dehydrogenase activity and ethanol oxidation[35,36]; insulin deficiency resulted in decreased ethanol dehydrogenase activity.[37] Therefore, the present observation of an increase in energy expenditure during nutrient administration may have been caused both by enhanced ethanol oxidation due to hyperinsulinemia and by insulin-induced sympathetic activation.[37]

Plasma NEFA concentrations decreased during ethanol administration as compared with saline in the present study, with no difference between the low and high ethanol dose, suggesting maximal effects of ethanol at moderate blood ethanol levels. Similar results have been reported by others.[26,38] Since an elevation of plasma NEFA concentrations results in decreased leucine oxidation,[25,39] it is likely that the ethanol-induced decrease of leucine oxidation observed in the present study was even underestimated. Since increased plasma leucine concentrations augment leucine oxidation,[40] the present observation that ethanol increased plasma leucine probably resulted in a further underestimation of the postulated protein-sparing effect. The abolished decrease of plasma insulin levels during ethanol infusion compared with saline reflects the well-known insulinotropic effect of ethanol.[8] Since increased plasma insulin concentrations have been observed to decrease leucine flux,[41] this

suggests that the ethanol-induced increase in leucine flux observed in the present study was underestimated.

To conclude, the present results indicate a protein-sparing effect of ethanol when administered alone in normal subjects at relatively high doses during postabsorptive conditions. The ethanol-induced increase in $\dot{V}O_2$ and energy expenditure during anabolic conditions (substrate administration) may have counteracted the protein-anticatabolic effect.

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