Effects of a 3-Day Fast and of Ethanol on Splanchnic Metabolism of FFA, Amino Acids, and Carbohydrates in Healthy Young Men

B. M. WOLFE, J. R. HAVEL, E. B. MARLISS, J. P. KANE, J. SEYMOUR, and
S. P. AHUJA

From the Cardiovascular Research Institute and Department of Medicine,
University of California School of Medicine, San Francisco, California 94143;
the Elliott P. Joslin Research Laboratory, Boston, Massachusetts 02215; the
Department of Medicine, University of Toronto, Toronto, Ontario, Canada;
and the Department of Medicine, University of Western Ontario,
London, Ontario, Canada

A B S T R A C T Splanchnic metabolism was studied to quantify changes underlying the fatty liver, hyperlipemia, and hypoglycemia produced by ethanol. Four subjects fasted for 15 h were compared with five subjects fasted for 69 h under basal conditions and during continuous intravenous infusion of sufficient ethanol to give a concentration of 3–5 mM in arterial blood plasma. Splanchnic storage of fatty acids was estimated from the difference between uptake of FFA and secretion of derived products. Basal values for splanchnic uptake of FFA were twofold higher after the 69-h fast while splanchnic storage of fatty acids and production of ketone bodies increased threefold. Values for basal secretion into the blood of triglycerides derived from FFA were similar in the two groups. In both nutritional states, the fraction of FFA taken up in the splanchnic region oxidized to ketone bodies and to CO_2 fell when ethanol was given because of preferential oxidation of ethanol to acetate, and the fraction esterified rose. However, systemic transport and splanchnic uptake of FFA fell with ethanol in subjects fasted 15 h, so that neither storage of triglycerides in splanchnic tissues nor secretion into the blood

increased. In subjects fasted 69 h, ethanol increased transport of FFA and splanchnic storage of fat. In all but one subject it also increased secretion of triglycerides into the blood. The concentration of glucose in blood fell during ethanol infusion in all five subjects undergoing the 69-h fast. Mean splanchnic glucose production was maintained at about one-half of the pre-ethanol value, despite virtual cessation of splanchnic uptake of lactate and of those amino acids that are metabolized via malate. Quantitative estimates of extrasplanchnic metabolism suggest that enhanced formation of α-glycerophosphate from glucose, in addition to impaired hepatic gluconeogenesis, may contribute to ethanol-induced hypoglycemia in man.

INTRODUCTION

The fatty liver, hyperlipemia, and hypoglycemia that may accompany ingestion of ethanol have led to many studies of its effects on synthesis, transport, and catabolism of fatty acids and glucose in experimental animals (2–4). However, metabolic pathways such as those of gluconeogenesis and lipogenesis differ among species (5) and there is a paucity of information about the effects of ethanol on hepatic metabolism in man. Moreover, there has been little effort to correlate the various metabolic effects of ethanol in vitro and to relate them to maintenance of caloric homeostasis.

Ethanol is usually ingested with other foodstuffs but in alcoholics it often serves as the major source of energy in otherwise undernourished individuals. The present

study was performed to obtain data that would permit quantitative estimates of the uptake and metabolic conversions of the major substrates of splanchnic metabolism, FFA, amino acids, and carbohydrates, in healthy men during the usual postabsorptive state and after a 3-day fast. To facilitate the attainment of a steady state, ethanol was infused intravenously at a rate sufficient to saturate the capacity for its oxidation but which provided a blood level well below that known to increase release of catecholamines (6).

Although splanchnic oxidation of ethanol uniformly replaced that of FFA, rates of transport of FFA from extrasplanchnic tissues to the splanchnic region changed in opposite directions in the two nutritional states when ethanol was given, thereby either magnifying or diminishing the effects of the latter on splanchnic fatty acid metabolism. These different effects of ethanol were also reflected in the metabolism of extrasplanchnic tissues. Our observations can explain the hepatic steatosis observed in poorly nourished alcoholics as well as the tendency for triglycerides to accumulate in the blood. They also provide some new insights into the mechanisms of ethanol-induced hypoglycemia.

METHODS

Subjects. The subjects were healthy young male college students (Table I). Apart from a subject studied after a 15-h fast who had a marginal elevation of plasma triglycerides (151 mg/100 ml) none had clinical evidence of metabolic or other disease and none had a family history of diabetes mellitus. The four men undergoing the 15-h fast and the respective screening tests have been described (7). All procedures and risks were carefully explained to each subject, and his consent was obtained.

Preparation and experimental protocol. All subjects were consuming a regular diet and body weight (by history) had not varied appreciably for over a year in all except one subject fasted 15 h, who had lost 7 kg in association with increased physical activity several months before he was accepted for the study. Intake of ethanol in alcoholic beverages did not exceed 30 ml/day during the preceding 3 wk. All were admitted to the metabolic research ward of the hospital for strict dietary control for 3 days before the

study. Four subjects who were studied after a 15-h fast were maintained on a standard diet (7). No ethanol was permitted. Five subjects who were studied after a 69-h fast received one multivitamin tablet and drank at least 1.5 liters of water daily while fasting; they consumed regular diets before fasting.

The procedure for hepatic venous and arterial catheterization and the experimental protocol were as described previously (8, 9) with the following modifications: The 4-h catheterization study, during which albumin-bound [1-^{14}C]-palmitate (New England Nuclear, Boston, Mass.) and indocyanine green were infused at a constant rate, was divided into a 120-min control period followed by a 120-min period of ethanol infusion. In two subjects fasted 69 h the control period was extended to 240 min and was followed by a 120-min period of ethanol infusion. A priming dose of 80 mmol/m² ethanol, as a 10% solution in 75 mM sodium chloride in water, was given over 10 min through a plastic catheter placed in a left antecubital vein. Thereafter, a constant infusion of ethanol at the rate of 1 mmol/min·m² was maintained for the remainder of the study. Values for the control period were based on four sets of simultaneous arterial and hepatic venous blood samples obtained at 60, 80, 100, and 118 min.[1] During administration of ethanol, five sets were obtained at 140, 160, 180, 210, and 240 min. About 10% of blood volume was withdrawn over the 4-h period and this was replaced with 0.15 M sodium chloride solution. Since the concentration of FFA fell abruptly when ethanol was given, the 140-min values were omitted from the calculations. Simultaneous samples of arterial and hepatic venous blood were obtained for analysis of amino acids at 118 and 240 min in three subjects fasted 15 h and in five fasted 69 h. In a subject fasted 15 h and in three subjects fasted 69 h samples of blood for analysis of amino acids were also obtained at 180 min. Radioactivity in acetoacetate (AcAc)[2] was determined in hepatic venous blood obtained at 118 min.

Analyses. Ethanol was measured in duplicate samples of plasma by a microfluorimetric modification of the method of Bonnichsen (10). Acetate was measured in duplicate samples of plasma by a microfluorimetric modification of the method of Bergmeyer and Moellering (11). For deter-

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[1] The timing of the specimens began 120 min later in the two subjects fasted 69 h who received a 6-h infusion of [1-^{14}C]palmitate.

[2] *Abbreviations used in this paper:* AcAc, acetoacetate; β-OHB, β-hydroxybutyrate; TGFA, triglyceride fatty acid(s); VLDL, very low density lipoprotein(s).

TABLE I
Characterization of Groups of Subjects*

Duration of fast	Age	Height	Weight	Surface area	Ponderal index	Packed volume of erythrocytes	Serum lipid concentrations‡		
							Total cholesterol	Phospholipids	Triglycerides
h	yr	cm	kg	m²	$\frac{Ht\ (in)}{Wt\ (lb)^{\frac{1}{3}}}$	%	mg/100 ml		
15	26±2	182±3	73±5	1.92±0.07	13.2±0.3	43±1	192±17	178±9	115±19
69	28±2	176±2	66±3	1.80±0.02	13.2±0.3	44±1	190±9	181±15	63±6

* Mean value±SEM for four healthy subjects fasted 15 h and five healthy subjects fasted 69 h before study of splanchnic metabolism.
‡ All specimens were obtained after a 12 to 15-h fast.

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mination of amino acids, proteins were removed from plasma with sulfosalicylic acid (12). Amino acids were estimated by automated ion exchange chromatography (13) with a Beckman 120 C amino acid analyzer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) modified for single-column analysis of basic as well as acidic and neutral amino acids (14). The remainder of the analyses, including estimation of plasma volume, were performed as described previously (7–9).

Calculations. The general equations used have been described (8). Average values for splanchnic exchange of metabolites (except amino acids) and for [1-^{14}C]palmitate converted to triglyceride fatty acid (TGFA) of plasma and very low density lipoprotein (VLDL) were the means of three or four samples obtained from each subject between 60 and 118 min for the control period and from 160 to 240 min for the ethanol period. Values for transport in blood plasma of TGFA produced in the splanchnic region were obtained from the product of total splanchnic uptake of FFA and the percent of FFA converted to plasma TGFA. In four subjects fasted 15 h in whom control samples were obtained only between 60 and 120 min after starting the infusion of radioisotope, the values obtained for conversion of [^{14}C]FFA to [^{14}C]TGFA were corrected for incomplete equilibration of plasma FFA with hepatic precursor pools of VLDL-TGFA by multiplying by a factor of 1.5 (7). No such correction was applied in subjects fasted 69 h because the percent of FFA converted to TGFA increased only slightly between 60 and 80 and 100 and 118 min after starting infusion of [1-^{14}C]palmitate (6.4±1.5 vs. 7.2±1.3%, $P > 0.5$, $n = 3$). Transport of plasma TGFA was also estimated from the rate of isotopic equilibration of [^{14}C]-TGFA of plasma VLDL with hepatic venous plasma [^{14}C]-FFA (8, 15).

Total splanchnic oxidation of FFA was calculated stoichiometrically from splanchnic oxygen uptake, by assuming that oxygen not used for ketogenesis or oxidation of ethanol to acetate was used for complete oxidation of fatty acids.[3] Extrasplanchnic metabolism of ethanol was estimated by assuming that ethanol was distributed uniformly through body water within 100–120 min and that the volume of total body water was 23.3 liters/m^2 (19).

With the assumption that the α-glycerophosphate used to synthesize triglycerides from FFA taken up into extrasplanchnic tissues is derived entirely from glucose, the requirement of glucose carbon (glucose-C) for this process was estimated from the following equation (see Appendix): microatoms glucose-C required for esterification of FFA = 0.961 [(microatoms FFA-C uptake/17)+(microatoms glucose-C uptake/24.5) − (micromoles O$_2$ used for oxidation of glucose-C and FFA-C/24.5)].

Since ethanol (20) and acetate (21) are distributed be-

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[3] The oxidation of 1 mol of ethanol to acetate requires 1 mol of oxygen. For the partial oxidation of 1 mol of monounsaturated fatty acid with 17 carbon atoms, 7 mol of oxygen is required when the product is AcAc and 5 when it is β-hydroxybutyrate (β-OHB). The stated assumption is subject to the following limitations: (*a*) oxygen consumed in hepatic production of ketone bodies is underestimated because some ketone bodies are oxidized in extrasplanchnic tissues (16); (*b*) extramitochondrial oxidative processes may account for as much as 20% of hepatic oxygen consumption (17); (*c*) as much as 4% of the FFA taken up in the splanchnic region in the postabsorptive state may be used for synthesis of phospholipids secreted into bile (18).

tween erythrocytes and plasma in proportion to water, the following relationship holds: splanchnic uptake (or production) = plasma flow × arterial-hepatic venous concentration × (milliliter water per milliliter blood/milliliter plasma water per milliliter blood).

Differences between groups were evaluated according to Snedecor and Cochran (22) for both paired and unpaired samples. Variance is expressed as standard error of the mean.

RESULTS

General information about the nine subjects is given in Table I. The essential findings are summarized[4] in Tables II–IV and Figs. 1–4.

Concentration of metabolites in plasma and whole blood

The mean arterial blood glucose concentration did not change during ethanol infusion in subjects fasted 15 h, but it fell to 50 mg/100 ml or lower in four of five subjects fasted 69 h (Table II and Fig. 1). When ethanol was administered, the concentration of lactate rose (Fig. 1) and the lactate-pyruvate ratio in hepatic venous blood increased from 10±1.5 to 395±215 after the 15 h fast and from 22±5 to 104±26 after 69-h fast ($P < 0.05$); the concentration of pyruvate in subjects fasted 15 h (14±7 μM) or 69 h (15±1 μM) was not significantly different from the control value. The concentration of AcAc fell abruptly with ethanol and the β-OHB-AcAc ratio in hepatic venous blood rose during ethanol administration from 1.5±0.1 to 6.9±1.1 ($P < 0.025$) in the group fasted 15 h and from 2.6±0.6 to 5.3±1.0 ($P < 0.005$) in the group fasted 69 h.

During administration of ethanol, the arterial plasma concentration of FFA fell moderately in all subjects fasted in 15 h (Table II and Fig. 1); however, the decrease did not persist consistently throughout the period of ethanol infusion. After an initial fall, there was an increase in plasma FFA and blood glycerol (mean 37 and 85%, respectively, $P < 0.05$) in the group of subjects fasted 69 h.

The arterial plasma concentrations of four major amino acids (alanine, glycine, serine, and threonine) that are considered to be metabolized in liver via pyruvate (23) were somewhat lower in the group fasted 69 h; however, the difference was significant for serine only (91±2 vs. 136±3 μM, $P < 0.05$). The plasma concentrations of amino acids whose glucogenic carbons are thought to be metabolized in liver via malate (phenylalanine, tyrosine, methionine, and proline) and that of citrulline were not significantly different in the two groups, whereas concentrations of the branch chain

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TABLE II

Arterial Concentrations of Metabolites and Other Values Obtained during Study

| Duration of fast | Period | Plasma volume | Splanchnic | | | Arterial concentrations | |
| | | | Plasma flow | O₂ consumption | RQ | Plasma | |
						FFA	TGFA
h		*liters/m²*	*ml/min·m²*	*mmol/min·m²*		*μmol/ml*	
15	C	1.36±0.06	364±42	1.20±0.17	0.57±0.04	0.75±0.05	4.34±0.71
	E	—	461±72	1.33±0.26	0.34±0.06	0.60±0.06	3.94±0.79
69	C	1.63±0.05‡	439±19	1.79±0.11‡	0.31±0.04‡	1.75±0.08‡	2.53±0.11
	E	—	604±17§	1.94±0.14	0.36±0.06	2.40±0.17§·‖	2.23±0.13§

* Mean value±SEM for four healthy subjects fasted 15 h and five healthy subjects fasted 69 h before study of splanchnic metabolism.
‡ Significantly different from C of subjects fasted 15 h, $P < 0.05$.
§ Significantly different from C of subjects fasted 69 h, $P < 0.05$.
‖ Significantly different from E of subjects fasted 15 h, $P < 0.05$.

amino acids and of α-aminobutyrate were significantly higher in the group fasted 69 h, as expected.

The arterial plasma concentration of glycine fell significantly when ethanol was given to subjects fasted 15 h (130±19 vs. 149±15 μM) or 69 h (92±6 vs. 131±4 μM); however, the concentrations of serine (51±2 vs. 91±2 μM) and threonine (48±4 vs. 75±5 μM) fell significantly only in subjects fasted 69 h. The concentrations of glucogenic amino acids metabolized via malate
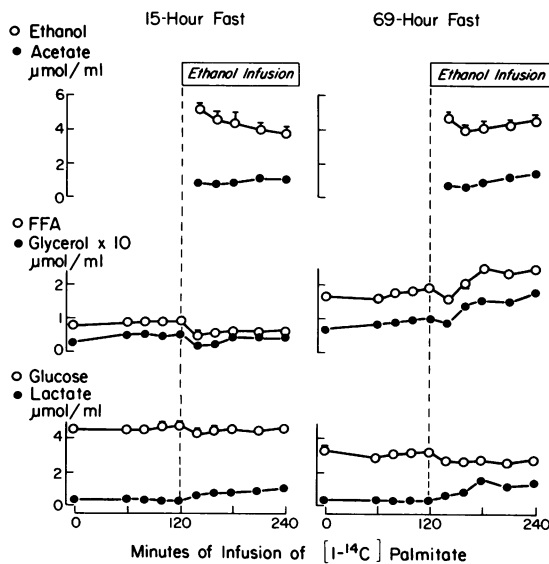

Figure 1 Arterial concentrations of some metabolites before and during infusion of ethanol in two nutritional states. Values are means obtained in four subjects fasted 15 h and five subjects fasted 69 h. Bars represent 1 SEM (where not shown, values were too small to be indicated). Concentrations of FFA, ethanol, and acetate are for plasma; others are for whole blood.

changed relatively little. The concentration of leucine of subjects fasted 69 h rose significantly when ethanol was given (310±14 vs. 270±16 μM), whereas that of citrulline fell (11±2 vs. 24±3 μM, $P < 0.05$). Arterial plasma levels of amino acids measured 1 and 2 h after starting ethanol in a subject fasted 15 h were within 10% of each other. In three subjects fasted 69 h the only differences were consistently lower concentrations of glycine, serine, threonine, and proline (21-31%) after 2 h; only the difference for serine was significant ($P < 0.05$).

Splanchnic metabolism of FFA

Effects of fasting. Total net inflow transport and splanchnic uptake of FFA were twofold higher in subjects fasted 69 h ($P < 0.05$, Table III). The percent of FFA taken up in splanchnic tissues that was converted to plasma TGFA in subjects fasted 69 h was significantly lower than in subjects fasted 15 h (8.3±1.1 vs. 16.4±2.2%, $P < 0.05$). Transport in blood plasma of TGFA produced from FFA in the splanchnic region (measured radiochemically) during the control period was similar in subjects fasted 15 and 69 h (20.4±2.9 vs. 21.6±3.7 μmol/min·m², respectively). From both radiochemical and chemical measurements, secretion of plasma TGFA was attributable entirely to secretion of plasma VLDL-TGFA. Values for transport of plasma VLDL-TGFA derived from FFA calculated from the rate of isotopic equilibration of [¹⁴C]TGFA of VLDL with [¹⁴C]FFA during the control period were similar to those obtained from radiochemical measurements of transsplanchnic gradients in ¹⁴C-labeled TGFA (data not shown).

Splanchnic oxidative metabolism of FFA was strikingly different after the 69-h fast (Fig. 2). The percent

Plasma	Blood					
VLDL-TGFA	AcAc	β-OHB	Glycerol	Glucose	Lactate	Pyruvate
μmol/ml			*μmol/ml*			
2.70±0.61	0.20±0.05	0.34±0.11	0.056±0.011	4.6±0.2	0.38±0.03	0.038±0.004
2.35±0.57‡	0.06±0.01	0.23±0.05	0.048±0.013‡	4.5±0.2	0.93±0.13‡	0.037±0.005
1.31±0.09	1.34±0.14‡	3.46±0.20‡	0.100±0.010‡	3.2±0.0‡	0.44±0.07	0.030±0.002
1.11±0.12§	0.85±0.09§·‖	3.94±0.22§·‖	0.185±0.017§·‖	2.7±0.1§·‖	1.16±0.14§	0.029±0.002

of FFA oxidized to β-OHB was significantly higher in subjects fasted 69 h (27±0.8 vs 19±3.8%, $P < 0.05$). Specific activity of the carbonyl carbon of blood AcAc was 80±14% ($n = 3$) of that of the [^{14}C]FFA carbon in hepatic venous blood plasma after the 69-h fast, a value similar to that of 84% obtained in a subject who fasted 15 h.[5] Splanchnic production of ketone bodies was threefold higher in subjects fasted 69 h than in those fasted 15 h (572±57 vs. 181±45 μmol/min·m², $P < 0.05$). The percent of FFA carbon taken up in the splanchnic bed that was converted to CO_2, not accurately reflected in conversion to $^{14}CO_2$ (17), was significantly lower after 69-h fast (14±2 vs. 31±5%, $P < 0.05$). Conversion of FFA to major secreted products accounted for a similar percent of FFA taken up in the

[5] Similar values (mean 82%) have been found in two other healthy subjects fasted for 15 h (8).

splanchnic region after 15- and 69-h fasts (81±10 vs. 76±8%, respectively); however, the amount of FFA not accounted for in secreted products was significant in subjects fasted 69 h (67±23 μmol/min·m²) and this was presumably stored in the liver.

Effects of ethanol after 15-h fast. The percent of FFA taken up in the splanchnic bed that was converted to plasma TGFA rose abruptly 40 min after infusion of ethanol was begun (Fig. 2). Although the mean increase after ethanol was 88%, this was not statistically significant because no change occurred in one of the four subjects. Net inflow transport of TGFA was unchanged because of decreased splanchnic uptake of FFA (Fig. 3). Values for the ratio (specific activity of arterial VLDL-TGFA)/(specific activity of hepatic venous FFA) after 240 min of infusion of [1-^{14}C]palmitate were 0.76 and 0.74 in two subjects.

TABLE III
Metabolism of FFA*

Duration of fast	Period	Arterial net inflow transport	Turnover rate	Splanchnic metabolism					Total net inflow transport	Total splanchnic uptake
				Extraction fraction	Uptake (arterial)	Net release	Uptake (from portal inflow)	Total uptake (arterial + portal)		Total net inflow transport
h		*μmol/min·m²*	*min⁻¹*		*μmol/min·m²*		*μmol/min·m²*		*μmol/min·m²*	
15	C	311±22	0.30±0.01	0.39±0.05	105±11	39±7	20±9	125±16	331±23	0.38±0.03
	E	254±29	0.32±0.03	0.33±0.02	88±13‡	16±3‡	6±1	94±14‡	260±30	0.36±0.03
69	C	584±77‡	0.20±0.01‡	0.31±0.02	241±17‡	57±13	20±4	261±18‡	603±76‡	0.44±0.03
	E	770±90§,‖	0.20±0.01‖	0.22±0.01§,‖	341±34§,‖	59±25	16±7	357±34§,‖	787±90§,‖	0.45±0.03

* Mean values ±SEM during control period (C) and subsequent 2-h infusion of ethanol (E) for four healthy subjects fasted 15 h and five healthy subjects fasted 69 h before study of splanchnic metabolism.
‡ Significantly different from C of subjects fasted 15 h, $P < 0.05$.
§ Significantly different from C of subjects fasted 69 h, $P < 0.05$.
‖ Significantly different from E of subjects fasted 15 h, $P < 0.025$.

15-Hour Fast   69-Hour Fast

Ketone-Carbon x 100
FFA Carbon

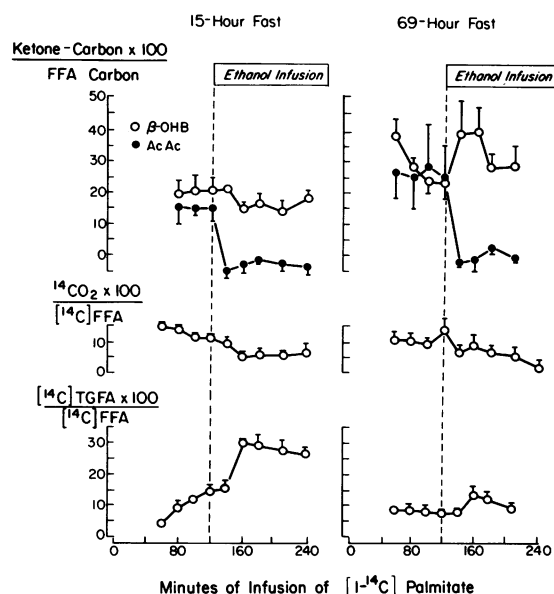Minutes of Infusion of $[1\text{-}^{14}C]$ Palmitate

FIGURE 2 Net splanchnic production of oxidized and esterified products of FFA expressed as percentage of splanchnic uptake of FFA. Values are means obtained in four subjects fasted 15 h and five subjects fasted 69 h. Bars represent 1 SEM.

Splanchnic oxidative metabolism of FFA was strikingly altered by ethanol. The percent of FFA oxidized to ketone bodies fell from 33±4 to 14±1% ($P < 0.05$). From stoichiometric calculations, complete oxidation of FFA also fell (25±10 vs. 39±6 μmol/min·m², $P < 0.05$; Fig. 3).

Effects of ethanol after 69-h fast. Net inflow transport and splanchnic uptake of FFA increased significantly, whereas their splanchnic extraction fell (Table III). Conversion of FFA to TGFA increased by an average of 35% (rising in four subjects, but falling 45% in another). Mean net inflow transport of TGFA increased 88% with increases occurring in four of five subjects (0.05 < P < 0.10, Fig. 3). FFA were not the sole precursor of fatty acids in VLDL-TGFA in subjects fasted 69 h. The mean value for the ratio (specific activity of arterial VLDL-TGFA)/(specific activity of hepatic venous FFA) after 225- to 240-min infusion of $[1\text{-}^{14}C]$palmitate in three subjects then receiving ethanol (0.58±0.04) was significantly lower than that (1.02± 0.03) of three previously reported postabsorptive subjects with similar concentrations of VLDL-TGFA (8) who were not given ethanol ($P < 0.025$). This ratio was somewhat higher (0.62 and 0.76) after 4-h infusion of radiopalmitate (before ethanol was given) in two other subjects fasted 69 h. Values in the latter two subjects for net splanchnic transport of TGFA derived from

FFA (based on determination of transsplanchnic gradients of ^{14}C-labeled triglycerides) were lower than respective rates of total splanchnic production of plasma TGFA (based on transsplanchnic chemical gradients). However, when a correction was made for the fraction of plasma VLDL-TGFA that was derived from precursors other than FFA, radiochemical values for total splanchnic production of plasma TGFA were similar to those obtained chemically.

As in subjects fasted 15 h, oxidation of FFA to AcAc was abolished with ethanol, but oxidation to β-OHB was unchanged (Fig. 2). The percent of FFA oxidized to $^{14}CO_2$ also fell (5.8±1.0 vs. 11±2.4%, $P < 0.05$, data not shown). Apparent storage of FFA in splanchnic tissues rose markedly (mean increase 160%) and accounted for 49% of splanchnic uptake (Fig. 3).

Splanchnic carbohydrate and amino acid metabolism

Effects of fasting. Mean net splanchnic production of glucose was 35% lower after the 69-h fast (167±31 vs. 257±67 μmol/min·m², 0.2 < P < 0.3) whereas formation of glyceride-glycerol was 360% higher (30±8 vs. 11±3 μmol/min·m², 0.05 < P < 0.10). Splanchnic uptakes of glycine (13.1±1.5 vs. 4.5±1.0), threonine (10.4±0.4 vs. 4.5±1.0), phenylalanine (4.2±0.3 vs. 0.3± 0.8), and tyrosine (6.8±0.7 vs. 2.6±0.6 μmol/min·m²) were significantly higher in subjects undergoing the 69-h fast, as was their splanchnic extraction fraction
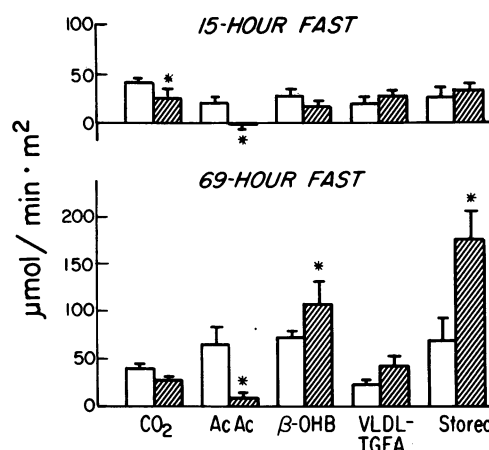
FIGURE 3 Conversion of FFA taken up in the splanchnic region to secreted and stored products. Values expressed in micromoles FFA equivalents (17 carbon atoms/molecule) are the mean±SEM during the control period (open bars) and subsequent 2-h infusion of ethanol (hatched bars) for four healthy subjects fasted 15 h and five healthy subjects fasted 69 h before study of splanchnic metabolism. * Significantly different from control. $P < 0.05$.

($P < 0.05$). Net uptake of all measured potential glucose precursors, including whole blood glycerol, lactate, and pyruvate and plasma amino acids (216 ± 30 μmol/min·m^2), was equivalent in carbon atoms to only 45% of net splanchnic carbohydrate formation (glucose secretion + formation of glyceride-glycerol) in subjects fasted 15 h. However, uptake of these potential precursors reached 85% (285 ± 13 μmol/min·m^2) in subjects fasted 69 h. Lactate was consistently the major single precursor of carbohydrates formed in the splanchnic region (mainly glucose) under basal conditions; however, uptake of glycerol, glycine, threonine, phenylalanine, tyrosine, and isoleucine rose in subjects fasted 69 h ($P < 0.05$).

Effects of ethanol. After the 15-h fast, uptake of lactate in splanchnic tissues ceased completely and was replaced by net production (-42 ± 9 vs. 123 ± 17 μmol/min·m^2, $P < 0.05$). By contrast, net uptake of pyruvate rose (17 ± 5 vs. 11 ± 3 μmol/m·m^2, $0.2 < P < 0.3$). Splanchnic uptake of glycerol fell because of a fall in its extraction fraction (0.77 ± 0.3 vs. 0.88 ± 0.2, $P < 0.05$; Fig. 4). Splanchnic exchange of amino acids was essentially unchanged. Net splanchnic production of glucose (340 ± 70 vs. 257 ± 67 μmol/min·m^2, $0.2 < P < 0.3$) and glyceride-glycerol (17 ± 3 vs. 11 ± 3 μmol/min·m^2, $0.2 < P < 0.3$) increased in all subjects, although the potential contribution of the measured glucogenic substrates fell from 45 to 12% ($0.10 < P < 0.2$).

Splanchnic exchange of lactate and pyruvate was similarly affected by ethanol after the 69-h fast. Splanchnic uptake of glycerol doubled ($P < 0.05$) as a result of its increased concentration in arterial blood (Table II and Fig. 4). Little change occurred in the summed splanchnic uptake of amino acids that are metabolized via pyruvate, but uptake of those that are metabolized via malate fell significantly and the branched-chain amino acids (valine, isoleucine, and leucine) were consistently released from the splanchnic region (Fig. 4). Net splanchnic glucose production fell 45% (91 ± 37 vs. 167 ± 31 μmol/min·m^2); however, there was wide variation between subjects from cessation of glucose production in one subject to partial or complete maintenance in each of two subjects. Splanchnic production of glyceride-glycerol more than doubled (72 ± 10 vs. 30 ± 8 μmol/min·m^2, $P < 0.05$). The mean potential contribution of the measured glucogenic substrates to estimated splanchnic carbohydrate synthesis (glucose + glyceride-glycerol) fell consistently when ethanol was given (54 ± 10 vs. $85\pm10\%$, $P < 0.05$). Glycerol became the principal glucogenic precursor, accounting for $83\pm19\%$ of the total splanchnic uptake of carbohydrate and amino acid substrates (average of five individual values).

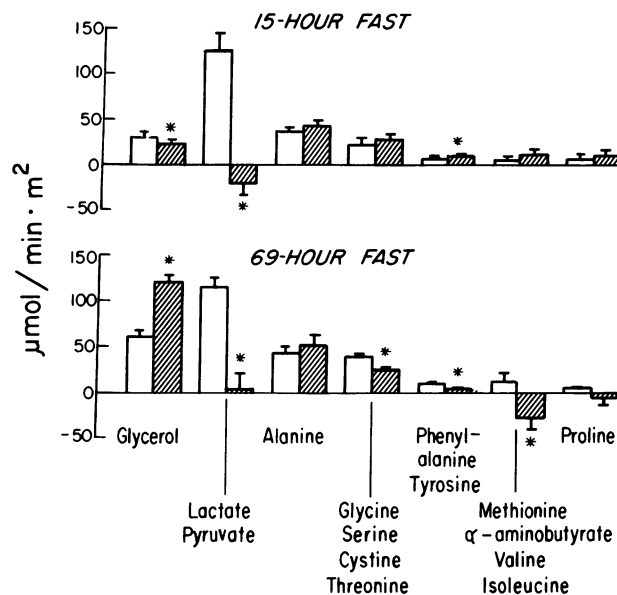The mean concentration of ethanol and arterial blood plasma was similar in the two groups, 4.2 and 4.3 mM

FIGURE 4 Splanchnic exchange of carbohydrates and amino acids. Values are means±SEM during the control period (open bars) and subsequent 2-h infusion of ethanol (hatched bars) for three healthy subjects fasted 15 h and five healthy subjects fasted 69 h before study of splanchnic metabolism. * Significantly different from control, $P < 0.05$.

(Fig. 1), as was splanchnic uptake of ethanol. Hepatic venous-arterial difference in concentration of plasma acetate was significantly lower than arterial-hepatic venous difference for ethanol in subjects fasted 15 h (0.69 ± 0.6 vs. 1.0 ± 0.14 mM, $P < 0.05$); these values were almost identical in subjects fasted 69 h (0.72 ± 0.06 vs. 0.74 ± 0.08 mM, respectively).

Splanchnic metabolism of oxygen

Utilization of oxygen for major oxidative processes in splanchnic tissues is shown in Table IV. Oxygen remaining after partial oxidation of FFA to ketone bodies and of ethanol to acetate largely represents the complete oxidation of substrates in the tricarboxylic acid cycle. During administration of ethanol, oxidative reactions were significantly reduced in subjects fasted 15 h ($P < 0.05$) and they fell in four of five subjects fasted 69 h.

DISCUSSION

The measurements made here provide estimates of the effects of short-term fasting on splanchnic oxidative metabolism in healthy, lean men. By contrast with the constancy of total body oxygen consumption (24), splanchnic oxygen consumption was significantly higher (49%) after the 69- vs. the 15-h fast and was similar to that reported for subjects fasted 80–86 h (25). Increased uti-

TABLE IV

*Splanchnic Oxygen Metabolism**

Duration of fast	Period	Total splanchnic O₂ consumption	O_2 consumed for oxidation of:				Percent total O_2 consumed for oxidation of:		
			FFA to:		Ethanol to acetate	Other substrates‡	Ethanol to acetate	FFA to ketones	Ethanol plus ketones
			AcAc	β-OHB					
h		μmol/min·m²			μmol/min·m²			%	
15	C	1,195±169	125±35	126±30	—	945±142	—	21±5	—
	E	1,330±263	0§	67±13‖	672±26	591±239§	56±9	5.1±0.7§	61±10
69	C	1,794±109§	439±130	357±29§	—	916±157	—	46±8§	—
	E	1,936±141	56±19¶**	528±115**	678±88‡‡	734±101	35±3	30±4**	65±4

* Mean values±SEM during control period (C) and subsequent 2-h infusion of ethanol (E) for four healthy subjects fasted 15 h and five healthy subjects fasted 69 h before study of splanchnic metabolism.

‡ Includes nonmitochondrial respiration (17).

§ Significantly different from C of subjects fasted 15 h, $P < 0.05$.

‖ Corrected for uptake of AcAc.

¶ Significantly different from C of subjects fasted 69 h, $P < 0.05$.

** Significantly different from E of subjects fasted 15 h, $P < 0.05$.

‡‡ Value based on three subjects.

lization of oxygen in the formation of ketone bodies (which trebled) accounted for at least 92% of this increase (Table IV).⁶

The decrease in the percent of FFA converted to plasma VLDL-TGFA after the 69-h fast contrasts with the augmented conversion to ketone bodies (Fig. 3). However, as a result of the doubling of splanchnic uptake of FFA, net splanchnic production of TGFA derived from FFA changed little after the longer fast. Since, in agreement with previous reports (25, 27), the concentration of VLDL-TGFA was not affected by fasting, peripheral removal of TGFA evidently was not impaired. Total production of plasma TGFA probably increased because only about two-thirds of VLDL-TGFA was found to be derived from FFA in the two subjects in whom production of TGFA was measured chemically (the remainder was probably derived from fat stored in the liver.)⁷ Because of this, our values for splanchnic storage of fat (Fig. 4) may be somewhat high. Accumulation of hepatic triglycerides with fasting has also been observed in rabbits (28). Increased oxidation of FFA to ketone bodies minimizes this accumulation.

Most of the glucose secreted from the liver in the postabsorptive state is derived from glycogen. This source is largely depleted after a 3-day fast (29) and our data show, in accordance with a previous study (25), that potential glucose precursors could account for over 85% of the glucose and glyceride-glycerol produced in the splanchnic region at this time. Our estimates of splanchnic flux of amino acids are based upon concentrations in plasma and thus are in error to the extent that red blood cells contribute to splanchnic flux of amino acids (in postabsorptive subjects, about 20%, reference 30). The only additional amino acid that would be expected to make a substantial additional contribution is glutamine (31), and recent data suggest that it is removed by extrahepatic splanchnic tissues rather than liver (32). Felig et al. (33) have previously observed increased splanchnic extraction of gluconeogenic amino acids in subjects undergoing cardiac catheterization after fasting 36-48 h.

Ethanol profoundly altered splanchnic oxidative metabolism, replacing other substrates as the main fuel in both nutritional states (Table IV). However, its effect upon fat mobilization from adipose tissue varied with duration of fast. Inhibition of lipolysis by ethanol in the postabsorptive state, evidently mediated by acetate (34), may be related to generation of ATP during oxidation of acetate in adipocytes, a process that could supply energy for reesterification of FFA. The enhancement of lipolysis by low concentrations of ethanol after a 3-day fast (Table III and reference 35) may result from the associated hypoglycemia (Fig. 1) and release of counter-regulatory hormones (36).

Net splanchnic production of plasma TGFA from FFA usually rose with ethanol particularly after the 69-h fast (Fig. 3). Although the value of 28.6% for

⁶ Increased plasma levels after a 69-h fast are likely to have increased extrahepatic splanchnic uptake of ketone bodies (26) leading to underestimation of hepatic ketone body production and of the requirement of oxygen for their formation.

⁷ It is apparent from the balance between splanchnic production of acetate and uptake of ethanol that acetate was not utilized for synthesis of plasma TGFA in subjects fasted 69 h.

TABLE V
Extrasplanchnic Metabolism (Mean Values)

	15 − h fast (n = 4)		69 − h fast (n = 5)	
	Control	Ethanol	Control	Ethanol
	μmol carbon/min·m²			
Estimated extrasplanchnic uptake				
FFA	3,498	2,826	5,821	7,307
Glucose*	915	1,785	655	542
AcAc	303	0‡	1,066	135
β-OHB	426	228	1,214	1,796
Ethanol	—	274	—	0§
Acetate	—	942	—	1,323§
	μmol/min·m²			
Oxygen consumption				
Splanchnic	1,200	1,330	1,794	1,936
Extrasplanchnic (estimated)	5,405	5,270	4,806	4,664
	μmol/min·m²			
Oxygen consumed in extrasplanchnic oxidation of:‖				
AcAc	303	0	1,066	135
β-OHB	480	257	1,365	2,019
Ethanol	—	411	—	0§
Acetate	—	942	—	1,323§
Glucose	863	1,699	393	154
FFA	3,757	1,962	1,981	1,033
	μmol carbon/min·m²			
Estimated				
Extrasplanchnic oxidation of FFA	2,609	1,361	1,375	716
Extrasplanchnic storage of FFA	889	1,466	4,446	6,590
Glucose (or equivalent) required for esterification of FFA	53	87	262	388

* Corrected for recycling of lactate and pyruvate.

‡ Slight splanchnic uptake of AcAc which was subtracted from β-OHB.

§ Value based on measurements in three subjects only.

‖ It has been assumed that all subjects had normal basal oxygen consumption, that fasting (24) and ethanol (41) did not change it, that any ethanol not accounted for by splanchnic oxidation or by distribution in body water was oxidized in the extrasplanchnic region, that glucose not recycled as lactate and pyruvate and AcAc, β-OHB, and acetate were oxidized in extrasplanchnic tissues at the rate at which they were released from the splanchnic bed, and that extrahepatic oxidation of other substrates was negligible. The assumption for ketone bodies overestimates actual oxidation, particularly in subjects fasted 69 h, since ketone bodies accumulated in their body fluids during the 2-h control period and an appreciable amount was excreted in urine (26). Oxygen use not attributable to these substrates was taken to be used for oxidation of FFA and glucose. FFA extracted in extrasplanchnic tissues in excess of that oxidized was assumed to be stored as triglycerides; this requires provision of ⅓ mol of α-glycerophosphate per mole of FFA, which was taken to be supplied by glucose. Glycogen in skeletal muscle, unlike that of liver, is not grossly depleted after a 2-day fast and its concentration at rest appears to be stable during more prolonged fasting (42).

percent conversion of FFA to plasma TGFA in subjects fasted 15 h after ethanol was not significantly higher than the control value of 16.4%, the following considerations leave little doubt that, as predicted by Nestel and Hirsch (37), ethanol promoted splanchnic conversion of FFA to plasma TGFA. First, fractional conversion increased abruptly 40 min after infusion of ethanol was begun (Fig. 2). Second, the value of 28.6 after ethanol is significantly higher than that of 18.4±

0.7% found previously (8) in four healthy young men given radiopalmitate infusion for 4 h (P < 0.05) whereas the control value in the present subjects (16.4%) is simliar. Splanchnic uptake of FFA increased with ethanol after the 69-h fast (Table III), and secretion of TGFA in VLDL more than doubled in three of the five subjects. In one it increased by 50% and in another it fell. Nevertheless, the concentration of VLDL-TGFA fell slightly (Table II). Although increased se-

cretion of VLDL appears to occur commonly with ethanol in poorly nourished subjects, a preexisting defect in plasma triglyceride catabolism may be a prerequisite for the development of hypertriglyceridemia (38).

Ethanol had a variable effect upon splanchnic glucose production depending upon the nutritional state. In subjects fasted 15 h, the rise in splanchnic glucose output (average 32%) was attributable to glycogenolysis since the measured splanchnic uptake of potential precursors fell by 59% (Fig. 4). The maintenance of splanchnic glucose production while its concentration fell in two of five subjects given ethanol after the 69-h fast suggests that hypoglycemia produced by ethanol may not be explained solely by inhibition of gluconeogenesis (39).

The inhibitory effect of ethanol on splanchnic uptake of glucose precursors was limited to lactate and (after the 69-h fast) to a group of amino acids entering glucogenic pathways via malate (whose contribution to glucogenesis is small) each requiring oxidized pyridine nucleotides, at least initially, for conversion to glucose. The virtual cessation of splanchnic uptake of lactate confirms the view that hyperlactacidemia produced by ethanol is at least partly attributable to inhibition of the Cori cycle (40).

The measurements of net splanchnic production of major oxidizable substrates, together with those for transport of FFA, permit an assessment of the effects of fasting and of ethanol upon oxidative metabolism in extrasplanchnic tissues. The results of this assessment, summarized in Table V, show that the effects of ethanol upon extrasplanchnic metabolism differed substantially in the two nutritional states. After the 15-h fast, extrasplanchnic uptake of ethanol and acetate compensated for the fall in provision of FFA and ketone bodies, so that storage of FFA rose little, while oxidation of glucose increased because of an interruption of the Cori cycle. After the 69-h fast, uptake of acetate was accompanied by increased provision of FFA, but essentially no change in that of ketone bodies, so that storage of FFA increased fivefold. Increased utilization of glucose for esterification could be a factor in the production of hypoglycemia by ethanol. The extent to which glucose, rather than other substrates, is used to synthesize the α-glycerophosphate needed remains to be determined. However, enhanced disposal of glucose during ethanol-induced hypoglycemia has been demonstrated recently by measurement of glucose kinetics in healthy, lean subjects fasted for 3 days (43). The large increase in estimated extrasplanchnic storage of FFA with ethanol is compatible with reports that it increases myocardial triglyceride content (44), augments incorporation of FFA into triglycerides in perfused heart (45), and increases uptake of glucose in the intact heart while diminishing oxidation of FFA (46).

APPENDIX

Derivation of the equation estimating glucose carbon or equivalent (glucose-C) requirement for the esterification of FFA carbon (FFA-C) stored in extrasplachnic tissues (1 μmol O_2 *is required to oxidize 1 μatom of glucose-C and 24.5 μmol O_2 is required to oxidize 17 μatoms of FFA-C).*

μatoms of glucose-C required for esterification of FFA

$$= \frac{\text{μatoms of FFA-C stored}}{17} \tag{1}$$

$$= \frac{\text{μatoms FFA-C uptake} - \text{μatoms FFA-C oxidized}}{17} \tag{2}$$

$$= \frac{\text{μatoms FFA-C uptake} - \frac{17}{24.5}\left(\begin{array}{c}\text{μmol } O_2 \text{ remaining for} \\ \text{oxidation of glucose-C and FFA-C}\end{array} - \begin{array}{c}\text{glucose-C available} \\ \text{for oxidation}\end{array}\right)}{17} \tag{3}$$

$$= \frac{\text{μatoms FFA-C uptake}}{17} - \frac{\begin{array}{c}\text{μmol } O_2 \text{ remaining for oxidation} \\ \text{of glucose-C and FFA-C}\end{array} - \begin{array}{c}\text{glucose-C available} \\ \text{for oxidation}\end{array}}{24.5} \tag{4}$$

$$= \frac{\text{μatoms FFA-C uptake}}{17} - \frac{\begin{array}{c}\text{μmol } O_2 \text{ remaining for oxida-} \\ \text{tion of glucose-C and FFA-C}\end{array} - \left(\begin{array}{c}\text{μatoms glucose-} \\ \text{C uptake}\end{array} - \begin{array}{c}\text{μatoms glucose-C required for} \\ \text{esterification of FFA-C}\end{array}\right)}{24.5} \tag{5}$$

$$= \frac{\text{μatoms FFA-C uptake}}{17} - \frac{\begin{array}{c}\text{μmol } O_2 \text{ remaining for oxida-} \\ \text{tion of glucose-C and FFA-C}\end{array}}{24.5} + \frac{\begin{array}{c}\text{μatoms glucose-} \\ \text{C uptake}\end{array}}{24.5} - \frac{\begin{array}{c}\text{μatoms glucose-C required for} \\ \text{esterification of FFA-C}\end{array}}{24.5} \tag{6}$$

$$\mu\text{atoms of glucose-C required for esterification of FFA-C} \times \left(1 + \frac{1}{24.5}\right)$$

$$= \frac{\mu\text{atoms FFA-C uptake}}{17} - \frac{\mu\text{mol O}_2 \text{ remaining for oxidation of glucose-C and FFA-C}}{24.5} + \frac{\mu\text{atoms glucose-C uptake}}{24.5} \tag{7}$$

μatoms of glucose-C required for esterification of FFA

$$= 0.961 \left(\frac{\mu\text{atoms FFA-C uptake}}{17} - \frac{\mu\text{mol O}_2 \text{ remaining for oxidation of glucose-C and FFA-C}}{24.5} + \frac{\mu\text{atoms glucose-C uptake}}{24.5} \right) \tag{8}$$

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