

# Hepatic Ketogenesis and Gluconeogenesis in Humans

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**ABSTRACT** Splanchnic arterio-hepatic venous differences for a variety of substrates associated with carbohydrate and lipid metabolism were determined simultaneously with hepatic blood flow in five patients after 3 days of starvation.

Despite the relative predominance of circulating  $\beta$ -hydroxybutyrate, the splanchnic productions of both  $\beta$ -hydroxybutyrate and acetoacetate were approximately equal, totaling 115 g/24 h. This rate of hepatic ketogenesis was as great as that noted previously after 5–6 wk of starvation. Since the degree of hyperketonemia was about threefold greater after 5–6 wk of starvation, it seems likely that the rate of ketone-body removal by peripheral tissues is as important in the development of the increased ketone-body concentrations observed after prolonged starvation as increased hepatic ketone-body production rate.

Splanchnic glucose release in this study was 123 g/24 h, which was less than that noted previously after an overnight fast, but was considerably more than that noted during prolonged starvation. Hepatic gluconeogenesis was estimated to be 99 g/24 h, calculated as the sum of lactate, pyruvate, glycerol, and amino acid uptake. This was greater than that observed either after an overnight fast or after prolonged starvation. In addition, a direct relationship between the processes of hepatic ketogenesis and gluconeogenesis was observed.

## INTRODUCTION

The accumulation of ketone bodies (acetoacetate [AcAc]<sup>1</sup> and  $\beta$ -hydroxybutyrate [ $\beta$ -OHB]) in the bloodstream is a consequence of a relative imbalance between production and removal rates. While the liver is the only organ in humans that makes a net contribution of ketone bodies to the bloodstream, muscle, kidney, and brain are quantitatively the most important tissues

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<sup>1</sup>Abbreviations used in this paper: AcAc, acetoacetate;  $\beta$ -OHB,  $\beta$ -hydroxybutyrate.

that remove ketone bodies from the blood (1–3). However, unlike kidney and brain, the utilization of ketone bodies by skeletal muscle is not dependent solely upon the arterial concentrations of these substrates (2, 4). After a 3- and 24-day fast, total ketone-body (AcAc plus  $\beta$ -OHB) venous blood concentrations in obese subjects were about 2.5 and 7.5 mmol/liter, respectively (1, 2, 5). It was reported that after 3–7 days of starvation, ketone bodies were the preferred fuels for skeletal muscle, accounting for 50–85% of muscle oxidative metabolism in obese and lean subjects (2, 6). However, it was observed in obese subjects that after more prolonged periods of starvation, free fatty acids (FFA) were preferentially utilized by muscle, and only a small net uptake of ketone bodies occurred (2, 7, 8). It is therefore possible that the decreased ketone-body utilization by muscle, rather than an increased ketone-body production by the liver, may in part be responsible for the heightened ketonemia observed after prolonged starvation. In order to investigate this possibility, hepatic ketone-body production rate in humans after a 3-day fast was determined. In addition, this study offered the opportunity to reassess hepatic gluconeogenesis and to delineate the relationship between ketogenesis and gluconeogenesis after a 3-day fast.

## METHODS

**Subjects.** Five patient volunteers requiring diagnostic cardiac catheterization were selected for admission to the General Clinical Research Center of the Temple University Hospital and are described in Table I. All patients were informed of the medical necessity for, and the associated risks of, cardiac catheterization. The additional risks of both hepatic venous catheterization and starvation were also explained. No patient had evidence of congestive heart failure, diabetes mellitus, hepatic or renal insufficiency, or thyroid and adrenal abnormalities. On admission, all patients had normal serum thyroxine measurements and urinalyses. Routine determinations of blood chemistries were performed by Technicon autoanalyzer procedures (Technicon Instruments Corp., Tarrytown, N. Y.) in the Clinical Laboratories of the Temple University Hospital. In all instances, normal SMA 6/60 profiles (serum electrolytes, blood urea nitrogen, and fasting glucose) were obtained.

TABLE I  
Clinical Data

Subject	Age	Sex	Height	Weight	Expected weight range*	Surface area	IVGTT‡	HCT§	Diagnoses
	<i>yr</i>		<i>cm</i>	<i>kg</i>	<i>kg</i>	<i>m</i> <sup>2</sup>	<i>k</i>	<i>%</i>	
J. J.	37	M	167.6	59.6	66.4–82.8	1.67	1.10	35.0	Essential hypertension; chronic dissection of aortic arch
L. S.	37	M	174.0	87.3	71.4–87.8	2.02	—	43.0	Normal cardiac anatomy
R. W.	41	M	180.4	94.6	74.5–90.9	2.15	2.99	42.0	Normal cardiac anatomy
R. B.	40	F	160.0	101.1	56.0–76.1	2.05	1.82	43.5	Normal cardiac anatomy
E. S.	42	F	167.0	60.5	58.7–79.1	1.64	1.31	39.0	Essential hypertension; ballooning of posterior leaflet mitral valve

\* Range of weights for approximately 50% of the population, corrected for age and height (U. S. Department of Health, Education, and Welfare, Public Health Service, *Vital and Health Statistics*, Series 11-No. 14.

‡ IVGTT, intravenous glucose tolerance test.

§ Venous hematocrit at the time of admission to the General Clinical Research Center.

SMA 12/60 profiles (total protein, albumin, calcium, inorganic phosphorus, total bilirubin, uric acid, cholesterol, creatinine, alkaline phosphatase, creatine phosphokinase, lactic dehydrogenase, and glutamate oxalacetate transaminase) during the prestarvation interval were normal with the exception of persistent mild elevations of creatine phosphokinase noted in patient R. W. This patient also had an extraordinarily large skeletal muscle mass. Chest and cardiac chamber radiographs and fluoroscopies were normal except for J. J. who demonstrated a widened and tortuous aortic arch. Electrocardiograms showed left ventricular hypertrophy (by the point score system) in J. J., R. W., and E. S. The latter patient also had a left atrial abnormality. No evidence of myocardial fibrosis was found in any patient. In the two patients with known essential hypertension (J. J. and E. S.) diastolic pressures did not exceed 100 mm Hg during their hospitalizations in the General Clinical Research Center.

During the prestarvation period, patients were maintained on diets containing about 2,400 calories/day, consisting of approximately 100 g protein, 85 g fat, and 300 g carbohydrate. An intravenous glucose tolerance test (9) was performed in each subject after an overnight fast, except in L. S., who had a normal 2-h postprandial blood glucose concentration.

During starvation, each patient was limited daily to 1,500 ml of water, 17 mEq of NaCl (sugar-free tablets), 17 mEq of KCl (gelatin capsules), and one multivitamin capsule (Unicap, The Upjohn Co., Kalamazoo, Mich.). All other medications were discontinued during the 3 days of starvation.

*Blood and urine collections.* Base-line blood samples were obtained from the antecubital veins after an overnight fast (day zero) and on days 1, 2, and 3 of starvation (day of catheterization) and analyzed immediately for glucose, AcAc, and  $\beta$ -OHB. Plasma was analyzed immediately for determining free FFA and triglyceride concentrations were kept frozen at  $-20^{\circ}\text{C}$  until assayed. Plasma specimens for determining free FFA and triglyceride concentrations were

frozen and thawed twice before analyses were completed.<sup>2</sup> Urine was collected in refrigerated plastic containers for 24-h periods. The volumes were measured and portions were frozen at  $-20^{\circ}\text{C}$ . Analyses of urinary AcAc,  $\beta$ -OHB, and total nitrogen were performed within 4 days of the catheterization.

*Catheterization, blood flow, and blood sampling.* All catheterizations were begun at 8:00 a.m. on the third day (80–86 h) of starvation. A Cournand catheter (no. 7) was inserted in an exposed deep antecubital vein and advanced to the main right hepatic vein under fluoroscopic guidance. The catheter tip was placed approximately 2–3 cm from the wedge position, and its location was checked immediately before and after each blood sampling period. A Cordis catheter (pigtail no. 8, Cordis Laboratories, Miami, Fla.) was inserted percutaneously into a femoral artery and advanced into the aorta. The catheters were kept patent by intermittent flushing with 0.5% sodium citrate in isotonic saline. The total amount of citrate received by each subject was less than 0.2 g for the entire procedure. Hepatic blood flow was measured by the primed continuous infusion technique (10) by using indocyanine green dye (11). After the catheters were placed and the dye infusion begun, no further manipulations were performed for about 30 min. At the end of this period, blood samples were collected simultaneously from the aorta and hepatic vein every 10 min for three sets. Immediately after withdrawal, blood aliquots were made for rapid analyses of respiratory gas contents, cardiac green concentrations, hematocrits, and appropriate substrates. 10 ml of fresh blood was injected into 10 ml of ice-cold 1 M perchloric acid and mixed. After

<sup>2</sup> Determinations of triglyceride, glycerol, and FFA concentrations in stored specimens frozen and thawed show: (a) reasonably consistent triglyceride and glycerol concentration, providing the specimens are not left thawed for too many hours, and (b) inconsistent fluctuations in FFA concentrations (probably due to technical limitations inherent in the titration method).

centrifugation at 4°C, supernatant portions were analyzed as quickly as possible for pyruvate and AcAc. The remaining supernate was stored overnight at -20°C and analyzed the next day for lactate and  $\beta$ -OHB. Plasma FFA and triglyceride concentrations were determined on specimens, stored at -20°C, but thawed twice before analyses. Plasma glycerol and alanine and blood glucose concentrations were determined from aliquots stored at -20°C until analyzed. Isovolumetric quantities of 5% human albumin in normal saline were administered simultaneously to each patient to replace blood withdrawn during the study.

At the conclusion of the sampling periods, the indocyanine green dye administration was interrupted, and the diagnostic catheterization was completed. Cardiac outputs were then determined by dye dilution curves after a single intravenous injection of 5 mg of indocyanine green (12). Cardiac outputs using the Fick principle were also estimated simultaneously by the arteriovenous oxygen differences (aortic-pulmonary) and the rate per minute of oxygen consumption by the patient.

**Chemical determinations.** The analytical methods and the precision for the determination of blood AcAc,  $\beta$ -OHB, lactate, pyruvate, glucose,  $O_2$  and  $CO_2$  content, plasma double-extracted FFA, serum insulin, and urinary AcAc and  $\beta$ -OHB, as performed in our laboratories, have been previously published (2). Total urinary nitrogen was determined in duplicate by the standard micro-Kjeldahl technique. Plasma triglycerides and glycerol were determined enzymatically by using glycerol kinase, pyruvate kinase, and lactate dehydrogenase (13, 14). Plasma alanine was determined spectrophotometrically by using bacterial alanine dehydrogenase and  $NAD^+$  in the Elliott P. Joslin Research Laboratories under the direction of Dr. George F. Cahill, Jr. (15). Glucose determinations were done in triplicate, and FFA and triglyceride determinations were done in quadruplicate. All other substrates and gas determinations were performed in duplicate. The precision for the determination of plasma triglyceride and glycerol was established by using pooled normal plasma. Assays on the pooled plasma sample were repeated 10 times and the values expressed as mean  $\pm$  SEM were  $0.161 \pm 0.001$  mmol/liter for glycerol and  $1.388 \pm 0.047$  mmol/liter for triglycerides.

**Statistics.** Differences in arterial and venous concentrations were evaluated by the paired and Student's *t* test (small sample method, two-tailed). Linear regressions were calculated by the method of least squares and their significance tested by determining the correlation coefficient (*r*). Values are expressed as the mean  $\pm$  SEM (16).

## RESULTS

**Circulating insulin and substrate concentrations and urinary losses during starvation.** Changes in venous blood concentrations of AcAc,  $\beta$ -OHB, FFA, triglycerides, glycerol, glucose, and immunoreactive insulin induced by 3 days of starvation in these five patients are shown in Fig. 1. The increases in circulating ketone bodies and FFA are comparable to those previously reported (1, 5). Plasma glycerol concentrations increased (5, 17), while the concentration of plasma triglycerides remained constant throughout the starvation period (18). Blood glucose and serum insulin concentrations both declined significantly (1, 5).

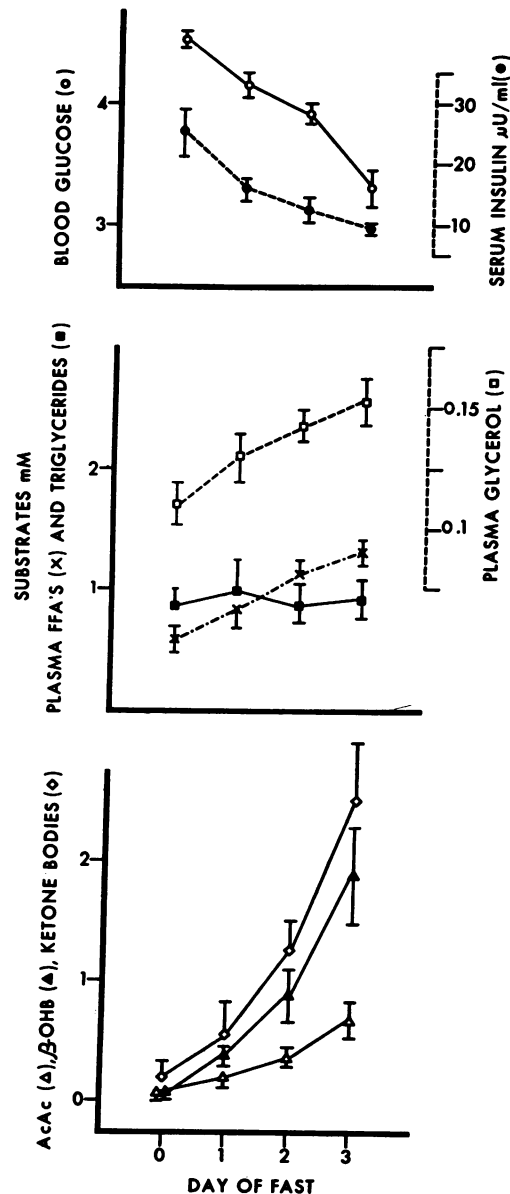


FIGURE 1 Circulating concentrations of substrates and insulin in the patients during the precatheterization fasting period. Concentration of glucose, AcAc,  $\beta$ -OHB, and total ketone bodies are given for whole blood, whereas FFA, glycerol, and triglycerides are reported as plasma concentrations. Serum insulin values are shown. Values are the means  $\pm$  SEM for all subjects and are expressed as mmol/liter, with the exception of insulin which is expressed as microunits per milliliter.

24-h urinary excretion of nitrogen products was determined for each patient, and this was found to increase from  $8.56 \pm 1.84$  g  $N_2$  on the first day to  $9.25 \pm 2.10$  g  $N_2$  on the second day of starvation. A slight decline to  $8.87 \pm 1.85$  g  $N_2$  was noted on day 3. A

TABLE II  
Substrates of Lipid Metabolism\*

Subject	Plasma FFA		Plasma triglycerides		Blood AcAc		Blood $\beta$ -OHB		Blood O <sub>2</sub>		Blood CO <sub>2</sub>	
	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†
J. J.	1.793	-0.299	1.616	-0.003	0.955	+0.159	2.145	+0.340	5.38	-1.92	19.33	+0.45
L. S.	1.105	-0.328	1.638	+0.152	0.686	+0.291	1.202	+0.227	6.69	-3.34	—	—
R. W.	1.754	-0.552	0.445	+0.025	0.503	+0.337	1.339	+0.304	6.92	-2.48	16.65	+0.23
R. B.	1.307	-0.424	1.286	+0.018	0.634	+0.306	1.260	+0.265	—	—	—	—
E. S.	1.318	-0.220	0.354	+0.053	0.936	+0.173	2.932	+0.196	5.54	-2.28	14.42	+1.02
Mean	1.455	-0.365	1.068	+0.049	0.743	+0.253	1.776	+0.266	6.13	-2.50	16.80	+0.57
±SEM	±0.135	±0.057	±0.280	±0.027	±0.088	±0.036	±0.336	±0.026	±0.39	±0.30	±1.42	±0.24

\* Values are expressed as millimoles per liter and are the means of three sampling periods.

† Hepatic venous minus arterial differences (HV-A); substrate uptake indicated by negative values (-).

transient elevation in urinary nitrogen loss on the second day of starvation has been observed previously (5). However, the changes noted here are not significant ( $P > 0.05$ , paired  $t$  test). Urinary ketone-body losses were  $1.78 \pm 0.21$  mmol/24 h on day 1,  $3.42 \pm 0.29$  mmol/24 h on day 2, and  $6.04 \pm 0.50$  mmol/24 h on day 3 of starvation, immediately before catheterization. Based on these data, the patients participating in this study appeared to behave metabolically in a manner similar to other groups of patients undergoing prolonged starvation for other purposes. The additional element of potential cardiac disease did not alter the pattern of substrate and hormonal response to the 3-day fast.

*Arteriovenous differences and estimated hepatic blood flow.* The arterial and hepatic venous concentrations of AcAc,  $\beta$ -OHB, lactate, pyruvate, glucose, FFA, triglycerides, glycerol, alanine, carbon dioxide, and oxygen in all subjects remained practically constant throughout the blood-sampling period of the study. The arterial concentrations and splanchnic arteriovenous differences for various substrates associated with lipid

metabolism are presented in Table II. All subjects showed splanchnic extraction of FFA and oxygen, and release of AcAc,  $\beta$ -OHB, and carbon dioxide. Splanchnic release of AcAc and  $\beta$ -OHB was approximately equal in amount, despite the fact that the arterial concentrations of  $\beta$ -OHB were more than twofold greater than that of AcAc. The splanchnic respiratory quotient was very low (0.23), which is consistent with a rapid rate of hepatic ketogenesis (19). The observed splanchnic fractional extraction of arterial plasma FFA (25%) was expected, and is in agreement with previous observations (1, 20-22). A small but statistically significant ( $P < 0.05$ ) splanchnic production of triglycerides was noted. Previous reports have shown a net release of triglycerides by the splanchnic beds from both animals and humans after brief fasts (21, 23, 24). Furthermore, Havel, Kane, Balasse, Segel, and Basso reported that part of the [<sup>14</sup>C]palmitate taken up by the liver was converted into plasma triglycerides (23).

Arterial concentrations and the splanchnic arteriovenous differences for a variety of substrates associated with carbohydrate metabolism are shown in Table III.

TABLE III  
Substrates of Carbohydrate Metabolism\*

Subject	Blood glucose		Blood lactate		Blood pyruvate		Plasma glycerol		Plasma alanine	
	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†	Arterial	HV-A†
J. J.	2.73	+0.36	0.557	-0.212	0.058	-0.021	0.223	-0.100	0.098	-0.049
L. S.	3.65	+0.27	0.438	-0.153	0.060	-0.013	0.124	-0.048	0.118	-0.059
R. W.	3.56	+0.49	0.804	-0.378	0.093	-0.024	0.150	-0.067	0.219	-0.128
R. B.	3.58	+0.37	0.598	-0.255	0.073	-0.017	0.111	-0.065	0.201	-0.103
E. S.	3.11	+0.26	0.537	-0.265	0.055	-0.016	0.187	-0.118	0.170	-0.085
Mean	3.33	+0.35	0.587	-0.253	0.068	-0.018	0.159	-0.080	0.161	-0.085
±SEM	±0.18	±0.04	±0.060	±0.037	±0.007	±0.002	±0.021	±0.021	±0.023	±0.014

\* Values are expressed as millimoles per liter and are the means of three sampling periods. Means  $\pm$  SEM values are the mean  $\pm$  SEM of the means.

† Hepatic venous minus arterial differences (HV-A); substrate uptake indicated by negative values (-).

TABLE IV  
Catheterization Hemodynamics\*

Subject	Cardiac blood output	Cardiac index	Hepatic blood flow	Hepatic blood flow / Cardiac blood output × 100
	<i>liter/min</i>	<i>liter/min/m<sup>2</sup></i>	<i>liter/min</i>	<i>%</i>
J. J.	7.7	4.6	1.38	18
L. S.	4.8	2.4	1.20	25
R. W.	10.6	4.9	1.79	17
R. B.	7.9	3.9	1.04	13
E. S.	4.8	2.9	2.25	47
Mean ± SEM	7.2 ± 1.1	3.7 ± 0.5	1.53 ± 0.22	24 ± 6

\* Methods for determination of cardiac blood output and hepatic blood flow are outlined in Methods.

All subjects showed splanchnic release of glucose and extraction of alanine, glycerol, lactate, and pyruvate. The fractional extraction of alanine, glycerol, and lactate amounted to approximately one-half of the arterial concentration for each substrate.

Hepatic blood flow remained relatively constant throughout the study period. Although the mean hepatic blood flow for the patients in this study (Table IV) was somewhat greater (1.53 liter/min) than reported previously, the fraction of the mean cardiac output which this hepatic flow represents is in good agreement with other estimations (25). The elevated hepatic blood flow observed in this report may be attributed to the increased cardiac outputs in this group of patients. Three of the subjects showed significant elevations in their resting cardiac outputs and in their cardiac indices. This may be accounted for, in part,

by the increase in cardiac output which accompanies the other circulatory changes of early essential hypertension (26). Two patients with elevated cardiac indices had essential hypertension as an underlying disease state.

*Production and utilization rates.* By combining arteriovenous differences with estimated hepatic flow rates, splanchnic substrate and respiratory gas production and utilization rates were calculated and are displayed in Table V. The total extrapolated ketone-body production rate after 3 days of starvation was 115 g/24 h. This mean value, as well as the individual values for each patient, are as great as the previously reported mean rate of 83 g/24 h for obese subjects after 4–6 wk of starvation (1).

By using palmitic acid as a model for calculating the conversion of FFA to ketone bodies, it can be estimated

TABLE V  
Daily Rates of Splanchnic Release and Uptake of Substrates and Respiratory Gases\*

Subject	AcAc	β-OHB	FFA	Triglycerides†	O <sub>2</sub>	CO <sub>2</sub>	Glucose	Lactate	Pyruvate	Glycerol‡	Alanine‡
J. J.	0.316 (32.23)	0.676 (70.30)	-0.416	-0.004	-3.82	0.89	0.715 (128.7)	-0.421	-0.042	-0.139	-0.065
L. S.	0.503 (51.30)	0.392 (40.77)	-0.368	+0.170	-5.77	—	0.467 (83.98)	-0.264	-0.022	-0.054	-0.066
R. W.	0.869 (88.61)	0.784 (81.54)	-0.882	+0.042	-6.39	0.59	0.832 (149.8)	-0.974	-0.062	-0.107	-0.205
R. B.	0.458 (46.72)	0.397 (41.29)	-0.377	+0.016	—	—	0.554 (99.70)	-0.382	-0.025	-0.058	-0.091
E. S.	0.561 (57.22)	0.635 (66.04)	-0.469	+0.098	-7.39	3.30	0.842 (151.6)	-0.052	-0.052	-0.251	-0.183
Mean ± SEM	0.541 ± 0.091 (55.22) (± 9.32)	0.577 ± 0.078 (59.99) (± 8.14)	-0.503 ± 0.094	+0.064 ± 0.031	-5.84 ± 0.75	1.59 ± 0.86	0.682 ± 0.075 (122.8) (± 13.5)	-0.580 ± 0.141	-0.041 ± 0.008	-0.122 ± 0.036	-0.122 ± 0.030

\* Values for each subject are the means of three sampling periods and are expressed as mol · g/24 h. Mean ± SEM values are the mean ± SEM of the means. Uptake of substrate or respiratory gas indicated by negative value (-).

† Triglyceride, glycerol, and alanine production or uptake rates were calculated from arterio-hepatic venous plasma concentration differences multiplied by hepatic plasma flow rates. All other values were calculated from arterio-hepatic venous blood concentration differences multiplied by hepatic blood flow rates.

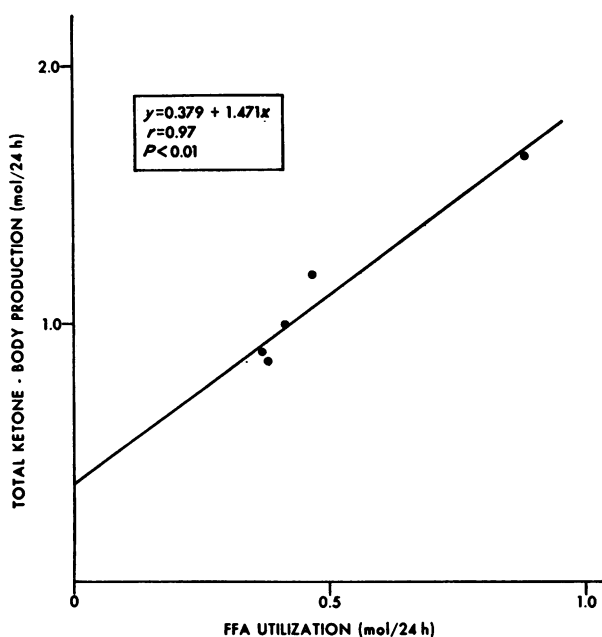


FIGURE 2 The relationship between splanchnic ketogenesis and FFA utilization. Individual determinations for these processes at each sampling period for every patient are given with values expressed as mol/24 h. A least squares linear regression line was calculated,  $r$  obtained, and both are shown in the figure together with the level of  $P$ .

that approximately 0.279 mol/24 h, or 55%, of FFA was consumed to form 1.118 mol/24 h of AcAc plus  $\beta$ -OHB (Table V). The error in measuring plasma triglycerides (see Methods) diminishes the significance of the mean hepatic release of these lipids shown in Tables II and V. Therefore, calculations aimed at estimating splanchnic conversion of FFA into metabolic products other than ketone bodies are unreliable.

Total splanchnic glucose release was approximately 123 g/24 h. Previous data have suggested optimal conversion of gluconeogenic substrates into glucose during prolonged starvation (1). If all the lactate, pyruvate, glycerol, and alanine removed by the splanchnic bed after 3 days of starvation were converted into glucose, then their respective contribution to hepatic glucose release would be about 52, 4, 11, and 10 g/24 h.

## DISCUSSION

Studies measuring arteriovenous differences combined with blood flow rates across the muscle compartments of forearms have shown that during starvation, ketone bodies function only transiently as important fuels for skeletal muscle metabolism. After an overnight fast, ketone-body utilization accounts for about 10% of the total oxygen consumption by muscle (2). However, after 3–7 days of starvation, ketone-body utilization

increases substantially, accounting for 50–85% of total oxidative metabolism in skeletal muscle (2, 6). With continuing starvation, a decline in ketone-body utilization by skeletal muscle has been observed, so that after 24 days of fasting, the net uptake of AcAc plus  $\beta$ -OHB accounts for 0–16% of muscle oxidative metabolism (2, 7, 8). The circulating arterial concentration of total ketone bodies in obese subjects increases from 2–3 mmol/liter after 3 days of starvation to 7–8 mmol/liter after 24–42 days of continuous starvation (1, 2). Thus, ketone-body removal by muscle paradoxically decreases despite the marked increase in the arterial concentrations of these substrates with progressive starvation. This is in marked contradistinction to other tissues, such as brain and kidney, which show increasing ketone-body uptake with increasing arterial concentrations of these substrates (27–29). The results of this study show that after 3 days of starvation, the total splanchnic production of ketone bodies was about 115 g/24 h. After 5–6 wk of starvation, splanchnic ketone-body production in obese subjects was about 83 g/24 h. At the latter time, blood concentration of ketone bodies are about threefold greater than that observed in the present study, which was performed on the third day of starvation. Furthermore, in this study, urinary losses of ketone bodies after 3 days of starvation (0.6 g/24 h) were approximately 5% of that observed after 5–6 wk of starvation (11.7 g/24 h) (1). Thus, if hepatic production rates in different populations can be compared, it would appear that after 3 days of starvation, hepatic ketogenesis is near maximum, despite the fact that the blood concentrations of AcAc and  $\beta$ -OHB continue to increase with more prolonged periods of fasting. Admittedly, we make the assumption that obesity per se does not diminish hepatic production rates of ketone bodies. Support for this assumption is given by the observation that differences in bloodstream concentrations of ketone bodies observed between lean and obese subjects may be due to various rates of clearance (e.g., for any given bloodstream concentration, obesity is associated with an increased rate of ketone-body oxidation and not a decreased rate of ketone-body production.<sup>3</sup>

The large splanchnic ketone-body production rate noted in this study was expected, since AcAc and  $\beta$ -OHB are the major fuels for muscle metabolism after a few days of starvation (2, 6). The caloric requirements of a 70-kg human in the basal state are approximately 1,700 kcal, and about 40% of this occurs in muscle (30). Assuming an approximate caloric value for ketone bodies of 4.5 kcal/g (31), it can be calcu-

<sup>3</sup> Unpublished observations of Dr. George A. Reichard, Jr., Lankenau Hospital, Philadelphia, Pa. 19151.

lated that ketone-body consumption by resting skeletal muscle after brief starvation must amount to at least 75 g/24 h. Furthermore, it has been shown that ketone-body utilization by tissues other than muscle, such as brain and kidney, is directly proportional to the blood concentrations of these substrates (27-29); therefore, these tissues consume additional quantities of ketone bodies during starvation. Thus, it seems likely that the hepatic ketone-body production rate of 115 g/24 h after a 3-day fast is a valid rate.

In view of the high ketone-body production rates and the relatively low circulating concentrations of these substrates after 3 days of starvation, it appears that the rate of ketone-body removal or utilization by peripheral tissues, especially muscle, is as important as the rate of hepatic ketone-body production for controlling the degree of starvation hyperketonemia. Support for this proposal can be obtained from the observations that the maximum rates of total ketone-body utilization (oxidation) and production were achieved at blood concentration of about 2-3 mmol/liter (32, 33), a value found in both lean and obese subjects after about 3 days of starvation (1, 2, 5).

A number of factors, such as substrate availability, the interrelationships of various hormonal effectors, such as insulin and glucagon, and end product inhibition, may influence the rate of hepatic ketogenesis (34). Flatt has pointed out that ketogenesis from FFA functions as the primary energy-yielding process for hepatic

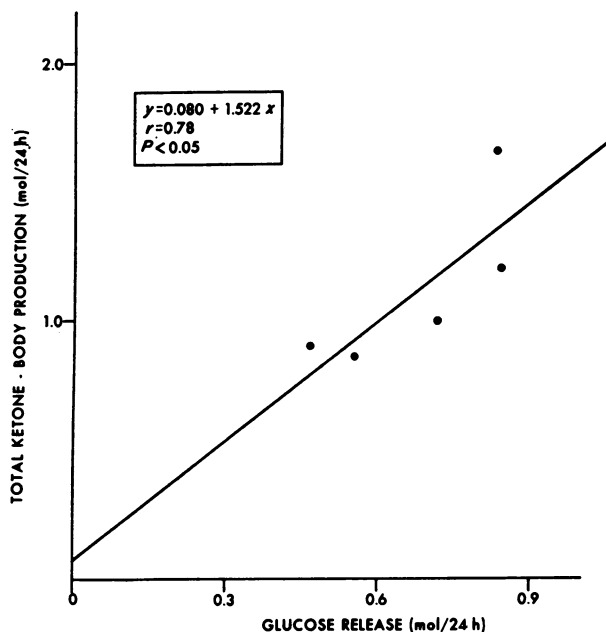


FIGURE 3 The relationship between splanchnic gluconeogenesis and ketogenesis.

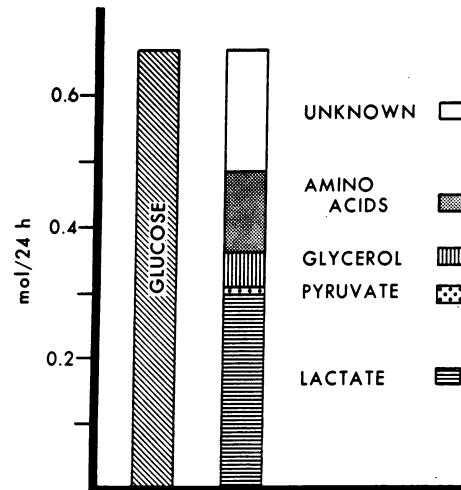


FIGURE 4 The balance of splanchnic glucose release and precursor uptake. Values given are the means and are expressed as mol/24 h.

activity (35). Fig. 2 shows the correlation between hepatic ketogenesis and FFA utilization ( $r = 0.97$ ;  $P < 0.01$ ). Since gluconeogenesis is a significant energy-requiring biosynthetic function of the liver, some relationship between ketogenesis and gluconeogenesis during starvation can be expected and as shown in Fig. 3, a significant correlation between splanchnic gluconeogenesis and ketogenesis was found ( $r = 0.78$ ;  $P < 0.05$ ). Thus, any increase in the rate of energy utilization, such as that created by an increased rate of gluconeogenesis, may be reflected by an increased rate of ketogenesis, all other factors remaining equal. A corollary of this contention has been published by Felig, Wahren, Hendler, and Brundin, who reported that the increased rate of gluconeogenesis observed in obesity was accompanied by elevated rates of splanchnic consumption of  $O_2$  and FFA (36).

The results in this report document the occurrence of increased gluconeogenesis after 3 days of starvation. The balance across the splanchnic bed of glucose released and precursor utilized is shown in Fig. 4. Lactate is the principle precursor, and approximately 46% of the daily amount of glucose released from the liver results from the recycling of lactate and pyruvate (56 g/day) arising primarily from the anaerobic metabolism of glucose. This rate of carbohydrate recycling does not appear to be significantly different from that noted in other studies involving different periods of fasting (30, 37). Plasma glycerol uptake accounts for an additional 11 g of precursor for glucose formation. This value is similar to that previously reported (1). However, it may be an underestimation of the total glycerol contribution, since plasma arterio-hepatic ve-

nous differences multiplied by estimated plasma flow rates were used to calculate the contribution made by glycerol. This does not include the possible contribution made by blood cellular elements. In addition, mesenteric lipolysis could also furnish glycerol for hepatic gluconeogenesis, but this is not detected by a determination of the splanchnic arterio-hepatic venous difference. The contribution of amino acids to gluconeogenesis may be estimated by the urinary nitrogen excretion rate using a D/N ratio of 3:65:1 (38).<sup>4</sup> From this data, a maximum of 32 g of glucose may be formed from amino acid catabolism. It is well established that alanine is the primary gluconeogenic amino acid accounting for 41–48% of total splanchnic extraction of amino acids (37, 39). In this study, the splanchnic fractional extraction of plasma alanine was 0.53 and was not significantly different from previously published data. Our observed splanchnic extraction of plasma alanine can account for about 10 g of glucose produced daily. Because the portal venous plasma alanine concentration exceeds the arterial plasma alanine concentration by approximately 11% (40), and since erythrocytes (41) may also contribute alanine to the liver for gluconeogenesis, determining arterio-hepatic venous plasma differences multiplied by estimated hepatic flow rates slightly underestimates the overall contribution of alanine to glucose. Nevertheless, after taking into account this underestimation and recognizing that alanine is the principal gluconeogenic amino acid, it is clear that the hepatic uptake of this substrate accounts for a maximum of no more than 10% of the glucose formed after 3 days of starvation. Despite the underestimations in the contributions of both glycerol and alanine for gluconeogenesis, the balance of substrate contributions in this study accounts for 99 g/24 h (81%) of the splanchnic glucose released. Small contributions of glucose from hepatic glycogenolysis may have occurred. However, it may be that accumulative errors in measuring arterio-venous differences multiplied by estimated hepatic flow rates and extrapolated to 24-h periods are responsible for the discrepancies noted here in the glucose balance.

These studies indicate (a) that hepatic production rates of ketone bodies after 3 days of starvation were as great as those previously reported in obese subjects after 5–6 wk of starvation; (b) that hepatic gluconeogenesis was increased approximately twofold over that observed either after overnight or a 5–6-wk fast (1, 37); and (c), that after 3 days of starvation, hepatic ketogenesis and gluconeogenesis were directly related.

<sup>4</sup>1 g of nitrogen from catabolized protein should yield about 3.65 g of glucose.

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