Protein, fat, and carbohydrate requirements during starvation: anaplerosis and cataplerosis[1–3]

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See corresponding editorial on page 1.

ABSTRACT The purpose of this work was to clarify the essentiality of glucose production from amino acids in obese subjects undergoing prolonged starvation and to provide an explanation for death after the depletion of lean body mass when some body fat is still available to meet body energy requirements. Five obese subjects fasted for 21 d. Nitrogen balance studies were combined with measurements of blood metabolite and hormone concentrations, indirect calorimetry, determination of body-composition changes, and catheterization techniques. Phenylacetate was administered from day 19 to day 21 to remove glutamine from the body and to assess this perturbation on energy requirements, ammoniagenesis, ureagenesis, gluconeogenesis, and ketogenesis. The obese subjects lost body fat and fat-free mass in parallel and resting metabolic energy requirements per mass remained constant during starvation. Urinary nitrogen excretion reflected continuous demands for amino acid oxidation. Phenylacetate administration decreased blood glutamine concentrations, increased plasma epinephrine concentrations, and increased urinary nitrogen loss through phenylacetylglutamine excretion; urinary excretion rates of urea, ammonium, urate, creatinine, and ketone bodies remained unchanged. The essentiality of amino acid oxidation was therefore shown. Late in prolonged starvation, aminogenic oxidation amounted to 7% and fat provided the remaining energy requirements. Hepatic and renal gluconeogenesis were not curtailed. Blood glutamate served as a vehicle for carbon and nitrogen transport; the contribution of glycerol to gluconeogenesis equaled that of all amino acids combined. The minimal quantities of amino acid (0.27 ± 0.08 and 0.52 ± 0.10 g) and fat (1.53 ± 0.21 and 2.98 ± 0.15 g) oxidized per kg body wt or fat-free mass/d, respectively, were determined. Included within amino acid and fat oxidation were the minimal amounts of precursors needed for synthesizing the essential quantity of glucose (0.34 ± 0.14 and 0.66 ± 0.20 g) oxidized per kg body wt or fat-free mass, respectively. *Am J Clin Nutr* 1998;68:12–34.

KEY WORDS Aminogen, gluconeogenesis, ketogenesis, ammoniagenesis, ureagenesis, fuel homeostasis, anaplerosis, cataplerosis, body-composition changes, energy requirements, fatty acid desaturation, starvation

INTRODUCTION

Humans have considerable metabolic versatility for fuel utilization to maintain energy requirements. However, the lower limits of flexibility for catabolism of one or another of the major fuel classes (carbohydrate, fat, and protein) have not been defined. This is an important issue because obese humans and animals subjected to prolonged starvation die after depletion of body proteins and death may be independent of the availability of fat for oxidative metabolism (1, 2). This suggests that substrates other than acetate derived from fatty acid oxidation are essential for maintaining energy requirements for vital functions.

The intermediate metabolites of the citric acid cycle are continuously being withdrawn or lost from the cycle through cataplerotic reactions and therefore must be continuously replenished through anaplerotic reactions (3). During total, prolonged starvation, amino acids may be the principal substrates for replenishing the 4-carbon intermediates of the citric acid cycle as well as supplying gluconeogenic substrates. The quantitative importance of amino acids in cataplerosis and anaplerosis can be estimated by measuring multiple regional exchange rates of the principal amino acids and from body nitrogen balance.

During starvation nitrogen is lost from the body after it is released from the amino and amide radicals originally derived from amino acids present in protein plus nonprotein (aminogen) materials. The amino acid carbon skeletons (carboxylic acids) are oxidized directly in the citric acid cycle or indirectly after conversion to glucose, which is subsequently oxidized in the citric acid cycle. The kidneys remove the nitrogenous waste products from the body through the excretion of urea, uric acid (urate), creatinine, ammonium (NH_4^+), and small quantities of other compounds (4). Urea and NH_4^+ excretion reflect the majority of the energy

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derived from catabolism of aminogens. After a few days of starvation, total urinary nitrogen excretion diminishes as the major nitrogenous excretory compound, urea, decreases. However, NH_4^+ excretion increases and late in fasting NH_4^+ becomes the principal nitrogenous excretory product (5), a condition that is highly correlated with ketonuria (6, 7).

Starvation hyperketonemia is accompanied by marked increases in the quantities of ketone bodies undergoing glomerular filtration (5, 6). A significant fraction of acetoacetate ($AcAc^-$) and β-hydroxybutyrate (β-OHB^-) escapes renal tubular reabsorption, resulting in heightened ketonuria. It is generally thought that these anions in tubular fluid obligate isomolar excretion of cations, mainly NH_4^+, to maintain near urinary electroneutrality (6–8). NH_4^+ is derived primarily from renal deamination and deamidation of glutamine (9). Therefore, persistent ketonuria results in the loss of valuable fuels derived from fat and has the potential to deplete protein stores to provide NH_4^+. However, we reported previously that after prolonged starvation the administration of small quantities of carbohydrate significantly reduced urinary excretion rates of $AcAc^-$, β-OHB^-, and NH_4^+ without changing blood ketone body concentrations (6). We attributed the decrease in NH_4^+ excretion to the decrease in $AcAc^-$ and β-OHB^- excretion, but the explanation for this diminished ketonuria and ammoniagenesis was inadequate. Subsequent studies showed that the kidneys can reabsorb more $AcAc^-$ and β-OHB^- than is filtered during starvation (10). Thus, from renal function assessments, persistent ketonuria does not make biological sense, and an explanation for its occurrence during starvation is needed.

The central nervous system (brain) and other organ systems with mitochondria may have minimum daily requirements specifically for glucose (11), but the exact minimum requirement has never been established. There is general consensus that during starvation amino acids, glycerol, and acetone are supplied from peripheral tissue stores for hepatic and renal gluconeogenesis to provide glucose for catabolism to carbon dioxide and water (11, 12). However, another logical but hypothetical perspective for evaluating hepatic and renal gluconeogenesis, ureagenesis, ammoniagenesis, and ketonuria can be developed: NH_4^+ excretion during catabolic states may be an essential mechanism for removing nitrogenous waste products from the body and generating glucose, a universal fuel, as a byproduct. Ketonuria may be a secondary event needed to maintain urinary electroneutrality. The loss of $AcAc^-$and β-OHB^- in obese individuals subjected to prolonged starvation is relatively trivial after considering the usual quantity of fat stored in the body (13). Although this hypothesis may appear iconoclastic, it is reasonable and should be investigated.

Urinary nitrogen excretion can be augmented by increasing the excretion of nitrogen-containing compounds other than urea, NH_4^+, urate, and creatinine. In the fed state, when nutrients are readily available, the administration of sodium phenylacetate promotes nitrogen excretion as phenylacetylglutamine and reduces nitrogen excretion as urea and NH_4^+ (14, 15). The glutamine acylation reaction is rapid and urinary excretion of phenylacetylglutamine is reported to be 81–98% completed in 24 h (16, 17).

If gluconeogenesis from amino acids is simply an economic process for conserving amino acid carbon skeletons, the rate of total urinary nitrogen excretion should not change after administering phenylacetate because the net effect of decreasing urea and NH_4^+ nitrogen excretion should match the increasing phenylacetylglutamine nitrogen excretion. On the other hand, an alternative hypothesis can be advanced that assigns a critical role to glutamine for survival during starvation. If there is a minimal amount of gluconeogenesis and obligatory ammoniagenesis and ureagenesis required from glutamine and other amino acids during the near steady state of prolonged starvation, the administration of phenylacetate will not induce significant decreases in the amount of NH_4^+ and urea excreted in the urine. Instead, total urinary nitrogen excretion will increase secondary to the added urinary excretion of conjugated glutamine with phenylacetate.

This work examines the orchestrated interplay among carbohydrate, fat, and protein to meet the energy requirements of starving humans. Blood concentrations of metabolites and hormones were determined to assess steady state measurements. Oxygen consumption and carbon dioxide production rates were coupled with urinary excretion rates of nitrogenous compounds to determine the quantities of carbohydrate, fat, and protein consumed. Changes in body composition were measured before and after prolonged starvation and changes in fat-free mass were related to urinary nitrogen excretion. Perturbations induced by administering phenylacetate were assessed by determining urinary excretion rates of nitrogenous compounds. The results of these studies were coupled with arteriovenous catheterization studies across the splanchnic, kidney, and lower-extremity vascular beds to calculate net regional exchange rates of glucose, urea, ammonia, phenylacetylglutamine, and related substrates and hormones. We quantified and linked multiple biophysiologic processes that provide fuels for maintaining citric acid cycle activity.

SUBJECTS AND METHODS

Subjects

Five obese subjects agreed to undergo research studies designed to characterize their metabolism, to better understand their energy requirements and body-composition changes during total starvation, and to contribute knowledge regarding fuel homeostasis. Informed, written consent was obtained from each individual after the protocol was approved by the Institutional Review Board of Temple University Health Sciences Center. The subjects were admitted to the General Clinical Research Center of Temple University Hospital for a 23-d study. They were instructed to consume a weight-maintaining diet with adequate carbohydrate and protein for several days as outpatients before the study began on day 0. Clinical characteristics are displayed in **Table 1**. All subjects had diseases in addition to obesity but had normal or compensated renal, hepatic, and thyroid function and normal hemograms and normal results on urinalyses.

Protocol

The research protocol is displayed in **Table 2**. All subjects served as their own controls. Because of the complexity of these studies, occasional values (≈3% of total) were not obtained, as indicated in the table. The missing data were estimated by the method of Snedecor and Cochran (18).

The subjects consumed a meal during the evening and then were studied the next morning, after a 10–12-h overnight fast represented as day 0. The subjects were awakened, instructed to urinate, weighed while nearly naked, and their heights measured while they were barefoot (19, 20). Indirect calorimetric meas-

TABLE 1
Clinical characteristics of the subjects

Subject[1]	Sex	Age	Height	Weight	Compounding disease	Therapy before hospitalization	Complications associated with starvation
		y	*cm*	*kg*			
1	M	25	170	170	Type 2 diabetes, hypertension	—	Dyspepsia, nausea, diarrhea
2	F	51	160	139	Type 2 diabetes, staghorn calculus, right nephrectomy	Regular insulin	Pyuria
3	M	27	183	136	—	—	Nausea, neurotic spitting
4	F	46	170	140	Type 2 diabetes, chronic obstructive pulmonary disease	Albuterol inhaler,[2] Theodur[3]	Lightheadedness
5	F	27	170	143	Migraine headaches, asthma	—	Iodine allergy claimed late in study

[1] Listed according to date of volunteering for the study.
[2] Alpharma, Baltimore.
[3] Key Pharmaceuticals Inc, Kenilworth, NH.

urements were taken (19–21), arterial blood samples were drawn, and underwater weighing for densitometric analysis of fat mass and densitometric analysis of fat-free mass (FFMD) was conducted in 3 of the 5 subjects (19–21). In the other 2 subjects body-composition studies were done on days 1 and 4. Day 1 represents the 10–12-h overnight fast plus the 24-h fast from day 0 to day 1. After most of the baseline valuation studies were complete, all subjects began a 21-d fast. They were encouraged to drink 1500 mL water and were prescribed 1 g NaCl and 1 g KCl to be taken orally daily (5). Subjects were weighed and 24-h urine collections were taken daily, and urine was kept on ice and under mineral oil. Daily urinary excretion of total nitrogen (22), urea nitrogen (23), NH_4^+ (24), urate (Auto Analyzer; Technicon Instruments, Tarrytown, NY), creatinine (25), $AcAc^-$ (24), and β-OHB^- (24) and pH (Accumet pH meter; Fisher Scientific Co, Pittsburgh) were measured. The nitrogen excreted as NH_4^+, urate, and creatinine was calculated from the measured excretion rates multiplied by the percentage composition of nitrogen in each compound.

Indirect calorimetry measurements were repeated during the mornings on days 0, 1, 2, 3, 4, 7, 14, 18, and 21 with our standardized techniques (19–21). The energy equivalents for respiratory exchange of oxygen and carbon dioxide and urinary nitrogen excretion were calculated by using the modern values reported by Jungas et al (26). We assumed that the Loewy value of 6.25 g protein/g N (27) provided a reasonable number for calculating the energy equivalents derived from urinary nitrogen because urinary nitrogen represented the sum of nitrogen derived from the oxidation of nucleotides and simple and conjugated proteins (28), some in the extracellular fluids, all initially formed from amino acids (*see* discussion below on the source of urinary nitrogen loss during starvation). The difference between urinary nitrogen excretion derived from catabolism of muscle or other lean cellular protein and that derived from catabolism of extracellular fluid protein plus other NH_2-containing compounds is only $\approx$10%. Nonetheless, to be consistent in this manuscript we reassigned the nonprotein respiratory quotient to the nonaminogen respiratory quotient (naRQ). We refer to urinary nitrogen losses as reflecting the oxidation of aminogenic materials. Corrections for changes in body urea pools were made (29); however, the effect of changes in the body urea pool on the quantity of fuels oxidized was trivial and subsequently omitted.

The energy equivalents of urinary nitrogen were considered when the composition of urinary nitrogenous compounds was changing during starvation (30). We concluded that the influence of different urinary nitrogenous compounds had an insignificant effect on the total energy requirements of our obese subjects during starvation (30). The influence of ketonuria (31) on the naRQ was <1% and was therefore disregarded.

Faced with naRQ values below the theoretical minimal value ($\approx$0.703) after correcting for ketonuria, we were forced to reconsider catabolism and energy requirements during protracted starvation of obese subjects. Fatty acids can undergo desaturation, consuming oxygen to form water and producing heat energy but no carbon dioxide. Thus, the RQ for desaturation of fatty acids is zero and could account for measured naRQs <0.703. When the naRQ values were below the RQ for complete fat oxidation (0.703), the total resting metabolic rate (RMR) corrected for fatty acid desaturation was calculated. Specifically, the calculations involved using nonaminogen carbon dioxide production ($naCO_2$) and the RQ value of complete fat oxidation (0.703) to partition nonaminogen oxygen consumption (naO_2) into oxygen consumed for complete fat oxidation (O_2 fat oxidation) and oxygen consumed in fatty acid desaturation (O_2 fat desaturation). This correction assumes that the $naCO_2$ is a more accurate measure of complete fat oxidation, and naO_2 in complete fat oxidation can be calculated from the following equation:

TABLE 2
Investigational protocol

Procedure and subject	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
24-h Urine collections																							
1	X[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X[1]	X	X	X	X	X	X	X	
2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[1]	
Indirect calorimetry measurements																							
1	X	X	X	X	X			X							X				X			X	
2	X	X	X	X	X			X							X				X			X	
3	X	X	X	X	X			X							X				X			X	
4	X	X	X	X	X			X							X				X			X	
5	X	X	X	X	X			X							X				X			X	
Arterial and venous blood collections																							
1	X				X											X			X			X	
2	X				X											X			X			X	
3	X				X														X			X	
4	X				X											X			X			X	
5	X				X											X			X			X	
Body-composition measurements																							
1	X																				X		
2	X																					X	
3	X																	X					
4					X																		X
5		X																					X
Phenylacetate administration																							
1																				X	X	X	
2																				X	X	X	
3																				X	X	X	
4																				X	X	X	
5																				X	X	X	
Hepatic, renal and lower extremity catheterizations																							
1																						X	
2																						X	
3																						X	
4																						X	
5																						X	

[1] Incomplete urine collection.

$$O_2 \text{ fat oxidation} = naCO_2/0.703 \quad (1)$$

The energy yielded from complete fat oxidation was assigned a value of 19.54 kJ/L O_2 (26).

The oxygen consumed in the process of desaturating fatty acids was calculated by the following equation:

$$O_2 \text{ fat desaturation} = naO_2 - O_2 \text{ fat oxidation} \quad (2)$$

The heat produced from fatty acid desaturation is 166.1 kJ/mol H_2 removed and double-carbon bond introduced into the fatty acid (30). Therefore, the kilojoule energy value of oxygen from fatty acid desaturation is 332.21 kJ/mol or 14.83 kJ/L. RMR was also calculated based on the $naCO_2$. The energy value of $naCO_2$ was calculated by the method of Elia and Livesey (32) by using the energy equivalents from Jungas et al (26). The assigned energy values of 17.7 kJ/g aminogenic material, 38.95 kJ/g fat, and 16.82 kJ/g carbohydrate were used (26).

Arterial blood samples were drawn on days 0, 4, 15, 18, and 21, and blood, plasma, or serum concentrations of glucose (33), fatty acids (34), $AcAc^-$ (24), β-OHB^- (24), amino acids (24, 35), insulin (36), C-peptide (C-Peptide RIA Kit; Serono Diagnostics, Inc, Braintree, MA), epinephrine, norepinephrine (Catecholamine Kit; Amersham Life Sciences, Inc, Arlington Heights, IL), thyroxine (T_4), triiodothyronine (T_3), reverse triiodothyronine (rT_3) (37), and urea nitrogen (23) were measured. Arterial blood pH (IL1301 Blood Gas Analyzer; Instrumentation Laboratory, Lexington, MA), lactate (24), pyruvate (24), glycerol (24), and ammonia were measured on days 18 and 21. In addition, on day 18 both arterial blood and plasma alanine, glutamine, and glutamate concentrations were measured (24, 35).

Body-composition studies were repeated after 17–21 d of starvation (19–21). Changes in total body weight, fat mass by densitometry, and FFMD were determined, and loss of FFMD was related to total urinary nitrogen excretion corrected for changes in the body urea nitrogen pool between the first and second densitometric measurement.

The source of urinary nitrogen loss during starvation was reconfigured because the classic value of 6.25 g protein/g N (27) is greater than the modern measurement of 5.57 g protein/g N in muscle (26). Therefore, the value of 6.25 g protein/g N must represent not only the 5.57 g protein/g N derived from lean structural tissues, eg, muscle, but also the 0.68 g of other aminogenic material derived from other sources, eg, extracellular proteins and glycoproteins. The total amount of urinary nitrogen excreted corrected for changes in body urea pools during the time between the first and second body-composition measurements was used to estimate the source of excreted nitrogen and to calculate losses of lean body tissue and extracellular fluid.

After 18 d of starvation, sodium phenylacetate was administered to the subjects. Obese humans usually excrete < 150 mEq (mmol) NH_4^+/24 h (5, 6). We assumed that ≈90% of the administered phenylacetate would be excreted as urinary phenylacetylglutamine with 2 nitrogen atoms per molecule that could have been excreted as NH_4^+ derived from glutamine. Thus, ≤83 mEq (mmol) phenylacetate should have conjugated enough glutamine to more than account for the estimated ≤150 mEq of urinary ammonia excreted after 18 d of starvation (5, 6). The molecular weight of phenylacetate is 136. The first subject took 21 mEq (2.9 g) sodium phenylacetate by mouth 4 times daily, but the unpleasant odor of this compound forced us to reconsider its delivery. Thereafter, it was mixed in water or hypotonic saline and administered intravenously at a rate of 65–94 mEq (9–13 g)/24 h. During the 2 d before (days 17 and 18) and the 3 d of administering sodium phenylacetate (days 19–21), urinary 24-h excretion of phenylacetylglutamine and phenylacetylglutamine nitrogen was measured (38).

After completing 21 d of fasting the subjects underwent catheterization studies so that net exchange rates of precursors and products across the splanchnic (hepatic), renal, and lower-extremity vascular beds could be measured. The subjects were studied in the resting supine state. A peripheral venipuncture was made for initially giving a primed (≈60 mg), continuous (≈12 mg/min) infusion of sodium *p*-aminohippurate and for subsequently adding a primed (≈2.5 mg), continuous (≈0.35 mg/min) infusion of indocyanine green dye mixed with 5% human albumin (39–43). Catheters were inserted into superficial antecubital, basilic, or common femoral veins and advanced to the right main hepatic vein ≈2–3 cm from the wedge position and to the right renal vein (left renal vein in subject 2) under fluoroscopic guidance (39–43). Another percutaneously inserted catheter was advanced from the contralateral femoral artery. After primed, continuous infusion of dyes was begun and the above-mentioned vessels catheterized, no manipulation was done for ≈30–60 min. Thereafter, 2 sets of blood samples were collected simultaneously at 10-min intervals from the femoral artery and hepatic and renal veins. Subsequently, the renal vein catheter was moved to the region below the common iliac vein and above the femoral vein. One pair of arterial and venous blood samples was collected simultaneously and ≈10 min later a second venous sample was collected. During the study, isovolumetric quantities of 5% human albumin in 0.9% saline were infused to replace blood withdrawn from the hepatic, renal, and extremity veins, and 0.9% saline was used to flush and replace blood from the arterial catheter. The hepatic and renal catheter positions were checked before and after each blood sampling period and later confirmed by dye and substrate-product measurements.

Immediately after withdrawal, blood samples were analyzed for indocyanine green dye and *p*-aminohippurate, and hematocrits were determined (39, 40). Regional blood flow rates for hepatic and renal beds were measured (41, 42) and peripheral blood flow rates to the extremities were estimated: it was assumed that hepatic plus renal blood flow rates accounted for 50% of cardiac output in the resting state and peripheral blood flow to the extremities accounted for 30% (44). Blood samples were used to measure arterial pH and glucose concentrations at each site. Ten milliliters of blood from each sampling site was also immediately injected into 10 mL ice-cold 1 mol perchloric acid/L and mixed. Supernatant fluids were rapidly analyzed for pyruvate and $AcAc^-$. The remaining supernatant fluids were analyzed for lactate, β-OHB^-, alanine, glutamine, glutamate, and glycerol. Other plasma or serum samples were stored at −20 °C until assayed for fatty acids, insulin, C-peptide, epinephrine, norepinephrine, T_4, T_3, rT_3, urea, ammonia, and phenylacetylglutamine. Arterial and venous metabolite and hormone concentrations were determined simultaneously in duplicate or triplicate. Single measurements were done for arterial pH values.

Data and statistical analyses

The variables measured were expressed as means ± SDs (45). Paired *t* tests were used to compare arteriovenous concentration

differences and selected mean values before (day 18) and after (day 21) administration of phenylacetate on days 19–21 (46). Differences between measurements of urinary excretion rates of total, urea, NH_4^+, urate, and creatinine nitrogen and $AcAc^-$ and β-OHB^- before and after phenylacetate administration were assessed by a paired *t* test (day 18 versus day 21). Linear regression analyses, including a fixed subject effect, were also used to test for differences in the intercepts and slopes of the lines describing total, urea, NH_4^+, urate, and creatinine nitrogen and $AcAc^-$ and β-OHB^- excretion rates before (days 9–18) and after (days 19–21) phenylacetate administration (47). Day 9 was chosen as the starting day for the pretreatment period because it was determined that linear approximations were most valid from this time point for all measurements. Trend lines (curves) were developed for mean urinary excretion rates before phenylacetate administration by using nonlinear regression analysis (KALEIDAGRAPH software; Abelback Software, Reading, PA). The time frames and types of models used were based on knowledge gained from another group of 10 obese subjects who were starved for a minimum of 36 d (OE Owen, KJ Smalley, RL Jungas, unpublished observations, 1998). It was assumed that the trend lines developed using these methods would be more valid if extended beyond the ranges of the observed data. The time frames used were days 5–18 for total urinary nitrogen and urea nitrogen, days 9–18 for NH_4^+ nitrogen, days 10–18 for urate nitrogen, and days 1–18 for creatinine nitrogen. The trend lines were extended to day 21 by using dashed lines. Linear regression analyses were also used to assess relations among indirect calorimetry measurements, weights, and the days of starvation; T_3 and carbon dioxide production; T_3 and NH_4^+ excretion; and T_3 and urea excretion. All paired *t* tests were two-tailed.

The probabilities of type I errors (rejecting a true null hypothesis) were increased in this study to reduce type II errors due to the small sample size and the study's exploratory nature (48). Controlling for one type of error increases the likelihood of the other (49). Nonetheless, metabolic events were considered statistically significant at a *P* value of 0.05. On occasion, we present information or supporting physiologic events regardless of their respective *P* values. For *P* values between 0.05 and 0.10, a power analysis was performed with the NQUERY ADVISOR power program (49) to determine the sample size required to reach the 0.05 level. All reported *P* values between 0.05 and 0.10 would have been ≤ 0.05 with a sample of 7 subjects.

RESULTS

Urinary excretion of metabolites, pH, and equations for urinary excretory compounds

One hundred nine 24-h urinary collections were made between day 0 and day 21. Incomplete 24-h urinary collections were taken on day 0. However, urine was collected during the time the indirect calorimetry measurements were made. Except for day 0, corrections for collection errors were made by estimating missing values (18). Twenty-four–hour urinary excretion rates of total, urea, NH_4^+, creatinine, and uric acid nitrogen; excretion rates of β-OHB^- and $AcAc^-$; and pH are shown in **Table 3**. Excretion rates for NH_4^+ and urea nitrogen were equal after 18 d of starvation. The mean excretion of β-OHB^- plus $AcAc^-$ was directly related to the excretion of NH_4^+ ($P = 0.0001$, $R^2 = 0.77$).

The exponential equations and curves for total urinary nitrogen (TUN) and 24-h nitrogenous components are displayed "unstacked" in **Figure 1**. The dotted lines were used between the data points after 18 d of starvation for easy visual inspection.

Linear regression, after controlling for the subject effect, was used to compare rates of TUN excretion before and after the administration of phenylacetate. The linear regression equation for days 9–18 is described by the following: TUN (g) = 10.94 − 0.31 d; $R^2 = 0.69$. There was a change in the magnitude of TUN excretion when phenylacetate was administered between day 19 and day 21. This change is displayed in **Figure 2** and is described by the following regression equation: TUN (g) = −0.38 + 0.33 d; $R^2 = 0.86$. The progressive increase after the administration of phenylacetate was a significant alteration in the relation between nitrogen excretion and day of starvation ($P = 0.03$, regression analysis). The usual downward drift in TUN excretion as starvation progresses was converted into an upward drift and, thus, increased negative nitrogen balance for TUN excretion. This augmentation in TUN excretion was induced by the acute, increased excretion of phenylacetylglutamine nitrogen. The TUN excretion rate on day 18 was probably lower than the TUN excretion rate on day 21 ($P = 0.10$). Insignificant changes were detected by regression analysis (days 9–18 versus days 19–21) and paired *t* test (day 18 versus day 21) in NH_4^+, urea, urate, or creatinine excretion rates, or in β-OHB^- or $AcAc^-$ excretion rates before and after phenylacetate administration.

Indirect calorimetry, weight, and body composition

The periodic, total RMR and contributions of energy supplied from oxidizing fat, carbohydrate, and aminogen compounds based on oxygen and carbon dioxide equivalents plus those calculated from fatty acid desaturation and the naRQ are shown in **Table 4**. The RMR after the overnight fast for day 0, based on oxygen consumption, was 6055 ± 984 J/min. On day 18 the RMR decreased to 5594 ± 812 J/min. This 8% decline in total energy requirement during the 18 d of starvation was not significant, but support for a decrease in RMR during prolonged starvation was obtained by correlating RMR with the duration of starvation: RMR = 6146 − 28.95 d; $P = 0.003$. When RMR/kg body wt on day 0 (6054 $J \cdot min^{-1} \cdot 146.0\ kg^{-1}$, or 42 ± 7.1 $J \cdot min^{-1} \cdot kg^{-1}$) was compared with that on day 18 (5594 $J \cdot min^{-1} \cdot 131.6\ kg^{-1}$, or 43 ± 7.5 $J \cdot min^{-1} \cdot kg^{-1}$), there was no decrease in energy requirements per unit mass on the basis of oxygen consumption. In addition, mean RMR versus total body weight [RMR (J/min) = −285 + 45 wt (kg); $P = 0.001$] and mean oxygen consumption versus total body weight [oxygen consumption (mL/min) = −24.50 + 2.37 wt (kg); $P = 0.001$] changed in parallel (**Figure 3**). The rise in RMR between day 18 and day 21 while phenylacetate was administered was not significant. Also, mean RMR by amount of oxygen consumed per kg body wt was not significantly correlated with day of starvation. Thus, the decrease in RMR via oxygen was proportionate to weight loss during the first 18 d of starvation.

Different values emerged when carbon dioxide production was used to calculate the indirect calorimetry results. The RMR for day 0 was 6048 ± 975 J/min and was indistinguishable from the value calculated by using oxygen consumption. In contrast, the RMR for day 4 was 5600 ± 528 J/min, significantly less than that based on oxygen consumption ($P = 0.02$). There was a continuous and significant decrease ($P = 0.01$) in RMR based on

TABLE 3
Urinary excretion of nitrogenous compounds and ketone bodies and urinary pH in obese subjects who fasted for 21 d[1]

	Day										
	0	1	2	3	4	5	6	7	8	9	10
Total nitrogen (g/total volume)	6.08 ± 2.83	12.70 ± 3.40	12.16 ± 2.73	12.22 ± 2.18	11.64 ± 2.98	11.66 ± 2.46	10.30 ± 1.42	8.26 ± 1.94	9.16 ± 2.74	8.46 ± 2.88	8.48 ± 1.71
Urea nitrogen (g/total volume)	3.63 ± 1.15	8.68 ± 2.75	7.72 ± 1.49	7.82 ± 1.92	7.28 ± 1.95	6.68 ± 0.76	6.22 ± 1.11	4.68 ± 0.88	5.26 ± 1.41	4.44 ± 1.56	4.20 ± 1.24
Ammonium nitrogen (g/total volume)	0.210 ± 0.148	0.636 ± 0.187	0.793 ± 0.264	0.813 ± 0.097	1.083 ± 0.361	1.276 ± 0.352	1.635 ± 0.680	1.504 ± 0.593	1.992 ± 1.215	1.774 ± 0.616	1.870 ± 0.931
Creatinine nitrogen (g/total volume)	0.244 ± 0.138	0.638 ± 0.293	0.637 ± 0.163	0.622 ± 0.203	0.706 ± 0.242	0.671 ± 0.208	0.690 ± 0.177	0.515 ± 0.076	0.666 ± 0.249	0.580 ± 0.179	0.642 ± 0.226
Uric acid nitrogen (g/total volume)	0.082 ± 0.036	0.211 ± 0.050	0.200 ± 0.071	0.163 ± 0.070	0.126 ± 0.043	0.119 ± 0.037	0.117 ± 0.048	0.088 ± 0.027	0.102 ± 0.033	0.115 ± 0.044	0.120 ± 0.041
β-OHB$^-$ (mmol/total volume)	0.02 ± 0.01	0.67 ± 0.50	6.34 ± 4.54	22.04 ± 19.46	45.09 ± 40.63	64.82 ± 41.56	99.10 ± 69.62	96.07 ± 64.12	116.29 ± 80.30	91.58 ± 47.91	95.74 ± 62.85
AcAc$^-$ (mmol/total volume)	0.04 ± 0.01	0.93 ± 0.30	5.24 ± 1.15	10.41 ± 3.90	14.61 ± 6.12	15.05 ± 3.68	19.71 ± 5.81	18.11 ± 5.57	19.92 ± 5.94	18.57 ± 6.90	18.02 ± 7.11
pH	5.68 ± 0.32	5.59 ± 0.26	5.53 ± 0.30	5.35 ± 0.16	5.26 ± 0.16	5.25 ± 0.18	5.30 ± 0.23	5.23 ± 0.25	5.31 ± 0.35	5.34 ± 0.44	5.44 ± 0.41

TABLE 3 (Continued)

	Day										
									Phenylacetate administration		
	11	12	13	14	15	16	17	18	19	20	21
Total nitrogen (g/total volume)	7.22 ± 2.82	6.54 ± 2.28	6.34 ± 1.99	6.76 ± 2.13	6.38 ± 1.62	6.74 ± 1.92	5.78 ± 1.77	5.20 ± 1.46	5.96 ± 1.78	6.32 ± 2.64	6.63 ± 2.57
Urea nitrogen (g/total volume)	3.40 ± 1.42	3.34 ± 1.07	3.20 ± 0.97	2.98 ± 1.26	2.86 ± 1.40	2.78 ± 1.40	2.12 ± 0.70	2.06 ± 1.01	2.30 ± 0.97	2.04 ± 1.29	1.95 ± 1.43
Ammonium nitrogen (g/total volume)	1.641 ± 0.720	1.856 ± 1.054	1.839 ± 0.857	1.962 ± 0.725	1.910 ± 0.652	1.730 ± 0.441	1.845 ± 0.749	1.664 ± 0.448	1.799 ± 0.593	1.863 ± 0.481	1.471 ± 0.497
Creatinine nitrogen (g/total volume)	0.539 ± 0.150	0.619 ± 0.239	0.633 ± 0.207	0.643 ± 0.209	0.619 ± 0.218	0.627 ± 0.240	0.579 ± 0.210	0.596 ± 0.233	0.615 ± 0.176	0.612 ± 0.182	0.613 ± 0.215
Uric acid nitrogen (g/total volume)	0.108 ± 0.033	0.129 ± 0.053	0.117 ± 0.038	0.111 ± 0.043	0.109 ± 0.042	0.112 ± 0.060	0.117 ± 0.067	0.121 ± 0.079	0.139 ± 0.080	0.143 ± 0.076	0.125 ± 0.096
β-OHB$^-$ (mmol/total volume)	81.97 ± 47.76	90.74 ± 62.02	92.54 ± 46.30	96.49 ± 32.81	97.17 ± 31.14	96.89 ± 27.17	82.47 ± 31.34	65.57 ± 12.00	64.07 ± 13.38	53.26 ± 7.45	56.15 ± 12.74
AcAc$^-$ (mmol/total volume)	16.46 ± 7.06	16.08 ± 6.85	17.38 ± 5.92	18.84 ± 5.51	18.74 ± 5.61	19.11 ± 5.63	16.29 ± 5.01	15.00 ± 5.70	16.451 ± 6.69	17.56 ± 3.74	17.07 ± 5.25
pH	5.49 ± 0.40	5.59 ± 0.41	5.60 ± 0.43	5.55 ± 0.48	5.55 ± 0.51	5.62 ± 0.35	5.72 ± 0.46	5.80 ± 0.39	5.73 ± 0.53	5.66 ± 0.41	5.64 ± 0.53

[1] $\bar{x}$ ± SD; n = 5. β-OHB$^-$, β-hydroxybutyrate; AcAc$^-$, acetoacetate.

carbon dioxide production by day 7 of the fast (5150 ± 653 J/min), and this diminution in carbon dioxide energy equivalents persisted throughout the starvation period. Energy requirements per unit mass on the basis of carbon dioxide production revealed no significant differences. However, the mean RMR by carbon dioxide production per kg body wt was significantly correlated with day of starvation ($P = 0.03$) and body weight ($P = 0.01$). Thus, the decrease in RMR via carbon dioxide production was disproportionate to weight loss during the first 18 d of starvation.

Carbohydrate oxidation based on oxygen consumption after an overnight fast (day 0) provided 1157 ± 565 J/min, supplying 19% of the total energy requirements (27% of the nonaminogen energy). Carbohydrate oxidation derived from glycogen stores was not detectable after 2–3 d of starvation. Oxidation of aminogenic compounds after an overnight fast provided 1795 ± 770 J/min, furnishing 30% of the total energy. Oxidation of aminogenic compounds declined in a curvilinear mode to 399 ± 112 J/min, supplying 7% of the total energy on day 18. There was no change in aminogenic compound oxidation between day 18 and day 21, after the heightened nitrogen excretion induced by and contained in excreted phenylacetylglutamine was corrected for. Fat oxidation after an overnight fast provided 3102 ± 1546 J/min (51%) and reached a value of 5240 ± 355 J/min after 2 d of starvation, when the quantity of fat oxidized provided 84% of the total energy requirements. After 18 d of starvation, fat oxidation furnished 93% of the total RMR and oxidation of aminogenic compounds provided the rest of the fuel requirements.

Oxidation of aminogenic compounds was based on urinary nitrogen excretion and, therefore, was the same for oxygen or carbon dioxide equivalents. Furthermore, differences in carbohydrate oxidation based on oxygen consumption or carbon dioxide production at different time intervals were insignificant. However, the decrease in fat oxidation on the basis of carbon dioxide production was significantly different after the fourth day of starvation ($P = 0.02$).

The naRQ after an overnight fast was 0.797 ± 0.077. It decreased to a nadir of 0.623 ± 0.041 after 7 d of starvation and remained at about that value throughout the rest of the study. There was no glycosuria in these starving subjects.

The energy equivalent for oxygen consumption was recalculated on the assumption that recycled fatty acids had undergone partial desaturation and caused the naRQ to fall below 0.703. Using this method to estimate the RMR gave values that were between those obtained with use of oxygen and carbon dioxide equivalents (Figure 3B). The naRQ values were below 0.703 between day 2 and day 21. Among these 7 measured values, the RMR corrected for desaturation showed no significant decreases in energy requirements expressed as $J \cdot min^{-1} \cdot kg^{-1}$ when compared with the baseline value on day 0. Also, mean RMR corrected for fatty acid desaturation per kg body wt was not significantly correlated with day of starvation or body weight. Thus, the decrease in RMR corrected for fatty acid desaturation was proportionate to weight loss during the first 18 d of starvation.

Body-composition changes and nitrogen balance data during prolonged starvation are shown in **Table 5**. Weight loss between the first and second body-composition studies was 11.7 ± 2.7 kg, partitioned as 5.9 ± 2.2 kg fat mass by densitometry and 5.9 ± 3.4 kg FFMD. Total urinary nitrogen excretion between the first and second body-composition measurements was 154 ± 26 g. Changes in the body pool of urea accounted for ≈7 ± 3 g of the

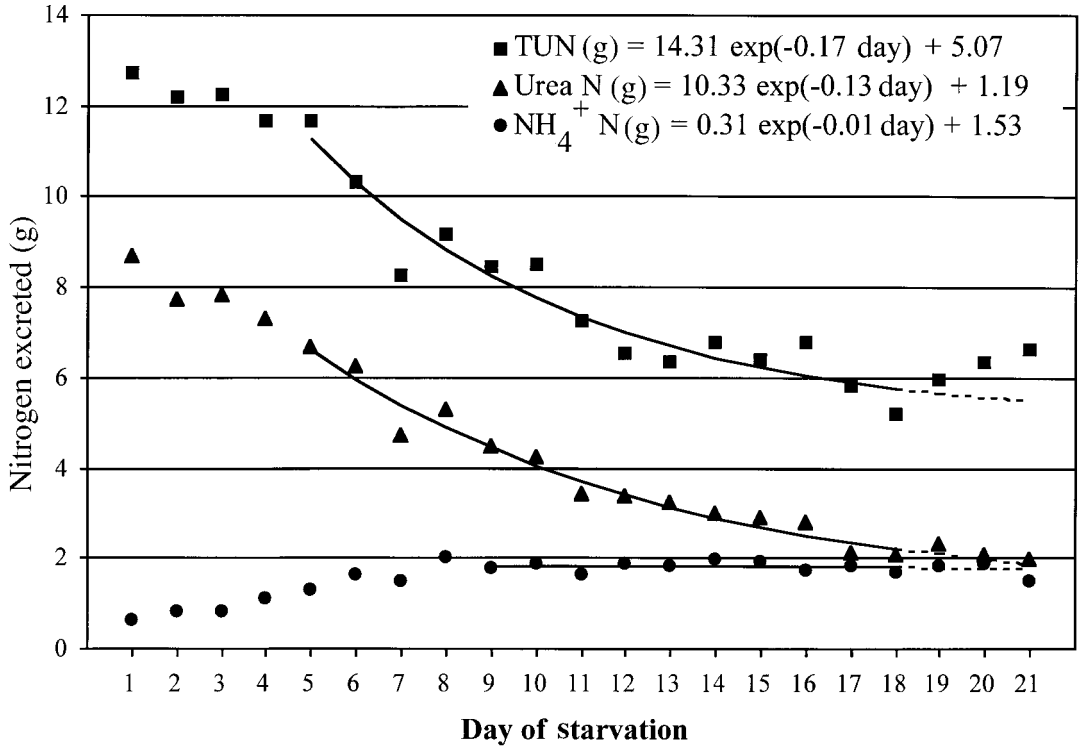

FIGURE 1. Daily quantities of total urinary nitrogen (TUN), urea nitrogen, and ammonium (NH_4^+) nitrogen excreted by 5 obese starving subjects. The quantities of nitrogenous compounds excreted were plotted unstacked to permit their analysis by exponential regressions. Note the time-dependent reciprocal relation between the quantities of urea and NH_4^+ nitrogen excreted.

TABLE 4

Indirect calorimetry: energy requirements and quantities of carbohydrate, fat, and aminogenic compounds oxidized during starvation[1]

	Day								
	0	1	2	3	4	7	14	18	21
RMR (J/min)									
Based on O_2	6055 ± 984	6340 ± 1108	6203 ± 445	6065 ± 655	5947 ± 475	5732 ± 640	5661 ± 710	5594 ± 812	5676 ± 756
Based on CO_2	6048 ± 975	6296 ± 1149	6050 ± 516	5858 ± 796	5600 ± 528	5150 ± 653	5189 ± 788	5045 ± 791	5156 ± 911
Corrected for desaturation	6055 ± 984	6330 ± 1119	6167 ± 460	6016 ± 681	5866 ± 481	5592 ± 630	5548 ± 726	5463 ± 803	5551 ± 783
P (O_2 versus CO_2)	0.498	0.192	0.067	0.180	0.015	0.013	0.004	0.003	0.026
P (O_2 versus desaturation)	—	0.229	0.071	0.185	0.020	0.013	0.005	0.003	0.026
P (CO_2 versus desaturation)	0.498	0.191	0.066	0.178	0.014	0.013	0.004	0.003	0.026
naRQ									
Uncorrected	0.797 ± 0.077	0.706 ± 0.022	0.683 ± 0.021	0.677 ± 0.046	0.655 ± 0.031	0.623 ± 0.041	0.637 ± 0.028	0.628 ± 0.025	0.632 ± 0.045
Corrected for desaturation	0.797 ± 0.077	0.713 ± 0.014	0.704 ± 0.002	0.706 ± 0.007	0.704 ± 0.001	0.703 ± 0.000	0.703 ± 0.000	0.703 ± 0.000	0.703 ± 0.000
P	—	0.258	0.071	0.196	0.024	0.012	0.006	0.002	0.024
Carbohydrate oxidized (J/min)									
Based on O_2	1157 ± 565	242 ± 392	29 ± 65	70 ± 156	26 ± 57	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Based on CO_2	1158 ± 566	241 ± 390	29 ± 65	70 ± 156	25 ± 57	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Corrected for desaturation	1157 ± 565	242 ± 392	29 ± 65	70 ± 156	26 ± 57	0 ± 0	0 ± 0	0 ± 0	0 ± 0
P (O_2 versus CO_2)	0.969	0.420	0.374	0.374	0.374	—	—	—	—
P (O_2 versus desaturation)	—	—	—	—	—	—	—	—	—
P (CO_2 versus desaturation)	0.969	0.420	0.374	0.374	0.374	—	—	—	—
Fat oxidized (J/min)									
Based on O_2	3102 ± 1546	5122 ± 991	5240 ± 355	5056 ± 577	5028 ± 473	5097 ± 672	5142 ± 697	5195 ± 721	5278 ± 570
Based on CO_2	3096 ± 1540	5080 ± 1043	5087 ± 452	4849 ± 685	4681 ± 535	4515 ± 625	4669 ± 746	4646 ± 713	4758 ± 756
Corrected for desaturation	3102 ± 1546	5113 ± 1003	5204 ± 377	5007 ± 592	4946 ± 482	4958 ± 649	5028 ± 705	5063 ± 715	5153 ± 602
P (O_2 versus CO_2)	0.398	0.199	0.067	0.180	0.015	0.013	0.004	0.003	0.026
P (O_2 versus desaturation)	—-	0.229	0.071	0.185	0.020	0.013	0.005	0.003	0.026
P (CO_2 versus desaturation)	0.398	0.198	0.066	0.178	0.014	0.013	0.004	0.003	0.026
Aminogenic compounds oxidized via urinary nitrogen excretion (J/min)	1795 ± 770	976 ± 261	934 ± 210	939 ± 167	894 ± 229	634 ± 149	519 ± 164	399 ± 112	399 ± 193

[1] The calculations of energy based on oxygen consumed and carbon dioxide produced and of energy based on grams of urinary nitrogen were performed by the method of Jungas et al (26) and of Loewy as described in Best and Taylor (27), respectively. RMR, resting metabolic rate; naRQ, nonaminogen respiratory quotient. $n = 5$.

[2] $\bar{x} \pm$ SD.

TABLE 5
Body composition, urea pool, nitrogen excretion, and ratio of fat-free mass to nitrogen loss before and after starvation [1]

	Value
Weight (kg)	
1	143.8 ± 15.2
2	132.1 ± 13.7
Change	11.7 ± 2.7
FATMD (kg)	
1	71.2 ± 13.4
2	65.3 ± 12.8
Change	5.9 ± 2.2
Fat (%)	
1	49.3 ± 5.8
2	49.3 ± 6.0
Change	0.0 ± 3.6
FFMD	
1 (kg)	72.6 ± 8.1
2 (kg)	66.7 ± 6.0
Change (kg)	5.9 ± 3.4
Total urinary nitrogen (g)	154.1 ± 26.0
Change in urea pool (g)	6.6 ± 2.5
Change in FFM N (g)	147.4 ± 25.1
ΔFFMD: ΔN	43 ± 31

[1] $\bar{x}$ ± SD; n = 5. 1 and 2 refer to the first and second body-composition measurements (*see* Table 2). FATMD, fat mass by densitometry; FFMD, fat-free mass by densitometry; FFM N, fat-free mass nitrogen.

nitrogen loss from the body. Therefore, the net fat-free mass nitrogen loss was ≈147 ± 25 g. The calculated ratio of FFMD to TUN loss corrected for changes in the body urea pool was 43:1.

The mean FFMD loss from lean structural protein was 147 g × 5.57/6.25 protein = 730 g protein. Lean tissue is ≈20% protein, or the ratio of lean tissue to protein is 5:1. Therefore, the mass of lean tissue loss equaled ≈730 g × 5 tissue = 3650 g tissue. The difference between total FFMD loss and lean tissue loss was calculated as 5880 g − 3650 g = 2230 g, which was assigned to extracellular fluid loss. The mean assumed simple and conjugated proteins (eg, glycoproteins) lost from the extracellular fluid were 147 g × 0.68 aminogenic material = 100 g aminogenic material. The percentage of extracellular fluid loss that was aminogenic materials was 100/2230 = 4.5%, or, the ratio of extracellular fluid to aminogenic material loss was ≈22:1.

Arterial concentrations, arteriovenous concentration differences, and exchange rates

In **Table 6** are shown the changes in the arterial blood or plasma concentrations of glucose, alanine, β-OHB^-, $AcAc^-$, and fatty acids that mimic the patterns known to occur in venous blood or plasma of nondiabetic subjects during prolonged starvation (5, 6, 50). The absolute values for blood glucose concentrations were slightly greater than those reported previously for obese starving persons with normal glucose tolerance (5). The perturbations induced by the administration of phenylacetate caused no significant differences in arterial blood or plasma concentrations of these metabolites between day 18 and day 21.

Arterial blood glutamine concentrations did not change significantly between day 0 and day 18 of the study; however, there was a significant decrease between day 18 and day 21 (P = 0.02). Arterial blood glutamate concentrations did not change significantly throughout the study. On day 18, arterial blood and plasma alanine and glutamine concentrations were indistinguishable, but concentrations of arterial blood glutamate (145 ± 34 μmol/L) and arterial plasma glutamate (102 ± 17 μmol/L) were significantly different (P < 0.01) (51, 52).

Typical decreases in blood glucose and serum insulin concentrations occurred, but the changes were not in parallel. Serum T_3 concentrations fell by 30% from day 0 to day 4 (P = 0.0003) and continued to decline through days 18–21 of fasting to values below the normal range. Serum T_4 concentrations decreased between day 4 and day 21 (P = 0.02) but remained above the lower limit of the normal value. The rise in rT_3 may not have been significant (P = 0.11). The morning serum T_3 concentrations were significantly correlated with the following day's urinary excretion of urea nitrogen (P = 0.03), NH_4^+ nitrogen (P = 0.05), and urea plus NH_4^+ nitrogen (P = 0.03) during starvation. In addition, the decreases in serum T_3 concentrations were also correlated with the decreases in respiratory carbon dioxide production (P = 0.007).

The decreases in arterial blood pH (P = 0.10) and plasma C-peptide (P = 0.08) from day 18 to day 21 (Table 6) were of borderline significance. The mean decrease in the arterial serum insulin concentration was of questionable significance and the norepinephrine concentration remained unchanged. The rise in the arterial concentration of epinephrine was clearly significant (P = 0.04).

There were considerable variances among the subjects regarding substrate arteriovenous concentration differences, flow rates, and, thus, net exchange rates, as reflected in the large SDs of the means and probability values. Substrate arteriovenous concentration differences showed the greatest variances, and practically every subject had some deviation from the expected finding among the 3 sampling sites, eg, hepatic uptake of glucose in 1 of 2 arteriovenous differences for subject 1.

Individual arteriovenous concentration differences were multiplied by individual regional blood (or plasma) flow rates to calculate net exchange rates (Table 6). The mean (± SD) hepatic blood (plasma) flow rate was 2295 ± 1112 (1419 ± 730) mL/min and the renal blood (plasma) flow rate was 1267 ± 450 (776 ± 278) mL/min. The calculated peripheral extremity blood (plasma) flow rate was 2137 ± 618 (1317 ± 422) mL/min.

The mean (± SD) hepatic (splanchnic) release of glucose (Table 6) for the 4 subjects who underwent catheterization stud-

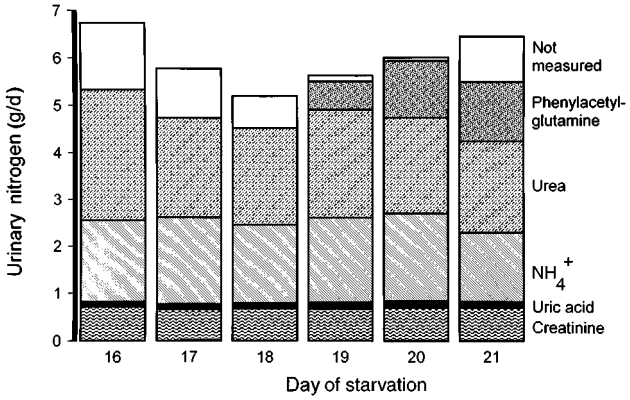

FIGURE 2. Daily excreted quantities of urinary total nitrogen; creatinine, uric acid, ammonium (NH_4^+), urea, and phenylacetylglutamine nitrogen; and nitrogen components not measured for starvation days 16–21 in 5 obese subjects before and during phenylacetate administration. Phenylacetate was administered from day 19 to day 21.

TABLE 6
Arterial blood, plasma, or serum metabolite, hormone, and hydrogen (pH) concentrations; arteriovenous concentration differences, and net exchange rates[1]

	Day					*P* (Day 18 versus 21)	Arteriovenous differences day 21			Net exchange rates day 21		
Metabolite or hormone	0	4	15	18	21		Hepatic	Renal	Extremity	Hepatic	Renal	Extremity
			mmol/L					*μmol/L*			*μmol/min*	
Blood glucose	7.06 ± 2.40	4.62 ± 1.28	5.33 ± 1.46	4.81 ± 1.41	4.39 ± 1.46	0.25	−143 ± 149	−206 ± 68	104 ± 100	−225 ± 310	−254 ± 86	249 ± 283
			μmol/L									
Blood lactate	—	—	—	—	605 ± 66	—	288 ± 49	64 ± 30	−117 ± 63	623 ± 197	87 ± 51	−225 ± 66
Blood pyruvate	—	—	—	—	62 ± 12	—	29 ± 17	16 ± 12	−11 ± 3	60 ± 26	17 ± 11	−23 ± 3
Blood glycerol	—	—	—	155 ± 44	111 ± 43	0.08	102 ± 34	49 ± 14	−65 ± 38	252 ± 189	64 ± 38	−144 ± 89
Blood glutamine	439 ± 69	454 ± 87	484 ± 108	528 ± 103	405 ± 78	0.02	1 ± 29	85 ± 52	−24 ± 35	−9 ± 69	88 ± 52	−46 ± 58
Blood glutamate	172 ± 40	155 ± 31	140 ± 45	145 ± 34	148 ± 36	0.75	−70 ± 9	−8 ± 10	36 ± 14	−156 ± 99	−8 ± 7	77 ± 35
Blood alanine	340 ± 54	234 ± 25	241 ± 43	198 ± 27	198 ± 36	0.98	86 ± 13	19 ± 24	−45 ± 22	189 ± 74	18 ± 21	−90 ± 32
			mmol/L									
Plasma FFAs	0.84 ± 0.36	1.13 ± 0.40	1.53 ± 0.27	1.36 ± 0.29	1.19 ± 0.18	0.08	616 ± 90	63 ± 45	−281 ± 161	842 ± 369	40 ± 31	−612 ± 342
Blood β-OHB^-	0.19 ± 0.18	2.44 ± 1.19	4.18 ± 1.12	4.85 ± 1.01	4.60 ± 1.19	0.34	−508 ± 118	98 ± 305	−132 ± 526	−1090 ± 347	−6 ± 110	−177 ± 1025
Blood $AcAc^-$	0.13 ± 0.07	0.88 ± 0.31	1.39 ± 0.20	1.43 ± 0.36	1.72 ± 0.18	0.12	−583 ± 198	−8 ± 37	272 ± 63	−1195 ± 219	−8 ± 37	565 ± 128
			nmol/L					*nmol/L*			*nmol/min*	
Serum T_4	86 ± 10	92 ± 9	75 ± 25	71 ± 24	59 ± 17	0.12	−3 ± 3	−2 ± 3	0 ± 1	−4 ± 4	−2 ± 3	0 ± 1
Serum T_3	1.47 ± 0.17	1.04 ± 0.25	0.90 ± 0.34	0.89 ± 0.37	0.76 ± 0.23	0.26	−0.02 ± 0.04	−0.03 ± 0.05	−0.04 ± 0.03	−0.04 ± 0.05	−0.01 ± 0.03	−0.05 ± 0.04
Serum rT_3	0.37 ± 0.13	0.47 ± 0.17	0.45 ± 0.19	0.38 ± 0.10	0.32 ± 0.11	0.13	0.05 ± 0.01	−0.00 ± 0.02	0.05±0.02	0.07 ± 0.02	0.00 ± 0.01	−0.06 ± 0.03
			pmol/L					*pmol/L*			*pmol/min*	
Serum insulin	118 ± 87	79 ± 50	113 ± 82	122 ± 86	72 ± 42	0.16	−23 ± 8	29 ± 11	38 ± 28	−34 ± 23	22 ± 7	38 ± 27
			μg/L					*μg/L*			*μg/min*	
Plasma C-peptide	0.83 ± 0.31	0.49 ± 0.13	0.59 ± 0.20	0.74 ± 0.36	0.55 ± 0.20	0.08	−0.18 ± .07	0.15 ± 0.05	0.03 ± 0.03	−0.29 ± .27	0.11 ± 0.03	0.04 ± 0.04
			nmol/L					*pmol/L*			*pmol/min*	
Plasma epinephrine	257 ± 146	243 ± 139	400 ± 324	358 ± 343	560 ± 478	0.04	500 ± 412	233 ± 184	472 ± 338	6427 ± 4299	1644 ± 1348	5754 ± 3509
								nmol/L			*nmol/min*	
Plasma norepinephrine	1.54 ± 0.56	1.88 ± 1.00	2.11 ± 0.81	2.02 ± 0.90	233 ± 1.50	0.75	2.0 ± 1.1	−1.6 ± 0.4	0.5 ± 0.2	2.5 ± 0.7	−1.2 ± 0.4	0.6 ± 0.2
Blood pH	—	—	—	7.36 ± 0.07	7.34 ± 0.04	0.10	—	—	—	—	—	—

[1] $\bar{x}$ ± SD; n = 5. FFAs, free fatty acids; β-OHB^-, β-hydroxybutyrate; $AcAc^-$, acetoacetate; T_4, thyroxine; T_3, triiodothyronine; rT_3, reverse triiodothyronine.

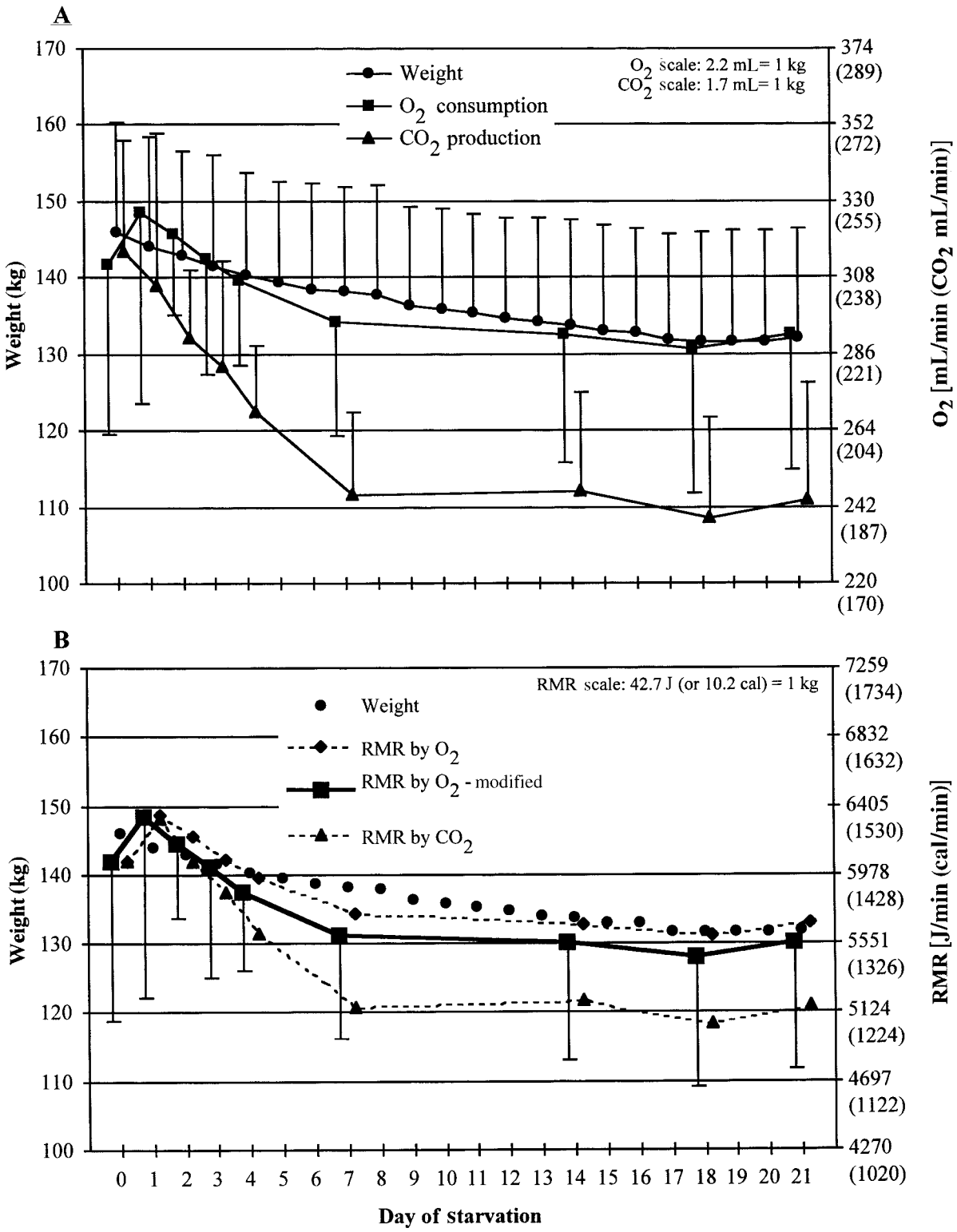

FIGURE 3. Mean (±SD) body weight, oxygen consumption, and carbon dioxide production (A) and resting metabolic rate (RMR) by oxygen consumption (26), oxygen consumption as modified for fatty acid desaturation (*see* Methods), and carbon dioxide production (26) (B) for 5 obese subjects who were studied during a 0–21 d fast. Carbon dioxide production decreased out of proportion to oxygen consumption, creating a mysterious nonaminogen respiratory quotient and a disparity in RMR according to oxygen consumption and carbon dioxide production that was rectified by correcting for assumed fatty acid desaturation during starvation. The symbols were slightly misaligned so that they could be distinguished from each other. The SDs for weight and RMR by oxygen consumption and carbon dioxide production were omitted in B to avoid clutter.

TABLE 7
Arterial concentrations, arteriovenous concentration differences, net exchange rates, and excretion rates of urea, ammonia or ammonium ion, and phenylacetylglutamine[1]

Metabolite	Day				Arteriovenous concentration differences			Net exchange rates			Urinary excretion rate
	0	4–7	14–15	18–21	Hepatic	Renal	Extremity	Hepatic	Renal	Extremity	
	mmol/L				*μmol/L*			*μmol/min*			*μmol/min*
Urea	4.47 ± 1.05	2.82 ± 0.52	1.73 ± 0.38	1.29 ± 0.69	−40 ± 116	95 ± 61	−28 ± 56	−41 ± 194	117 ± 76	−54 ± 94	53 ± 40
	μmol/L										
NH_3 or NH_4^+	—	—	—	71 ± 46	20 ± 36	−47 ± 36	9 ± 27	20 ± 67	−59 ± 46	17 ± 47	82 ± 16
Phenylacetylglutamine[2]	—	—	—	167 ± 92	−54 ± 26	78 ± 33	4 ± 15	−62 ± 21	60 ± 33	3 ± 21	36 ± 6

[1] $\bar{x}$ ± SD; n = 5.

[2] Plasma hepatic and renal arteriovenous concentration differences were multiplied by plasma hepatic and renal flow rates. Renal excretion rates were calculated from urine collected 0–24 h before the catheterization study. There was a lag between initiating the administration of phenylacetate and the urinary excretion of phenylacetylglutamine and there was persistent urinary excretion of phenylacetylglutamine after phenylacetate intake was stopped (data not shown).

ies was 225 ± 310 μmol/min and was not significantly different from zero. Net renal release of glucose was 254 ± 86 μmol/min (P = 0.01). The combined hepatic and renal glucose production was 479 ± 248 μmol/min (P = 0.03). The uptake of lactate (P = 0.01), pyruvate (P = 0.05), glycerol (P = 0.07), and alanine (P = 0.01), minus the release of glutamine and glutamate (P = 0.05) as α-ketoglutarate equivalents (39), could readily account for hepatic glucose production. The uptake of glutamine (P = 0.04), glycerol (P = 0.04), lactate (P = 0.02), and pyruvate (P = 0.05) could not account for renal glucose production. Only half of the hepatic glutamate release (156 ± 99 μmol/min, P < 0.05) was extracted by the extremities (77 ± 35 μmol/min, P < 0.02), suggesting extraction of glutamate by the brain or other tissues.

Net hepatic (splanchnic) extraction of plasma fatty acids (842 ± 369 μmol/min, P = 0.02) could easily account for the net production of β-OHB^- (1090 ± 347 μmol/min, P = 0.01) plus $AcAc^-$ (1195 ± 219 μmol/min, P = 0.01). There was a net release of fatty acids from the extremities (612 ± 342 μmol/min, P = 0.04) and a net extraction of $AcAc^-$ (565 ± 128 μmol/min, P = 0.01). The net release rate for β-OHB^- across the extremities was not significantly different from zero. There was a trend for renal extraction of plasma fatty acids (40 ± 31 μmol/min, P = 0.08), but the renal arteriovenous differences and, thus, the net exchange rates for β-OHB^- and $AcAc^-$ were not significantly different from zero despite large urinary excretion rates of β-OHB^- and $AcAc^-$ in every subject: β-OHB^-, 39 ± 9 μmol/min, P = 0.0005; $AcAc^-$, 12 ± 4 μmol/min, P = 0.002 (calculated from Table 3).

Regional exchange rates for T_4, T_3, rT_3, insulin, C-peptide, epinephrine, and norepinephrine are also shown in Table 6. No significant arteriovenous differences in the concentration of T_4 were detected across the hepatic, renal, or lower-extremity vascular beds. There were no detected arteriovenous concentration differences of T_3 across the liver or kidneys, but differences suggesting production of T_3 across the lower extremity were of borderline significance (P = 0.10). Unanticipated findings were lower-extremity production of rT_3 (P = 0.03) and hepatic arteriovenous concentration differences reflecting extraction of rT_3 (P = 0.003). All subjects probably released insulin from the splanchnic bed (34 ± 23 pmol/min, P = 0.06) and extracted insulin by the renal (22 ± 7 pmol/min, P = 0.01) and lower-extremity (38 ± 27 pmol/min, P = 0.07) beds. There were trends for splanchnic release of C-peptide (0.29 ± 0.27 nmol/min, P = 0.12) and lower-extremity extraction of C-peptide (0.04 ± 0.04 nmol/min, P = 0.11). C-peptide was clearly removed by the kidneys (0.11 ± 0.03 nmol/min, P = 0.007). Plasma epinephrine was extracted by the lower-extremity bed (5754 ± 3509 pmol/min, P = 0.05), and probably extracted by the hepatic (6427 ± 4299 pmol/min, P = 0.06) and renal (1644 ± 1348 pmol/min, P = 0.09) tissues. There was a definite release of norepinephrine into the renal veins (1.2 ± 0.4 nmol/min, P = 0.006) and clear hepatic (2.5 ± 0.7 nmol/min, P = 0.005) and lower-extremity (0.6 ± 0.2 nmol/min, P = 0.008) extraction of norepinephrine.

TABLE 8
Phenylacetate dose and phenylacetylglutamine excretion[1]

Measurement	Day of study				
	17	18	19	20	21
Phenylacetate dose (g/d)	—	—	11.5 ± 0.72	11.4 ± 0.94	11.0 ± 1.41
Urinary phenylacetylglutamine excretion	0.102	0.128	4.439	7.322	6.990
(g/d)	0.038	0.056	1.201	2.180	1.109
(mmol/d)	0.7 ± 0.2	1.0 ± 0.4	32.6 ± 8.7	53.8 ± 16.1	51.4 ± 8.1
Percentage of dose excreted (%)	—	—	39 ± 9	65 ± 18	64 ± 13
Urinary phenylacetylglutamine N excreted (mg/d)	21 ± 9	26 ± 11	914 ± 246	1508 ± 450	1439 ± 228
Phenylacetylglutamine excretion − control excretion × 2 − NH_4^+ (mmol/d)	—	—	65 ± 34	27 ± 56	10 ± 20

[1] $\bar{x}$ ± SD; n = 5.

Arterial blood urea concentrations (**Table 7**) from day 0 to days 18–21 decreased progressively ($P = 0.007$). The values for net regional exchange of urea showed insignificant hepatic and lower-extremity production rates. Renal extraction (117 ± 76 μmol/min, $P = 0.06$) and urinary excretion (53 ± 40 μmol/min, $P = 0.08$) rates were statistically frustrating because they were of only borderline significance. This was especially true for urea excretion because every subject excreted urinary urea but the variance among the 4 individuals who underwent catheterization was too large to give a $P < 0.05$. However, when the urinary urea excretion rates for the 5 subjects were used to calculate significance, the P value was 0.04.

There was a trend for renal release of ammonia into the blood (59 ± 46 μmol/min, $P = 0.08$). Urinary excretion of NH_4^+ was 82 ± 16 μmol/min ($P = 0.002$). Thus, of the 141 μmol ammonia/min produced by the kidney, 58% was excreted in the urine as NH_4^+ and probably 42% was released into the blood as ammonia. Renal extraction of glutamine (Table 6) was 88 ± 52 μmol/min ($P = 0.04$) and could account for the total production of ammonia and NH_4^+ by the kidneys during the prolonged starvation period (Table 7).

Arterial plasma phenylacetylglutamine concentrations on day 21 were 167 ± 92 μmol/L (Table 7). The net hepatic production rate was 62 ± 21 μmol/min ($P = 0.01$) and the net renal extraction rate was 60 ± 33 μmol/min ($P = 0.01$). These values were based on plasma concentrations and plasma flow rates. There was no significant arteriovenous concentration difference across the lower extremity for phenylacetylglutamine. Urinary excretion was 36 ± 6 μmol/min ($P = 0.001$). The excretion rate reflects the time period of ≈24 to 0 h before the catheterization studies were done. The difference between urinary excretion and renal extraction rates can be explained, at least in part, by the lag between intake of phenylacetate and excretion of phenylacetylglutamine, ie, phenylacetylglutamine excretion continued for 2–3 d after phenylacetate intake was stopped (data not shown).

The mean doses of phenylacetate for days 19, 20, and 21 and the percentage of the dose excreted as phenylacetylglutamine are shown in **Table 8**. The heightened TUN during days 19–21 could be accounted for by the amount of nitrogen contained in urinary phenylacetylglutamine. The urinary phenylacetylglutamine excretion, corrected for control excretion, multiplied by 2, minus urinary NH_4^+ excretion, provided a mean (± SD) residual quantity of NH_4^+ not covered by phenylacetylglutamine excretion that amounted to 65 ± 34, 27 ± 56, and 10 ± 20 mmol/d on days 19, 20, and 21, respectively.

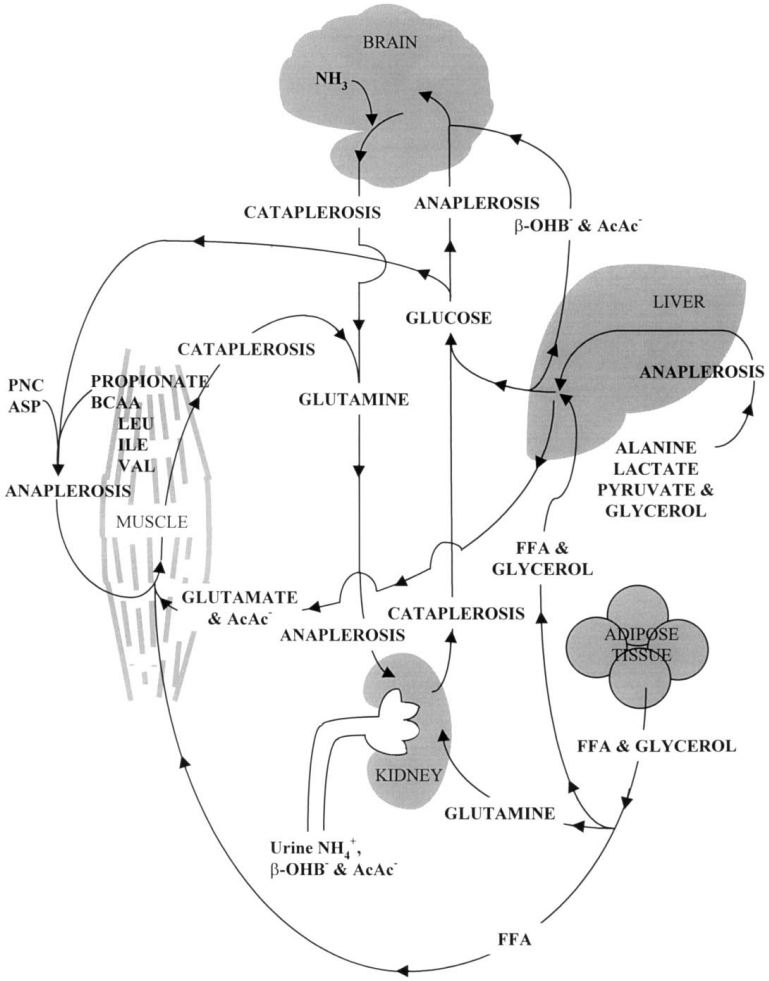

FIGURE 4. Anaplerotic and cataplerotic reactions in the major organ systems of the body. The interorgan fluxes of fuels are highlighted by glucose and glutamine. $AcAc^-$, acetoacetate; ASP, aspartate; β-OHB^-, β-hydroxybutyrate; BCAA, branched-chain amino acid; FFA, free fatty acid; LEU, leucine; ILE, isoleucine; NH_3, ammonia; NH_4^+, ammonium; PNC, purine nucleotide cycle; VAL, valine.

DISCUSSION

In this study we combined nitrogen balance studies with measurements of blood metabolite and hormone concentrations, indirect calorimetry, measurements of body-composition changes, and catheterization techniques to gain insight into the essentiality of amino acid utilization during starvation. We assessed the influence of administering phenylacetate on ammoniagenesis, ureagenesis, gluconeogenesis, ketogenesis, and resting metabolic energy requirements during the near steady state of prolonged starvation. After 3 d of phenylacetate administration, practically all NH_4^+ excreted could have been excreted as phenylacetylglutamine and no NH_4^+ needed to be excreted to rid the body of this nitrogenous waste product. However, we found that robbing the body of a cardinal gluconeogenic amino acid, glutamine, resulted in augmented aminogenolysis, as reflected by the increased urinary excretion of nitrogen as phenylacetylglutamine while ammoniagenesis, ureagenesis, and gluconeogenesis remained constant. This compensatory response of proteolysis (or aminogenolysis) increased urinary nitrogen excretion and was accompanied by a modest increase in plasma epinephrine concentrations, which may have also augmented hepatic ketogenesis. These results show that there was a minimum urinary nitrogen loss of NH_4^+ and urea (and creatinine and urate) per unit body mass during a given time of total starvation and suggest that augmented proteolysis occurred to maintain a consistent supply of amino acid intermediates or precursors to provide substrates for fuel homeostasis. Glutamine specifically provided precursors for gluconeogenesis and ammoniagenesis. Glutamine mobilization was essential and persisted in grossly obese persons even though their bodies were being compromised by protein depletion. RMR remained unchanged by the perturbation.

These results confirm that energy demands, which are generated mostly in the citric acid cycle of mitochondria, are relatively constant in the resting state and have precedence over all other body requirements (53). Furthermore, they clearly show that even in the resting state the body must have a continuous supply of amino acids or glucose for oxidation to accompany fatty acid oxidation to maintain functional activity of the citric acid cycle. Summarily, the data suggest that morbidly obese persons subjected to prolonged periods of starvation can die from protein depletion because there is constitutive amino acid oxidation for energy production, which can lead to depletion of vital proteins before the huge fat stores are consumed.

Urinary excretory products

The daily urinary excretion rates for total, urea, NH_4^+, and urate nitrogen and for β-OHB^- plus $AcAc^-$ underwent progressive changes during starvation (Table 3). During the first 18 d of starvation total daily urinary nitrogen excretion is curvilinear and the persistent loss of nitrogen from the body is unfortunate for the starving person. As the duration of starvation progresses into months, persistent nitrogen excretion in the presence of adequate fat stores (5, 54) can cause death from gross fragmentation of myofibrils (1) and depletion of other lean body mass organs (2).

Throughout starvation, urea and NH_4^+ were the dominant excretory nitrogenous compounds. Whereas urea excretion decreased in an exponential manner, NH_4^+ excretion increased to equal urea excretion by 18 d of starvation. During prolonged starvation, urinary excretion rates of NH_4^+, β-OHB^-, and $AcAc^-$ rose acutely and grossly until day 9 of starvation; thereafter, they slowly declined in parallel and in an exponential manner. Urate excretion plummeted during the first few days of starvation and developed an exponential decay curve after day 10 of starvation. Creatinine excretion also decreased in an exponential manner as starvation progressed, but the duration of starvation was too short to show a significant reduction.

The roles urate and creatinine played in providing energy during this starvation study were not investigated in detail. However, the purine nucleotide cycle is interconnected with the citric acid cycle, providing intermediate metabolites via anaplerosis for sustained energy production (55–57). Persistent urinary excretion of uric acid during prolonged starvation indicates persistent de novo synthesis of purines and maintenance of the purine nucleotide cycle, which contributes to energy production in muscle, especially during strenuous exercise (55). In skeletal muscle the purine nucleotide cycle is involved in the anaplerotic supply of citric acid cycle intermediates for maintaining a high average ATP-to-ADP ratio. AMP deaminase, adenylosuccinate synthetase, and adenylosuccinate lipase form a functional unit of enzymes in skeletal muscles (57–59) that convert aspartate and guanosine triphosphate into fumarate, ammonia, and guanosine diphosphate. It is reasonable to assume that the aspartate anaplerotic reaction contributing fumarate is dragged into the 4-carbon pool of the citric acid cycle intermediates by the glutamine cataplerotic reaction that drains α-ketoglutarate from the citric acid cycle. Giving phenylacetate during starvation augmented urate excretion by 21% (Table 3), suggesting a heightened contribution of the purine nucleotide cycle. However, there was no significant difference in uric acid excretion between day 18 and day 21. Assuming an energy value of ≈109 kJ/g urate nitrogen (60), which is probably twice the actual value (30), these quantities of uric acid equal only 13–21 kJ/d derived from protein catabolized to uric acid and excreted in the urine (60). Therefore, the overall quantitative contribution of energy to the body from the purine nucleotide cycle during rest was minute.

Creatine phosphate also provides muscle with ATP. The carbon-nitrogen structure of creatine phosphate is synthesized from arginine, glycine, and methionine. The metabolism of these amino acids is commingled with that of the amino acids of the urea and purine nucleotide cycles (26). Creatine kinase liberates the free energy of creatine phosphate as it is converted into ATP and creatinine (61). Creatinine is the anhydride of creatine and its urinary excretion reflects the energy provided from creatine phosphate. Because of the length of this starvation study, the decline in creatinine excretion was not significant. Assuming an energy value of 56.1 kJ/g creatinine nitrogen excreted daily (30), creatinine excretion reflected a contribution of ≈29–42 kJ/d. Thus, the combined urate and creatinine contributions to total energy requirements were small; collectively, they provided no more than ≈8–11% of the energy derived from aminogen catabolism after prolonged starvation.

Body composition

Obese humans lose body fat and fat-free masses in parallel during the initial 3 wk of total starvation. The ratio of FFMD loss to urinary nitrogen loss corrected for changes in the urea body pool was 43:1 in the present study, suggesting that 43 g of fat-free body mass was lost for every 1 g of urinary nitrogen excreted. The changes in fat-free mass included fluid and electrolyte losses, primarily during early the phases of starvation, and simple and conjugated protein losses during all phases of

starvation. Diuresis and natriuresis may cause an overestimation of muscle wasting and other readily identifiable structural proteins in lean body organs according to results obtained from hydrodensitometry measurements during starvation (50). The following data give credence to this claim. The classic value of 1 g of urinary nitrogen equaling the loss of 6.25 g protein (27) does not agree with the modern value of 1 g muscle nitrogen equaling 5.57 g muscle protein (26). However, assigning the 6.25 value to 1 g urinary nitrogen can be used to quantitate the loss of structural proteins and the loss of visceral proteins and glycoproteins in the extracellular fluids during starvation. On the basis of body-composition changes in FFMD of ≈5.9 kg (Table 5), the following calculations were made to estimate the source of urinary nitrogen. The total quantity of structural protein loss (730 g) could account for ≈3.7 kg lean tissue or ≈62% of the measured FFMD changes. It is likely that the remaining loss of ≈2.2 kg or ≈38% of the FFMD came from extracellular fluids containing nitrogen in simple and conjugated proteins and other aminogenic compounds. This is plausible because a sizable portion of body aminogenic components are extracellular. These deductions are supported by the findings of Nurjhan et al (62), who combined arteriovenous concentration differences and regional blood flow rates with kinetic analyses of traces to show that the release of the 2 most prominent amino acids, alanine and glutamine, from body muscle accounted for only 46% and 71%, respectively, of their appearance in plasma.

The urinary nitrogen loss from the body was curvilinear, suggesting that the loss of fat-free mass was persistent although not absolutely constant during starvation. These nearly equal and parallel losses of body fat and fat-free mass in obese starving persons whose physical activity was limited to short walks in hospital rooms or hallways were astonishing findings and should be worrisome to those who need to predict survival time for obese individuals deprived of food.

Indirect calorimetry

When the RQ is < 0.7 during starvation, the energy values for respiratory gaseous exchange rates (coupled with urinary nitrogen excretion rates and corrected or not corrected for ketonuria, changes in the urea body pool, or source of nitrogen) are indeterminate when using the table of Cathcart and Cuthbertson in Best and Taylor (27). We and others previously proposed interpretations that fail to explain the naRQ of 0.63 observed in this study (31, 63, 64). A reassessment of the data presented here provides a novel explanation for an naRQ ≤ 0.7.

Although the energy value for a liter of oxygen consumed to oxidize carbohydrate, protein, and fat varies (26, 27), the energy value of O_2/L is constant when a constant mixture of fuels is oxidized, eg, fat, aminogens, or both. After days 0–1 (ie, during days 2–21), fat oxidation accounted for ≈84–93% of the oxygen consumed and practically all of the remaining oxygen was consumed by the oxidation of aminogenic compounds. Thus, for practical purposes the energy value of O_2/L should be relatively constant because the mixture of fuel oxidized was relatively constant. Although it is alleged that thermogenesis and energy expenditure are predominantly dependent on oxygen rather than carbon dioxide respiratory exchange rates (27, 30, 60), the basis for this claim is elusive. We present data showing that not making corrections for desaturation of fatty acids when the naRQ is < 0.7 may moderately overestimate the energy value of oxygen consumption and uncouple thermogenesis from carbon dioxide production.

Daily changes in body weight and periodic measurements of oxygen consumption and carbon dioxide production are shown in Figure 3A. The energy values for O_2/L for protein (aminogen) and fat oxidation were based on measurements made by Loewy (27) and Jungas et al (26), respectively. Body weight and energy requirements based on oxygen consumption decreased in parallel. Thus, the small decrease in RMR during prolonged starvation was directly related to body size. The RMR per kg body wt on the basis of oxygen consumption and urinary nitrogen excretion in this study remained constant at ≈42 $J \cdot kg\ body\ wt^{-1} \cdot min^{-1}$ during the first 18–21 d of fasting. This constancy in thermogenesis during starvation among obese subjects agrees with the classic study of Benedict, who reported that the RMR (heat production/24 h) and body weight of a lean man who fasted for 31 d decreased in parallel during the first 18 d of total starvation (65). In contrast, Keys et al (66) subjected 12 lean men to 24 wk of semistarvation and reported that the RMR/kg body wt decreased progressively. These subjects' RQs did not fall below 0.7. Keys et al qualified their data from semistarved subjects by writing that among the control subjects there was also a progressive fall in RMR independent of weight loss, which was attributed to "the relaxation that occurred with training" (66). Our subjects were familiarized with the equipment used to measure respiratory exchange rates and we did not detect a drop in oxygen consumption (RMR)/kg body wt.

Another perspective can be developed by calculating RMR from carbon dioxide production rates. Use of carbon dioxide production to estimate RMR/kg body wt generated results that agreed with those obtained with use of oxygen consumption when the naRQ was ≥ 0.7. However, as starvation progressed to day 4 and the naRQ fell below 0.7, we recorded disparate results. Energy requirements based on carbon dioxide exchange and urinary nitrogen excretion rates were $41.8 \pm 7.1\ J \cdot kg^{-1} \cdot min^{-1}$ on day 0 of the fast and fell to $40.2 \pm 3.3\ J \cdot kg^{-1} \cdot min^{-1}$ by day 4 and then remained relatively constant per kg body wt during days 4–21 of the starvation period. The carbon dioxide data between day 4 and day 21 were significantly lower than the oxygen data for RMR ($P = 0.02$).

Carbon dioxide production fell disproportionately to oxygen consumption, creating a mysterious situation in which naRQ values were below the theoretical minimum of 0.7 after ketonuria was corrected for. It is likely that oxygen consumption and carbon dioxide production rates during starvation that yield naRQ values ranging from 0.62 to 0.65 reflect important but incompletely explored biochemical and physiologic adaptations for survival. In the past, such low naRQs were attributed to the conversion of fat to glucose (31, 60). When this occurs during diabetic ketoacidosis (31) and the glucose carbon derived from acetone (or propanediol) is excreted in the urine, the naRQ is ≤ 0.7. During starvation, any glucose derived from fat is oxidized to carbon dioxide and water. Therefore, the naRQ is not in the range of 0.62–0.65 during starvation because of conversion of fat to glucose.

Another perspective for explaining naRQs ≤ 0.7 is possible. It is reasonable to assume that naRQs ≤ 0.7 occur during a long but undefined, time-limited process for a non–steady state condition of catabolism during starvation. The higher oxygen consumption in relation to the lower carbon dioxide production could occur if some of the fatty acids released from stored triacylglycerol underwent desaturation (30), eg, in the peroxisomes or microsomes (61), before being recycled to adipose tissue. Oxidative

desaturation enzymatic activities in liver (66, 67), intestine (68, 69), and lung (70) are modulated by the nutritional status of animals (71). During the winter in hibernating dormice, unsaturated fatty acid concentrations increase in plasma cholesteryl esters, triacylglycerol, and phospholipids. On arousal in the spring, the oleic acid content decreases in total fatty acids of liver and cardiac muscle (72). Thus, desaturation of fatty acids has been shown to occur during starvation (30). The desaturation process consumes oxygen and produces heat but releases no carbon dioxide. The RQ of desaturation of fatty acids is zero and the algebraic sum of total body catabolism could be responsible for yielding naRQs of 0.62–0.65 during a protracted period of a non–steady state condition. If this is true, the naRQ should rise before death once the unsaturated fatty acids undergo remobilization and oxidation. Although other possibilities do exist—lower-than-expected naRQs may be the net effect of ketonuria, uric acid excretion, glucose formation from glycine, and other trivial events that collectively affect the naRQ—we believe that fatty acid desaturation is the most reasonable explanation for naRQs in the range of 0.62–0.65 during prolonged starvation.

The differences in naO_2 and $naCO_2$ rates were used to calculate the energy equivalents derived from desaturation of fatty acids (30). The mean (± SD) energy equivalents derived from desaturation of fatty acids ranged from 29.04 ± 45.74 kJ/d on day 1 to a maximum of 439.57 ± 229.58 kJ/d on day 7, and thereafter remained relatively constant. The RMR corrected for fatty acid desaturation was significantly lower ($P = 0.02$) than the RMR based on oxygen consumption and significantly higher ($P = 0.02$) than the RMR based on carbon dioxide production between day 4 and day 21 (Table 4 and Figure 3B). However, the actual mean difference between the RMR based on oxygen consumption and that corrected for desaturation was < 1%. On the other hand, the difference between the RMR based on carbon dioxide production and the RMR corrected for fatty acid desaturation amounted to ≈6–8% of the total energy requirements. Thus, the amount of energy that can be derived from desaturation of recycled fatty acids was about equal to that provided by oxidation of aminogenic materials during the late phase of starvation.

Oxygen consumption is directly related to thermogenesis (27, 30, 60), thermogenesis in the fed state is proportionally related to carbon dioxide production, and both are dependent on the nature of the fuel oxidized, eg, glucose, fat, or protein. We suggest that during prolonged starvation heat production is more closely reflected by oxygen consumption, being generated not only from energy released from carbon-carbon bonds but also from hydrogen-carbon bonds and associated oxidative processes. The cleavage of hydrogen-carbon bonds dissociates carbon dioxide production from oxygen consumption. Carbon dioxide production reflects cleavage of carbon-carbon bonds and terminal oxidation of the carbon skeleton of metabolic fuels.

The decreases in respiratory production rates of carbon dioxide were accompanied by parallel decreases in serum T_3 concentrations and next day decreases in urinary urea and increases in NH_4^+ excretion rates. This suggests that T_3 influenced the body to conserve stored body fuels, specifically aminogens (proteins), during prolonged starvation (73, 74) and may initiate the reversion in humans from ureotelic to ammonotelic nitrogen excretion after ≈18 d of starvation.

Arterial concentrations and interrelations of catheterization and urinary excretion data

Serum T_4 concentrations decreased between day 4 and day 18. Serum T_3 concentrations decreased abruptly by 30% from day 0 to day 4 and thereafter descended gradually to below normal concentrations by the 18th day of starvation. Arterial serum rT_3 concentrations remained relatively constant during the fast. This is contrary to the generally accepted responses of rT_3 measured in venous blood during fasting (37).

Most of the catabolism of branched-chain amino acids is initiated in skeletal muscle (26), and skeletal muscle is one of the primary target organs for T_3 (73). The decreases in serum T_3 concentrations during starvation were directly or indirectly correlated with urinary excretion rates of urea, NH_4^+, and urea plus NH_4^+ nitrogen. Overall, these decreases agree with the postulate that a decrease in serum T_3 attenuates proteolysis and amino acid oxidation (73, 74). This adaptation to energy deprivation could have the advantage of conserving vital lean body tissues and enhancing survival in humans subjected to starvation.

T_3 is supposedly produced by deiodination of T_4 in the liver and kidney (73), but arteriovenous hepatic-renal concentration differences were too small to be detected in this study. There was a trend toward peripheral release of T_3 and significant and near equal rates of peripheral production and splanchnic removal of rT_3. The results of thyroid hormone exchanges showed that skeletal muscle was the primary organ that converted T_4 into T_3 and rT_3 and the liver was the primary site for removing rT_3 and thus iodothyronines from the blood during prolonged starvation.

Arterial plasma epinephrine concentrations remained constant between day 0 and day 18 but rose significantly after phenylacetate intake and probably maintained or promoted gluconeogenesis (75); increases in arterial plasma norepinephrine concentrations during the 21-d starvation period were not significant. The liver, kidney, and extremities probably all extracted arterial epinephrine. Norepinephrine was clearly released into renal venous blood and there was also definite hepatic and lower-extremity extraction of plasma norepinephrine.

Decreases in serum insulin and blood glucose concentrations were not correlated because of the dysfunctional state of the β cells in 3 of the 5 subjects (5, 76). The splanchnic bed released insulin and probably C-peptide, and the renal beds extracted insulin and C-peptide. The lower-extremity beds extracted arterial insulin and possibly C-peptide.

Fatty acid and ketone body blockade of glucose transport, phosphorylation, glycolysis, and oxidation (77, 78) in peripheral tissues is incomplete during prolonged starvation (79) and the glucose-lactate, glucose-alanine (75, 80), and glucose-glutamine (81) cycles persist. In addition, our results reconfirm the glucose-glycerol cycle (82) and suggest that there is a greater glutamate-glutamine cycle among liver, muscle, and other organs than generally appreciated (83).

The mean rate of glucose production by the liver was 225 ± 310 μmol/min and was indistinguishable from the value reported 3 decades ago for obese human subjects undergoing prolonged starvation (5). However, net kidney production was 254 ± 86 μmol/min and amounted to twice the historical production rate (5). Physiologic increases in the concentrations of epinephrine increased renal glucose release twofold (84); if renal gluconeogenesis was augmented during this study, it was probably secondary to the heightened secretion of epinephrine in response to trapping glutamine. Total hepatic and renal glucose production

was 479 ± 248 μmol/min. This value agrees closely with the [^{14}C]glucose turnover value reported by Bortz et al (82) for starving obese men.

Lactate and glycerol were the predominant precursors extracted by the liver. Recycled lactate (615 ± 228 μmol/min) plus pyruvate (77 ± 32 μmol/min) provided 692 ± 242 μmol/min or 346 ± 121 μmol glucose equivalents/min. Generally, none of this was for terminal oxidation, but the glucose derived from recycled lactate and pyruvate is essential for powering cells that lack mitochondria.

In recent years the importance of glycerol as the dominant precursor for glucose that can be terminally oxidized during starvation was cast aside. We reaffirm the significance of glycerol (82), which supplied 316 ± 196 μmol/min (Table 6) or 154 ± 95 μmol glucose equivalents/min. By combining the catheterization data with indirect calorimetry data, the proportion of glycerol converted to glucose that was then oxidized to carbon dioxide and water and recycled to the periphery, primarily to adipose tissue, for reconversion into α-glycerol 3-phosphate for reesterification of fatty acids can be estimated. Stored triacylglycerols that are mobilized and oxidized during starvation are ≈10% glycerol. During the near steady state of prolonged starvation (days 18–21) glycerol oxidation based on oxygen consumption was 151 ± 16 μmol/min (14.0 ± 1.5 mg/min), which equals 48% of the glycerol converted to glucose being oxidized. The remaining glucose derived from glycerol was recycled. The net contribution of glycerol to glucose for oxidation amounted to ≈20.1 ± 2.2 g/d. This quantity was approximately the same as that for all amino acids combined.

Glutamine and alanine together account for ≈80% of the amino acids released from muscle (83), and only they and glutamate are displayed in Table 6. It is known that alanine is the major nitrogenous compound released from muscle (26, 75) and extracted by the splanchnic bed (26, 75) and that glutamine is the primary amino acid extracted by the kidneys (26, 75). However, the magnitude of the splanchnic release and the lower-extremity uptake of glutamate was unexpected, and suggests a pivotal role for glutamate in the interorgan transport of nitrogen from muscle and other peripheral tissues. Branched-chain amino acids (leucine, isoleucine, and valine), along with aspartate and propionate, are catabolized in muscle and other organs, providing acetate, succinate, fumarate, and oxaloacetate (26). The carbon skeletons of these substrates are oxidized in the citric acid cycle or converted primarily to alanine and glutamine for secretion into the blood. Although the synthesis of glutamine in mammalian tissue was first described by Krebs (85) in 1935 and in 1995 Perriello et al (81) showed that ≈13% of plasma glutamine is derived from glucose, and the interplay of glutamine and glutamate is widely recognized, the obvious hepatic release and lower-extremity extraction of whole-blood glutamate in this study should refocus interest on this amino acid not only in muscle but also in brain metabolism (86, 87).

From the arteriovenous balance data, it is inferred that glutamate may play a key role as a precursor for glutamine. Part of the ammonia released from muscle, brain, lung, adipose tissue, and other tissues during amino acid catabolism is presumably transiently docked by glutamine synthetase (88) to glutamate and then released back into the blood as glutamine, which dumps its nitrogen and mixes with other carbon skeletons to be reformed and released as glutamate for recycling. This concept is at variance with the 4-h glutamate-glutamine tracer studies done in men who had fasted overnight, in which very little plasma glutamine was derived from plasma glutamate (83). However, the difference in glutamate concentrations between whole blood and plasma, the hormonal milieu, the large pool sizes of these amino acids, and the compartmentalization of glutamine and glutamate have limited the usefulness of currently available tracer data in which plasma served as the vehicle for investigating transport. The red blood cells of humans actively participate in amino acid transport (51) and a system exists that concentrates the intracellular content of glutamate (52). We were able to show large arteriovenous concentration differences by measuring glutamate in whole blood.

The uptake of gluconeogenic precursors could readily account for hepatic glucose production. However, the extraction of lactate, pyruvate, glycerol, α-ketoglutarate equivalents, and alanine failed to adequately account for the precursors needed for renal glucose release (Table 6). This suggests that unidentified precursors (eg, glycoproteins and acetone) furnished the remaining precursors for renal gluconeogenesis. On the other hand, renal glutamine extraction (88 ± 52 μmol/min) could account for renal ammoniagenesis.

We reported previously that acetone provided ≈11% of the plasma glucose in obese patients who fasted for 21 d (89). In the present group of starving subjects this would equal 53 ± 27 μmol glucose equivalents/min. Summing the mean exchange rates of alanine (207 ± 82 μmol/min) plus glutamine (80 ± 109 μmol/min) minus glutamate (−164 ± 97 μmol/min) across the hepatic and renal beds (Table 6) amounts to 122 ± 108 μmol/min or 61 ± 54 μmol glucose equivalents/min. The collective contribution of lactate, pyruvate, glycerol, amino acids, and acetone equals 591 ± 157 μmol glucose equivalents/min, which exceeds the measured rate of 479 ± 248 μmol/min for glucose production. Conjoint measurement errors or the generation of triacylglycerols for recycling fatty acids could account for some of this imbalance.

The precursor-product relations of alanine, glutamine, and glutamate to glucose and waste nitrogenous compounds should agree and they did. The net mean hepatic and renal extraction rates of these amino acids equaled ≈16 g glucose/d. This was in excellent agreement with the 24-h urinary nitrogen data. If we assume a 3.57:1 ratio for glucose production to urinary nitrogen excretion corrected for phenylacetylglutamine nitrogen (Tables 3 and 7), collectively, amino acids should contribute ≈20 g glucose/d. This implies that alanine plus α-ketoglutarate equivalents provided ≈80% of the glucose carbon skeleton derived from amino acids.

The peripheral release of lactate, pyruvate, and glycerol as well as of glutamine and alanine could not account for the hepatic and renal extraction of these substrates. Some amino acid substrates, specifically glutamine and alanine as well as other amino acids, were derived from sources other than skeletal muscle (26, 51, 75, 88). Net splanchnic release rates of β-OHB$^-$ (1090 ± 170 μmol/min) and AcAc$^-$ (1195 ± 219 μmol/min) were twice the rates reported previously with use of catheterization techniques (5, 43) but in reasonable agreement with data from kinetic analyses of radioactive tracer techniques (90, 91). The heightened blood concentration of epinephrine after phenylacetate administration may have augmented ketogenesis (92).

Net renal exchange of β-OHB$^-$ and AcAc$^-$ after 21 d of starvation could not account for ketonuria, which suggests that the kidneys, like the liver, are ketogenic as well as gluconeogenic

organs. The quantity of fatty acids extracted (Table 6) from plasma (40 ± 31 μmol/min) could account for the renal excretion of $AcAc^-$ (12 ± 4 μmol/min) plus β-OHB^- (39 ± 9 μmol/min) as well as the renal synthesis and consumption of ketone bodies.

In 1945 Medes et al (93) wrote that ketone body formation was not exclusively a liver function, but may occur in tissues that metabolize acetate. They subsequently reported that kidney tissue preparations readily synthesized $AcAc^-$ and β-OHB^- from [^{13}C]acetate. However, the concurrent rate of ketone body oxidation was high and they surmised that renal ketogenesis would not yield ketone bodies to the bloodstream (94). In 1969 Weidemann and Krebs (95) reported that kidney cortex slices from starved rats removed more [^{14}C]oleate than could be oxidized by the amount of oxygen consumed. Oleate increased ketogenesis six- to sevenfold, but the maximum rates of renal ketogenesis were ≈20% of the maximum rates observed in the liver on a weight-to-weight basis. This would equate with the renal ketogenic rate being ≈2% of the hepatic ketogenic rate, which is in perfect agreement with our results. In addition, Weidemann and Krebs reported that renal ketogenesis and gluconeogenesis were coupled. Brady et al (96) studied the distribution of ^{14}C in β-OHB^- and concluded that a minimum of 11–17% of the ketone bodies formed in diabetic ketotic rats were derived from extrahepatic tissue, presumed to be the kidneys. Subsequent work from Scofield et al (97) reported the ketone bodies presumed to be produced in the kidneys entered the bloodstream before being excreted in the urine.

Our results show that the kidneys of a starving human may extract or release $AcAc^-$, β-OHB^-, or both, as reflected by the large SDs in Table 6. However, the mean renal exchange rates for the 4 obese subjects who starved for 21 d and underwent catheterization studies were not significantly different from zero but were different from values we reported previously for obese individuals fasting for 35–41 d (5). Because there was no mean renal removal of $AcAc^-$ or β-OHB^- from the arterial blood in the presence of gross ketonuria, net renal ketogenesis was ≥51 μmol/min or ≈2% of net hepatic ketogenesis. We have no data pertaining to the renal parenchyma or blood-borne origin of urinary ketone bodies but show that net renal synthesis and release were near zero. If the kidneys are producing, utilizing, and excreting $AcAc^-$ and β-OHB^-, simultaneous net balance and isotopic techniques will have to be combined to quantify these processes in humans in states of augmented ketogenesis.

The urinary NH_4^+ excretion rate (82 ± 16 μmol/min) accounted for 58% of renal ammoniagenesis. Urinary β-OHB^- (39 ± 9 μmol/min) and $AcAc^-$ (12 ± 4 μmol/min) excretion provided the anions needed to partially neutralize urinary NH_4^+ (Table 3). Unmeasured sulfate and phosphate anions probably filled the remaining urinary anion gap. The kidneys released ammonia (-59 ± 46 μmol/min) into the renal veins (Table 7). The liver, brain, and extremities probably extracted ammonia from the arterial blood and synthesized urea and glutamine (26, 75, 88, 98). Circumferentially, part of the nitrogen in glutamine will be converted into urea. The 59 ± 46 μmol ammonia/min released into the renal venous blood will eventually be converted into 30 ± 24 μmol urea/min and subsequently excreted in the urine; this possible interplay between ammonia and urea could account for about one-half of the total urinary urea excretion (53 ± 40 μmol/min).

Phenylacetate trapping of glutamine was accompanied by a fall in arterial glutamine and a rise in epinephrine concentrations. Concurrently, metabolic acidosis, a standard consequence of the hypoglycemia and hypoinsulinemia of starvation, may have been accentuated. Metabolic acidosis stimulates the gluconeogenic and anaplerotic enzyme, pyruvate carboxylase (99), and increases proteolysis, muscle loss, and body nitrogen depletion (100–104). These acidotic and hormonal compensatory responses most likely aided the body in maintaining fuel homeostasis after phenylacetate administration. It is reasonable to assume that branched-chain and other amino acids enter the citric acid cycle via anaplerotic reactions to replenish intermediate substrates (and ammonia) lost to the carbon skeleton (and amino and amide nitrogen) of glutamine, which exits the citric acid cycle via cataplerotic reactions to furnish the kidneys with a gluconeogenic precursor and the byproduct ammonia.

The interaction of glucose, amino acid, and fatty acid oxidation results in the preferential utilization of fatty acids, $AcAc^-$, and β-OHB^- as the major energy sources for most tissues with mitochondria during starvation. Nonetheless, fatty acid and ketone body blockage of amino acid (105) and glucose oxidation (11, 77, 79) is incomplete. The final stage of oxidation of fats, amino acids, and carbohydrates occurs during 9 substrate-product reactions of the citric acid cycle. Fatty acids and ketone bodies enter this catabolic cycle primarily as acetyl-CoA. This substrate alone, however, is inadequate to maintain full cycle activity for normal cellular functions, especially when these functions require large quantities of energy from the citric acid cycle (106–109). Small amounts of the substrate-product intermediates are lost from the cycle by useful cataplerotic reactions and 4-carbon skeleton units need to be replenished from amino acids, glucose, and propionate by balanced anaplerotic reactions.

Minimum requirements for fat, protein, and carbohydrate oxidation

The lower limits of oxidative catabolism for fat, protein, and carbohydrate per unit mass during the near steady state of this study were defined at 18–21 d of starvation. However, lengthening the starvation period further reduces urinary nitrogenous compound excretion per kg body wt (OE Owen, KJ Smalley, RL Jungas, unpublished observations, 1998), but how this relates to body composition (fat-free mass) is unknown. Therefore, minimal rates for utilization of the major fuels may not be absolutely constant but instead may be dependent on starvation time and body composition and may be modified as starvation progresses.

We previously validated the accuracy of indirect calorimetry for measuring the nature and quantity of fuels oxidized by integrating the results of indirect calorimetry with [^{14}C]palmitate tracer analyses and catheterization techniques (110). The minimum requirements given in this section were based on urinary nitrogen excretion rates, respiratory gaseous exchange rates, and catheterization data derived from the 4 subjects who were catheterized. Therefore, some of the results were slightly different from those based on 5 subjects (eg, the results for urinary nitrogen excretion).

In this study, fat oxidation peaked after 1–2 d of total starvation and remained relatively constant thereafter. Based on oxygen consumption and assuming an energy value of 38.95 kJ/g fat (triacylglycerol), the minimum fat oxidation amounted to $\approx 1.53 \pm 0.21$ g · kg^{-1} · d^{-1} (2.98 ± 0.15 g · kg FFM^{-1} · d^{-1}). This minimum quantity for fat oxidation is a trifle above that calculated for fatty acid oxidation plus desaturation.

The minimum requirement for aminogenic compound oxidation was 0.27 ± 0.08 g $\cdot$ kg$^{-1} \cdot$ d^{-1} (0.52 ± 0.10 g $\cdot$ kg FFM$^{-1} \cdot$ d^{-1}) and that for nitrogen was 44 ± 13 mg $\cdot$ kg$^{-1} \cdot$ d^{-1} (83 ± 15 mg $\cdot$ kg FFM$^{-1} \cdot$ d^{-1}) based on the TUN excretion of 5.62 ± 1.28 g/24 h during the 18th day of total starvation (Table 3). Assuming an energy value of 17.7 kJ/g protein (26), this equaled ≈7% of the RMR (Table 4) and is probably unabated during more protracted periods of starvation. The further decrease in TUN known to occur during starvation (5, 54) is probably related to the loss of fat-free mass (66). Our measured rate of urinary nitrogen excretion was comparable with the obligatory loss of 37–60 mg $\cdot$ kg body wt$^{-1} \cdot$ d^{-1} reported by others for healthy adult subjects consuming diets sufficient to balance energy requirements but containing low-protein or protein-free nutrients (111).

The minimum amount of glucose that must be available for fuel homeostasis after 18–21 d of starvation equals ≈0.99 ± 0.55 g $\cdot$ kg$^{-1} \cdot$ d^{-1} (1.91 ± 1.04 g $\cdot$ kg FFM$^{-1} \cdot$ d^{-1}). In the 4 subjects who underwent catheterization studies, the measured quantity of glucose derived from amino acids [as reflected in urinary nitrogen excretion (20 ± 5 g/d) plus glycerol (20 ± 2 g/d) plus the estimated quantities of acetone (7 ± 4 g/d)] that must be terminally oxidized was ≈0.34 ± 0.14 g $\cdot$ kg$^{-1} \cdot$ d^{-1} (0.66 ± 0.29 g $\cdot$ kg FFM$^{-1} \cdot$ d^{-1}). This amount reflects the net utilization of newly formed glucose synthesized from precursors derived from aminogen and fat stores and needs to be qualified because the epinephrine response to phenylacetate could have induced augmented renal gluconeogenesis (84) and slightly inflated the essential quantity of glucose needed. However, a more reasonable analysis is that the epinephrine response maintained fuel homeostasis and, thus, that the overall influence of epinephrine was a corrective action rather than an augmentation of gluconeogenesis. This perspective is supported by the urinary nitrogen excretion rates reported in the present study after phenylacetylglutamine was corrected for, which agree perfectly with urinary nitrogen excretion rates not affected by epinephrine reported previously (5, 6, 54).

Anaplerosis and cataplerosis

After 18–21 d of starvation, glucose synthesized from lactate, pyruvate, amino acids, glycerol, and acetone is released by the liver and kidney to be catabolized in the brain and other tissues. Some of the glucose converted in the brain to pyruvate undergoes carboxylation to oxaloacetate (11) via the anaplerotic reaction catalyzed by pyruvate carboxylase (108). Subsequently, some of the α-ketoglutarate formed in brain mitochondria undergoes amino and amido nitrogen fixation to remove toxic ammonia from the central nervous system. Glutamine synthesized from α-ketoglutarate and ammonia via cataplerotic reactions executed by glutaminases (88, 98) is released into the blood and returned to the kidney where the precursor-product cycle is repeated, probably driven in part by the energy released by fatty acids during renal ketogenesis. This glutamine-glucose cycle has some similarities to the Cori and alanine-glucose cycles (75), except that the carbon skeleton of glutamine appears to originate mainly from branched-chain amino acids (and maybe glutamate) rather than from glucose (80, 112, 113).

In the glutamine-glucose cycle there is a balance between cataplerotic reactions that deplete the citric acid cycle of intermediate metabolites that serve to transport nitrogen and carbon from tissues and provide precursors for gluconeogenesis (62) and anaplerotic reactions that replenish intermediate metabolites (oxaloacetate from glucose, branched-chain amino acids, aspartate, and propionate) for maintenance of the citric acid cycle. The number of carbon atoms in metabolites and carbon dioxide exiting the citric acid cycle equals the number of carbon atoms in metabolites and carbon dioxide entering the cycle. This balance is displayed in **Figure 4**. Metabolites that are routed through anaplerotic and cataplerotic reactions are schematically highlighted by the interorgan transport of glutamine and glucose. The synthesis of data obtained from this and other catheterization studies creates and closes a newly recognized carbon-nitrogen cycle among muscle, liver, kidney, and brain, powered primarily by oxidation of fatty acids derived from adipose tissue. The gut is an important organ for amino acid metabolism in the fed state (26) but during starvation its role in glucose uptake and amino acid release is minor (41); thus, it was not included in our simplified view of the glutamine-glucose cycle. To avoid congestion, the alanine-glucose cycle was omitted (75). Adipose tissue was shown but not emphasized and the lung was not included because in nonstressful situations its behavior has not been defined (98).

The cataplerotic and anaplerotic reactions taking place in the human body during starvation can be reasonably estimated from the results of this study and from our results published previously. Partial removal of glutamine by phenylacetate conjugation and excretion from the body augmented TUN but did not diminish urea or NH_4^+ excretion. Therefore, the quantity of amino acid provided for gluconeogenesis remained constant and catheterization studies showed no curtailment in net hepatic or renal glucose production. Different results occurred after administering carbohydrates to individuals of comparable body weights undergoing similar periods of prolonged starvation, however (6). Those previous studies showed the tight relation between urinary nitrogen excretion and carbohydrate ingestion (6, 54). Oral intake of 7.5 g carbohydrate reduced daily urinary urea excretion by ≈1.5 g and NH_4^+ excretion by ≈0.6 g (6). This 2.1-g reduction in 24-h urinary nitrogen excretion after ingestion of 7.5 g carbohydrate gave a glucose-to-nitrogen ratio of 1:3.57, which was in good agreement with the classic studies of Stiles and Lusk as given in Kleiber (60). Increasing the daily carbohydrate ingestion to 15–25 g (6) or to 54–108 g (54) had no detectable effect on further reduction of nitrogen excretion, suggesting that some amino acid oxidation in addition to glucose was essential to yield energy for body functions.

The net effect of administering 7.5 g carbohydrate to starving obese subjects was an ≈40–50% reduction in urinary nitrogen excretion. Therefore, it is reasonable to assume that about one-half of urinary nitrogen excretion represents amino acids oxidized directly to carbon dioxide, water, urea, ammonia, urate, and creatinine in the appropriate metabolic pathways after anaplerotic entry; the other one-half is partially oxidized after cataplerotic exit and subsequently converted to glucose, which is then completely oxidized to carbon dioxide and water in the citric acid cycle. These data coupled with our previously published information (6) also provide an explanation for how after prolonged starvation small quantities of ingested carbohydrates diminish urinary excretion rates of $AcAc^-$, β-OHB^-, and NH_4^+ without changing blood ketone body concentrations.

Gluconeogenesis, ammoniagenesis, and ketogenesis

Proteolysis, aminogenolysis, or both provide amino acids and perhaps small quantities of carbohydrates for glucose synthesis. Ammonia and urea are byproducts. Hepatic ketogenesis con-

sumes bicarbonate and limits the need for liver ureagenesis to maintain whole-body acid-base balance. Nitrogen derived from catabolism is carried by glutamine to the kidneys, where it is excreted as NH_4^+ (26). Lipolysis provides the fatty acids needed for renal ketogenesis to yield the ATP needed for kidney functions and to permit the energy released from partial amino acid oxidation to be used to synthesize glucose and release ammonia from glutamine. Renal ketogenesis could also furnish hydronium cations (114) to trap ammonia as NH_4^+ in the renal intraluminal spaces and $AcAc^-$ and β-OHB^- to neutralize the trapped NH_4^+ and promote NH_4^+ excretion. Small quantities of carbohydrates ingested during starvation provide the minimal glucose requirements from amino acids and *1*) curtail renal gluconeogenesis, ketogenesis, and ammoniagenesis; *2*) reduce NH_4^+, $AcAc^-$, and β-OHB^- urinary excretion; and *3*) diminish hepatic ureagenesis. This speculation is highly likely.

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