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Research Article

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Regulation of Leucine Catabolism by Caloric Sources

Role of Glucose and Lipid in Nitrogen Sparing during Nitrogen Deprivation

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Abstract

Previously we showed that hypocaloric amounts of glucose reduce leucine catabolism while an isocaloric amount of fat does not (1985. *J. Clin. Invest.* 76:737.). This study was designed to investigate whether the same difference exists when the entire caloric need is provided either as glucose or lipid. Rats were maintained for 3 d on total parenteral nutrition (350 cal/kg per d), after which the infusion of amino acids was discontinued and rats received the same amount of calories entirely as glucose or lipid for three more days. A third group of rats was infused with saline for 3 d. In comparison to glucose, lipid infusion resulted in higher urinary nitrogen excretion (55±3 vs. 37±2 mg N/24 h, $P < 0.05$), muscle concentrations of tyrosine (95±8 vs. 42±8 μM, $P < 0.01$), and leucine (168±19 vs. 84±16 μM, $P < 0.01$), activity of BCKA dehydrogenase in muscle (2.2±0.2 vs. 1.4±0.04 nmol/mg protein per 30 min, $P < 0.05$), and whole body rate of leucine oxidation (3.3±0.5 vs. 1.4±0.2 μmol/100 g per h, $P < 0.05$). However, all these parameters were significantly lower in lipid-infused than starved rats. There was no significant difference between leucine incorporation into liver and muscle proteins of lipid and glucose-infused rats. On the other hand, starved rats showed a lower leucine incorporation into liver proteins. The data show that under conditions of adequate caloric intake lipid has an inhibitory effect on leucine catabolism but not as great as that of glucose. The mechanism of this difference may be related to a lesser inhibition of muscle protein degradation by lipid than glucose, thereby increasing the leucine pool, which in turn stimulates leucine oxidation.

Introduction

Studies in recent years have suggested a number of key roles for branched-chain amino acids (BCAA)[1] and their keto acids (BCKA) in health and disease (1–3). There is evidence that BCAA metabolism is highly sensitive to nutritional alterations (4–6); for example, several studies have shown that dietary

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1. *Abbreviations used in this paper:* BCAA, branched chain amino acids; BCKA, branched chain keto acids.

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protein content has a profound effect on BCAA catabolism (7–11). In contrast, the effect of dietary carbohydrate and fat on BCAA metabolism has not been adequately investigated. In a previous study (11) using patients who needed caloric restriction for treatment of obesity, we investigated the effect of one week of starvation and hypocaloric diets (300 or 500 cal/d) composed entirely of either carbohydrate or fat on whole body rate of BCAA catabolism. We found that brief starvation significantly increased the rate of leucine catabolism. The same occurred with the fat diet. In sharp contrast, the carbohydrate diet significantly reduced the rate of leucine catabolism. Our observation that brief starvation increases leucine oxidation in man has recently been confirmed (12).

These results prompt the following questions: (*a*) would the differential effects of carbohydrate and fat persist if their intakes were sufficiently high to meet the total caloric requirement, (*b*) where in the body does the caloric source influence the catabolism of BCAA, and (*c*) in view of the intimate relationship between metabolism of BCAA and body proteins, could the effects of carbohydrate and fat on BCAA catabolism be due to their effects on protein metabolism? Since ethical and practical considerations preclude the use of human subjects in these investigations, we have used rats for our present studies. There is evidence that alteration in BCAA metabolism in response to nutritional and metabolic perturbations in the rat is similar to man; for example, starvation and diabetes increase BCAA oxidation in both species (11–16). Furthermore, it is well established that both rats and man have a substantial loss of nitrogen during brief starvation (17).

Oral feeding of a diet composed entirely of fat or carbohydrate may affect intake or absorption of calories. To avoid these potential problems, we administered lipid and glucose by the technique of parenteral nutrition.

Methods

Animal preparation. Male Sprague-Dawley rats weighing ~ 150 g were obtained from Zivic-Miller Laboratories (Allison Park, PA). To allow acclimatization to the laboratory conditions, they were maintained in individual cages for one week before they were used in the study. During this period they consumed water and laboratory rat chow (Ralston Purina Co., St. Louis, MO) ad lib. At the end of one week, rats were anesthetized with an injection of 0.25 mg ketamine i.p. (Parke-Davis Co., Morris Plains, NJ) and a silastic catheter (0.02 in. ID; Dow Corning Corp., Midland, MI) was introduced into the superior vena cava through an incision made in the external jugular vein. The catheter was tunneled and exteriorized subcutaneously in the midscapular region through a trocar puncture wound. For protection, the catheter was passed through a flexible spring and the proximal end attached to a flow-through swivel apparatus (Instech Laboratories, Horsham, PA) mounted at the top of the cage. The protective spring was sutured to the rat skin using a stainless steel anchor button and stainless steel sutures. At the end of the surgical procedure, rats received 10 mg each of sodium ampicillin (Wyeth Laboratories, Philadelphia, PA) and

gentamicin sulfate (Schering Pharmaceutical Corp., Manati, PR) by an intraperitoneal injection. Rats were then transferred to individual metabolic cages that allowed collection of urine and feces. On the day of the surgery, rats were allowed water and rat chow ad lib. and in addition received half of their daily nutrient requirements by parenteral nutrition (see below). From the day after surgery to the end of the study, rats received all their nutrients parenterally.

Experimental design. During a 4-d recovery period rats received total parenteral nutrition that provided 350 cal/kg per d and 1.35 g N/kg per d. The composition of calories was 85% dextrose, 5% lipids, and 10% amino acids. The amounts of calories and nitrogen fall within the recommended intake for rats (18) and the caloric composition simulated the usual rat diet. The composition of the amino acid solution (grams per liter) was as follows: L-arginine 4.14, L-asparagine 2.38, L-histidine 2.03, L-isoleucine 3.75, L-leucine 5.10, L-lysine 6.07, L-methionine 4.05, L-phenylalanine 1.80, L-proline 2.70, L-threonine 3.45, L-tryptophan 1.05, L-valine 4.13, glycine 4.13, L-glutamic acid 26.83, and glycyl-L-tyrosine 5.31. In addition, appropriate amounts of minerals, trace elements, and electrolytes were added to the solution. The parenteral solution was infused with a Harvard infusion pump at a rate of 2.3 ml/h. Multivitamins were given daily as a 0.5-ml intravenous bolus. Solutions were prepared fresh each day.

After 4 d of total parenteral nutrition rats were divided into three groups. In one group all sources of calories were discontinued and rats were infused intravenously with a solution containing only minerals and electrolytes (starved group). In the other two groups parenteral infusion of amino acids was discontinued but each group received parenterally the same amount of calories as in the recovery period (350 cal/kg per d). Calories were provided entirely as glucose in one group and as lipid in the other. All three groups received daily vitamins as in the control period. The duration of each nutritional treatment was 3 d. The reason for the 3-d duration was based on the results of a preliminary study that showed that the starved rats had increased mortality after this period.

At the end of the third day, the parenteral infusion was discontinued and rats were anesthetized with ether. Within 2 to 3 min after stopping the infusion, rats were weighed, and blood was drawn by cardiac puncture. The liver, kidneys, and gastrocnemius muscles were quickly removed. These three tissues were selected for the study because they are the main sites for BCAA metabolism (5). Gastrocnemius muscle is composed of both white and red fiber types, and therefore, was chosen as a representative of skeletal muscle. A portion of each tissue was saved for measurement of leucine transaminase and BCKA dehydrogenase activities; the remainder was immediately frozen in liquid nitrogen. In addition to those rats killed for plasma and tissue analysis, a separate group of rats in each nutritional treatment was used for in vivo studies of leucine oxidation and incorporation into tissue protein.

Leucine metabolism in vivo. At the end of each nutritional treatment, while still receiving the parenteral solutions described above, rats were transferred to a plexiglass metabolic cage (Plas-Labs, Lansing, MI) to enable the collection of expired air. Rats were allowed $\sim$ 30 min to acclimatize to the new cage before receiving a priming dose of 3 μCi of 1-[^{14}C]leucine given intravenously over a 3-min period. Immediately after the priming dose, the parenteral solution was changed to an identical one, but containing 1-[^{14}C]leucine (2 μCi/ml). The amount of 1-[^{14}C]leucine infused was $\sim$ 0.08 μCi/min. After 180 min of infusion, expired air was collected at 20-min intervals for determination of ^{14}CO$_2$ production rate as described previously (19). After 240 min of infusion, rats were anesthetized with ether and blood was drawn in a heparinized syringe from the inferior vena cava for the determination of leucine specific activity. In addition, samples of liver and gastrocnemius muscle were obtained and immediately frozen in liquid nitrogen for determination of leucine incorporation into protein. We have previously shown that by 180 and 240 min of 1-[^{14}C]leucine infusion the specific activity of leucine in plasma and tissues reaches a near constant value, respectively (19).

Leucine incorporation into proteins. The incorporation of leucine

into muscle and liver proteins was measured by a previously described method (20). In brief, $\sim$ 0.5 g of frozen tissue was homogenized in cold 6% sulfosalicylic acid (1:10, wt/vol) using a ground glass homogenizer. The homogenates were centrifuged at 1,000 g for 10 min in a refrigerated (4°C) centrifuge. The supernatant fraction was decanted and stored at −20°C for later analysis of leucine specific activity. The pellet was washed three times with 5 ml of 5% TCA that contained 10 mM leucine. The supernatant fraction of these washes was discarded. Random analysis of these washes showed that they contained negligible (< 1%) amounts of the initial radioactivity. The washed pellet was solubilized in 2 ml of 2 N NaOH by heating at 80°C for 30 min. From the solubilized protein a 0.2-ml aliquot was taken to measure protein concentration and 0.2-ml aliquots were added to 2 ml of cold 10% TCA to re-precipitate protein. The protein precipitate was collected over a celite mat that had been previously formed over a filter paper (2.5 cm diam). The protein over the celite mat was washed in succession with 10 ml each of 5% TCA, absolute ethanol, and ether. The celite mat containing the protein was transferred to a scintillation vial and 1 ml of Soluene-350 (New England Nuclear, Boston, MA) was added. The vials were heated at 60°C for 30 min to solubilize protein. 10 ml of Liquifluor (New England Nuclear)-toluene scintillation mixture was added to each vial and the radioactivity measured by liquid scintillation spectrometry.

Leucine specific activity. The concentration of radioactive leucine was measured by the method outlined by Hutson et al. (21). Briefly, 0.5 ml of 6% sulfosalicylic acid extract (1:1, vol/vol) or 4 ml of 6% sulfosalicylic acid extracts of tissues (1:10, wt/vol) were applied to a 4 × 0.9-cm column containing Dowex 50 W-X8 cation exchange resin (Dow Chemical Co., Indianapolis, IN). The column was washed with 10 ml of 0.01 N HCl followed by 10 ml of distilled water to remove ketoacids. Subsequently, 1-[^{14}C]leucine was eluted from the column with 6 ml of 4 N NH$_4$OH and collected directly into 20 ml scintillation counting vials. Samples were evaporated to dryness by gently blowing air into the vial and redissolved in 1 ml distilled water. 10 ml of Aquasol-2 (New England Nuclear) was added to each vial and the radioactivity measured by liquid scintillation spectroscopy. The recovery of 1-[^{14}C]-leucine added to plasma or tissue and carried through the above procedure was 95%. Leucine concentration in plasma and tissue was measured by ion exchange chromatography using an amino acid analyzer.

Tissue preparation. For measurement of enzyme activities, liver and kidney were homogenized (1:9, wt/vol) in cold 0.25 M sucrose/10 mM Tris-HCl, pH 7.4, and gastrocnemius muscle in Chappell-Perry medium (22) as previously reported (20).

Leucine transaminase activity. Leucine transaminase activity was determined according to a previously published method (23). Briefly, tissue homogenates were incubated with L-1-[^{14}C]leucine (1.0 mM, 1,000 dpm/nmol) to produce ^{14}CO$_2$ and α-keto-1-[^{14}C]isocaproate. After this reaction was complete, α-keto-1-[^{14}C]isocaproate was oxidized with ceric sulfate and ^{14}CO$_2$ collected. The sum of enzymatically and chemically produced ^{14}CO$_2$ represents the rate of transamination.

BCKA dehydrogenase activity. BCKA dehydrogenase activity was determined by measuring the release of ^{14}CO$_2$ from α-keto-1-[^{14}C]-isocaproate (0.20 mM, 500 dpm/nmol) incubated with tissue homogenates as described previously (20). Incubation studies were carried out in 5 ml of previously described buffer (13). Reactions were initiated by the addition of 0.50 ml tissue homogenate. This amount of tissue homogenate provided 0.38 nmol of α-ketoisocaproate and 4.5 nmol of leucine. These amounts of endogenous substrates are < 0.5% of the exogenously added α-ketoisocaproate (1,000 nmol). To determine the maximal activity of BCKA dehydrogenase, the enzyme was fully activated by incubating tissue homogenates with 5 mM Mg^{2+} for 15 min at 37°C before measurement of activity. We and others (10, 24, 25) have previously shown that this method of activating the enzyme is effective.

Analytical methods. Nitrogen content of urine and feces was determined by a micro Kjeldahl method. Protein concentration in tissues was analyzed by the method of Lowry et al. (26). Plasma and urinary

β-hydroxybutyrate was determined by an enzymatic method (27). Plasma insulin concentration was measured by radioimmunoassay. Urinary acylcarnitine excretion was measured by an enzymatic radioisotopic method (28). Plasma and urinary glucose was determined by the glucose oxidase method (29). Plasma triglycerides were determined enzymatically (30). Plasma and tissue concentrations of BCAA were determined by an ion-exchange chromatography technique utilizing an automated amino acid analyzer. Concentration of 3-methylhistidine was measured by high performance liquid chromatography (31).

Materials. Dextrose (50%) was obtained from Abbott Laboratories (Chicago, IL), lipid emulsion (Intralipid, 20%) from Kabivitrum (Alameda, CA), and amino acid solution (7.5%) from Pfrimmer Co. (Erlangen, West Germany). Multivitamins (multivitamin concentrate-9 + 3) were obtained from Lypho-Med (Chicago, IL). 1-[^{14}C]acetyl-CoA (50 mCi/mmol) and L-1-[^{14}C]leucine (50 mCi/mmol) were purchased from Amersham Corp. (Arlington Heights, IL). α-Keto-1-[^{14}C]-isocaproate was prepared in this laboratory from L-1-[^{14}C]leucine by incubating with L-amino acid oxidase as described by Rudiger et al. (32). Radiochemical purity of 1-[^{14}C]KIC was verified by paper chromatography using a solvent system of 1-butanol-0.1 M borate buffer, pH 8.6, acetone (10:5:4, vol/vol) (7). A single radioactive spot was observed. The sodium salt of α-ketoisocaproate, NAD$^+$, thiamine pyrophosphate, and CoA were purchased from Sigma Chemical Co. (St. Louis, MO). Catalase and L-amino acid oxidase were obtained from Boehringer-Mannheim (Indianapolis, IN). Acetyl-CoA and carnitine acetyltransferase were obtained from Sigma. Anion-exchange resin (AG2-X8, 200–400 mesh, Cl$^-$ form) was obtained from Bio-Rad Laboratories (Richmond, CA). All other chemicals were of reagent grade.

Calculations and statistics. Nitrogen balance (in milligrams per 24 h) was calculated as the difference between nitrogen intake and nitrogen output (the sum of nitrogen excreted in urine and feces). Intracellular amino acid concentration was calculated using the following formula: $A_i = (A_t - A_p \times W_e)/(W_t - W_e)$, where A_i is intracellular amino acid concentration (μmol/ml water), A_t, amino acid concentration in the tissue (μmol/g), A_p, amino acid concentration in plasma water (μmol/ml), W_e, tissue extracellular water (ml/g) and W_t, total tissue water (ml/g). Tissue extracellular water was measured as described previously (33). Total tissue water was calculated as the difference between weights of tissues before and after drying in an oven overnight at 100°C.

Intracellular 1-[^{14}C]leucine radioactivity was calculated by the above formula, except for the substitution of amino acid concentrations by 1-[^{14}C]leucine radioactivities. The incorporation of leucine into protein (μmol/mg) was calculated by dividing the measured radioactivity (dpm/mg protein) by the intracellular leucine specific activity (dpm/μmol).

Fractional excretion of ^{14}CO$_2$ from 1-[^{14}C]leucine and rates of leucine oxidation by the whole animal were calculated by the following formulas: E = PR × 100/I × FR; OX = PR/SA$_{pl}$ × FR, where E is fractional excretion rate of ^{14}CO$_2$ from 1-[^{14}C]leucine (%/100 g); PR, ^{14}CO$_2$ production rate (dpm/h); I, infusion rate of 1-[^{14}C]leucine (dpm/100 g per h); FR, fraction of infused NaH^{14}CO$_3$ recovered as ^{14}CO$_2$ under steady state conditions; OX, oxidation rate of leucine (μmol/100 g per h); and SA$_{pl}$, specific activity of leucine in plasma (dpm/μmol).

Data are presented as mean±SEM. Multiple comparisons among the three groups were made by one-way analysis of variance followed by the Bonferroni *t* test (34). The level of significance was set at $P < 0.05$.

Results

Calorie utilization. Rats that were starved lost 22±3 g of their body weight after 3 d of starvation ($P < 0.01$). In contrast, there was no significant change in body weight of lipid ($-4±2$ g) and glucose-infused ($-2±3$ g) rats.

To assess the efficiency of calorie utilization we determined plasma concentration and urinary excretion of glucose and β-hydroxybutyrate and plasma triglyceride concentration. These data are summarized in Table I. Plasma concentration and urinary excretion of glucose were similar in starved and lipid-infused rats, but were significantly higher in glucose-infused rats. Plasma concentration and urinary excretion of β-hydroxybutyrate were also similar in starved and lipid-infused rats, but were significantly lower in glucose-infused rats. On the other hand, plasma triglyceride concentration was similar in starved and glucose-infused rats, but was significantly higher in lipid-infused rats.

Carnitine is involved in the oxidation of fatty acids and branched-chain amino acids (35–37). The oxidation of these substrates results in formation of acylcarnitine, which may be excreted in the urine. We therefore measured the urinary excretion of acylcarnitine (Table I). The excretion of acylcarnitine was highest in lipid-infused rats, intermediate in starved rats, and lowest in glucose-infused rats.

The above data provided evidence for efficient utilization of glucose and lipid under our experimental conditions. For example, urinary loss of glucose was quite small compared to the amount of glucose infused ($\sim$ 1%) and the lipid-infused rats had the highest rate of acylcarnitine excretion.

In view of the importance of insulin in regulation of substrate metabolism, we measured plasma insulin concentration. Plasma insulin concentration was significantly higher in glucose-infused than either starved or lipid-infused rats (Table I). There was no significant difference between insulin concentrations in plasma of starved and lipid-infused rats.

Protein metabolism. Initially, we investigated the effect of caloric source on urinary nitrogen excretion (Table I). The nitrogen excretion was highest in starved rats, intermediate in lipid-infused rats, and lowest in glucose-infused rats. Nitrogen

Table I. Fuel-hormone Metabolism

Parameter	Calorie source		
	None	Lipid	Glucose
Plasma concentration			
Glucose (*mg/dl*)	130±6* (6)	153±10* (6)	278±46‡ (5)
Triglyceride (*mg/dl*)	37±7* (6)	292±24‡ (6)	40±3* (5)
β-hydroxybutyrate (*mM*)	1.3±0.1* (6)	1.9±0.5* (6)	<0.02‡ (5)
Insulin (*μU/ml*)	19±2* (6)	19±7* (6)	66±11‡ (5)
Urinary excretion			
Nitrogen (*mg/24 h*)	74.8±3.6* (6)	54.6±3.3‡ (6)	37.2±2.1§ (6)
Glucose (*mg/24 h*)	1.6±0.8* (6)	1.5±0.6* (6)	19.0±3.0‡ (5)
β-hydroxybutyrate (*μmol/24 h*)	4.4±1.0* (6)	5.5±0.8* (6)	1.0±0.04‡ (5)
Acylcarnitine (*nmol/24 h*)	262±14* (6)	443±30‡ (6)	175±17§ (5)

All rats were maintained for 4 d on total parenteral nutrition receiving 350 cal/kg per d. The caloric composition of the infused solution was 85% glucose, 10% amino acids, and 5% lipid. After the 4th d, rats were divided into three groups. One group no longer received any calories but continued to receive the same amount of fluid containing only electrolytes and vitamins (starved group). The other two groups continued to receive the same amount of calories (350 cal/kg per d) but all the calories were restricted either to lipid or glucose. Plasma measurements were made after 3 d of each nutritional treatment. Urinary measurements were made during the last day of each treatment. The data are presented as mean±SEM. Means within a row not sharing a common superscript symbol are significantly different ($P < 0.05$). The number of rats in each group is shown in parentheses.

balance during the control and experimental periods is shown in Fig. 1. Rats in all three groups were in a positive and similar nitrogen balance during the control period when parenteral nutrition included adequate amounts of amino acids. Starvation or infusion of lipid or glucose alone without amino acids resulted in a decline in nitrogen balance (Fig. 1). The nitrogen balance became negative for all three groups, but significantly ($P < 0.05$) more negative for the starved rats than either lipid; or glucose-infused rats. The nitrogen balance was significantly ($P < 0.05$) less negative in glucose than in lipid-infused rats. In addition, the data summarized in Fig. 1 also show that for several consecutive days before and during each nutritional treatment rats were in a state of nitrogen equilibrium.

It is pertinent to note that because of increased mortality in the starved rats the duration of nutritional treatment was limited to 3 d. Not including the starved rats, a preliminary experiment on the effect of a longer period of treatment on urine nitrogen excretion showed that even after 7 d of treatment nitrogen excretion was still greater in lipid-infused than in glucose-infused rats (data not shown).

To investigate whether differences in protein degradation accounted for differences in nitrogen balance, we measured urinary excretion of 3-methylhistidine (38) and muscle and plasma concentrations of tyrosine (39). The urinary excretion of 3-methylhistidine (μmol/24 h) of starved rats (2.64 ± 0.11, $n = 10$) was significantly ($P < 0.01$) greater than that of either lipid-(2.16 ± 0.09, $n = 9$) or glucose (1.93 ± 0.11, $n = 8$)-infused rats. However, there was no significant difference in 3-methylhistidine excretion between lipid- and glucose-infused rats.

In contrast, all three groups displayed significant differences in their muscle and plasma concentrations of tyrosine (Fig. 2). Muscle and plasma concentrations of tyrosine were highest in starved rats and lowest in glucose-infused rats. Muscle and plasma concentrations of tyrosine in lipid-infused rats were significantly ($P < 0.05$) higher than that of glucose-infused rats, and significantly ($P < 0.05$) lower than that of starved rats. To investigate whether the differences in plasma concentrations were related to the differences in muscle concentrations we investigated tyrosine concentrations in the liver of the three groups of rats (Fig. 2). In contrast to muscle and plasma, there was no significant difference between tyrosine concentration in the liver of starved and lipid-infused rats.
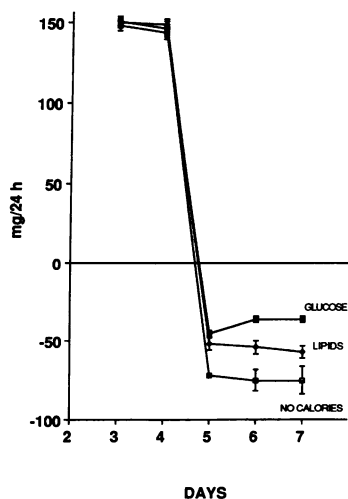

Figure 2. Tyrosine concentrations in tissues and plasma. The values (mean±SEM, six to seven rats) represent intracellular concentration in tissues or in plasma water after 3 d of treatment. For details of treatments see legend to Table I. In muscle and plasma, all treatments produced concentrations of tyrosine that were significantly different ($P < 0.05$ or lower) from each other. In liver, concentrations of tyrosine in glucose-infused rats were significantly different ($P < 0.05$) from lipid-infused rats but not from starved rats.

Protein degradation may be increased to meet the demand for gluconeogenic precursors. Alanine is a key precursor for gluconeogenesis (40), and its hepatic concentration usually reflects alterations in gluconeogenesis (41, 42). Therefore, we determined the intracellular concentration of alanine in liver of the three groups of rats (Fig. 3). Alanine concentration was greater by twofold in liver of glucose-infused rats than either starved or lipid-infused rats. There was no significant difference between alanine concentrations in liver of starved and lipid-infused rats. To determine whether the difference in hepatic alanine concentration reflected a difference in gluconeogenesis, we also measured alanine concentration in gastrocnemius muscle, a nongluconeogenic tissue. In contrast to a pronounced difference in liver, there was no significant difference in alanine concentrations in muscle of the three groups of rats (Fig. 3). Similarly, plasma alanine concentrations were also not significantly different (Fig. 3).

BCAA metabolism. Previous studies in man and rats have shown that plasma and tissue concentrations of BCAA are quite sensitive to changes in dietary intake (4, 6). Therefore, initially we determined plasma and intracellular concentrations of BCAA in liver and skeletal muscle (gastrocnemius). These data are summarized in Fig. 4. Muscle and plasma concentrations of leucine, isoleucine and valine were highest in starved, and lowest in glucose-infused rats. The lipid-infused rats displayed BCAA concentrations that were significantly (P

Figure 1. Nitrogen balance. (mean±SEM in six to seven rats). The values at day 6 and 7 were significantly different from each other ($P < 0.05$). For details on nutritional treatments see legend to Table I.
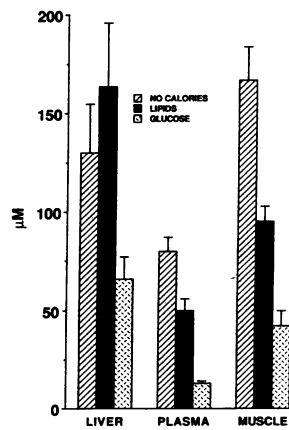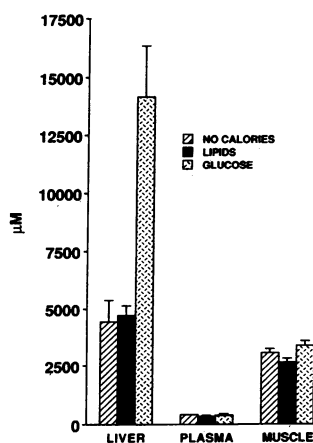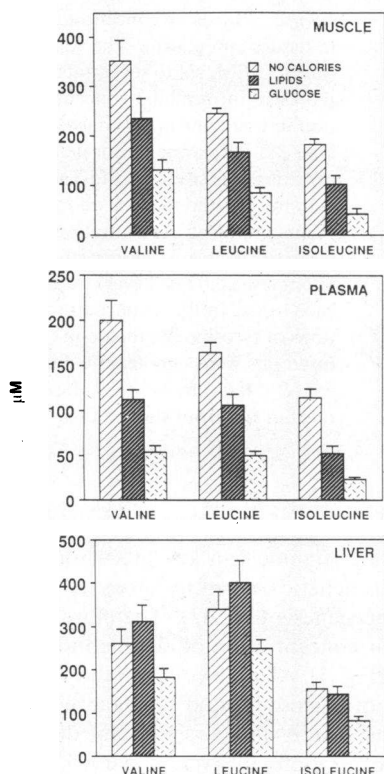

Figure 3. Alanine concentrations in tissues and plasma. The values (mean±SEM in 9–11 rats) represent intracellular concentration in tissues or in plasma water after 3 d of treatment. For details on treatments see legend to Table I. The only significant difference ($P < 0.01$) was between liver concentration of glucose-infused and either lipid-infused or starved rats.

Figure 4. BCAA concentrations in tissues and plasma. The values (mean±SEM in 9–11 rats) represent intracellular concentration in tissues or in plasma water after 3 d of treatment. In muscle and plasma, all treatments produced concentrations of BCAA that were significantly different ($P < 0.05$ or lower) from each other. In liver, concentrations of BCAA in glucose-infused rats were significantly different ($P < 0.01$) from lipid-infused and starved rats. There was no significant difference between liver BCAA concentration of lipid-infused and starved rats.

cose-infused than either starved or lipid-infused rats. There was no significant difference between the leucine transaminase activity in kidney of the three groups. There was also no significant difference between the transaminase activity in liver of starved and lipid-infused rats. However, leucine transaminase activity was significantly lower in liver of glucose than lipid-infused rats.

The next step in leucine catabolism is α-decarboxylation of α-ketoisocaproate, which is catalyzed by BCKA dehydrogenase. We therefore, measured BCKA dehydrogenase activity in tissues studied above. Among the tissues studied, muscle displayed major differences among the three groups. BCKA dehydrogenase activity was lowest in muscle of glucose-infused rats and highest in muscle of starved rats. The activity in muscle of lipid-infused rats was significantly ($P < 0.05$) higher than that of glucose-infused rats but significantly ($P < 0.05$) lower than that of starved rats. There was no significant difference between activities of BCKA dehydrogenase in kidney of the three groups of rats nor between the activities in liver of starved and lipid-infused rats. However, BCKA dehydrogenase activity was significantly ($P < 0.05$) lower in liver of glucose than lipid-infused rats.

To investigate the physiological significance of the above observations, we determined the whole body rate of leucine oxidation by the three groups of rats. The steady state specific activities of leucine in plasma of starved, lipid and glucose-infused rats were 227±21, 234±16, and 363±62 dpm/nmol, respectively. Fractional excretion of $^{14}CO_2$ (%/100 g) during the infusion of 1-[^{14}C]leucine was highest in starved rats (9.5±0.5, $n = 8$) and lowest in glucose-infused rats (3.8±0.2, $n = 9$) and intermediate in lipid-infused rats (7.1±1.6, $n = 5$). The differences between fractional excretion of $^{14}CO_2$ were each statistically significant ($P < 0.05$) from the others. These values were used to calculate rates of leucine oxidation, which are shown in Fig. 5. The rate of leucine oxidation was highest in starved rats and lowest in glucose-infused rats. The rate of leucine oxidation by lipid-infused rats was significantly ($P < 0.05$) higher than that of glucose-infused rats, but significantly lower ($P < 0.05$) than that of starved rats.

Finally, we investigated the other pathway of leucine metabolism, namely leucine incorporation into tissue proteins. These data are shown in Table III. There was no significant difference between leucine incorporation into muscle proteins of the three groups of rats or into liver proteins of lipid and glucose-infused rats. However, leucine incorporation into liver

< 0.05) higher than those of glucose-infused rats, but significantly ($P < 0.05$) lower than those of starved rats. Liver, on the other hand, showed no significant difference in BCAA concentrations between lipid-infused and starved rats. However, glucose infusion resulted in significantly ($P < 0.05$) lower liver BCAA concentrations than lipid infusion.

Next, we investigated the effect of caloric source on enzyme activities concerned with leucine catabolism. The first step in leucine catabolism is transamination, and liver, kidney, and muscle, are considered the major sites for leucine transamination (5). Therefore, we measured leucine transaminase activity in these tissues (Table II). The most pronounced effect of nutritional treatments was on leucine transaminase activity in muscle. The activity was significantly lower in muscle of glu-

Table II. Activities of Leucine Transaminase and BCKA Dehydrogenase (nmol/mg protein/30 min)

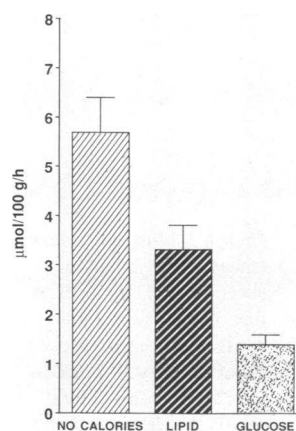|		Calorie source		
	Tissue	None	Lipid	Glucose
Leucine transaminase	Liver	3.4±0.2*‡ (6)	3.7±0.1* (6)	3.0±0.1‡ (5)
	Kidney	59±5* (6)	56±3* (6)	63±3* (5)
	Muscle	44±4* (6)	41±2* (6)	34±1‡ (5)
BCKA dehydrogenase	Liver	37±4* (6)	40±1* (6)	17±2‡ (5)
	Kidney	49±3* (6)	48±3* (6)	49±2* (5)
	Muscle	4.0±0.4* (7)	2.2±0.2‡ (7)	1.4±0.04§ (7)

For nutritional treatment, see legend to Table I. Activities of leucine transaminase and BCKA dehydrogenase were determined after 3 d of each nutritional treatment. The data are presented as mean±SEM. Means within a row not sharing a common superscript are significantly different ($P < 0.05$). The number of rats in each group is shown in parentheses.

Figure 5. Whole body rate of leucine oxidation (mean±SEM in six to seven rats). These rates were measured during a primed continuous infusion of 1-[^{14}C]leucine. All measurements were made after 3 d of treatment. For details on treatments see legend to Table I. Each rate was significantly different from each other ($P < 0.05$).

*Table III. Leucine Incorporation into Tissue Proteins
(pmol/mg protein)*

Tissue	Calorie source		
	None	Lipid	Glucose
Liver	271±36* (6)	400±55‡ (5)	416±31‡ (6)
Muscle	27±3* (6)	26±4* (5)	29±3* (6)

For nutritional treatment, see legend to Table I. Incorporation into protein was measured after 3 d of each nutritional treatment. The data are presented as mean±SEM. Means within a row not sharing a common superscript are significantly different ($P < 0.05$). The number of rats in each group are shown in parentheses.

protein of starved rats was significantly ($P < 0.05$) lower than either lipid or glucose-infused rats.

Discussion

The results of the present study show that both lipid and glucose individually, when given in amounts sufficient to meet the daily caloric need, inhibit catabolism of BCAA in the body. Among the tissues examined, muscle appears to be the site of this inhibition. For example, lipid infusion had no significant effect on the activity of BCKA dehydrogenase in liver and kidney, but significantly decreased it in muscle (Table II). The results further show that lipid is not as effective as glucose in inhibition of whole body rate of leucine oxidation (Fig. 5). The mechanism of this difference in metabolic effect appears to include a greater inhibition of muscle proteolysis by glucose than by lipid.

Evidence supporting a difference in muscle proteolysis is suggested by a significant difference in concentration of tyrosine in muscle and plasma. Previous studies have shown that during starvation and protein deprivation changes in plasma tyrosine concentration reflect changes in muscle tyrosine concentration, and moreover, there is no muscle uptake but release of tyrosine during these nutritional alterations (40–43). Tyrosine is neither synthesized nor catabolized by muscle (44), and its intracellular concentration during starvation and protein deprivation is governed by rates of muscle protein synthesis and degradation. Since amino acid incorporation into muscle protein was similar in both groups of rats (Table III), the twofold greater intracellular tyrosine concentration in muscle of lipid than glucose-infused rats suggests a greater muscle proteolysis. This suggestion receives further support by the observation on intracellular concentration of BCAA in muscle. For example, the concentration of leucine was twofold greater in muscle of lipid than glucose-infused rats (Fig. 4). This increase occurred despite lack of any significant difference between leucine incorporation into muscle protein (Table III) and a greater leucine oxidation in muscle of lipid than glucose-infused rats (Table II). The suggestion of a difference in muscle proteolysis was not supported by the 3-methylhisti-dine excretion data since both glucose and lipid-infused rats excreted the same amount of 3-methylhistidine. However, urinary 3-methylhistidine represents breakdown of myofibrillar protein, largely that of skeletal muscle (38). There is evidence that the rate of turnover as well as susceptibility to change by nutritional alterations are greater for nonmyofibrillar (e.g.,

myoglobin, enzymes) than myofibrillar proteins in the muscle (45–47).

Whether glucose or lipid influences muscle proteolysis directly or indirectly, such as by stimulation of insulin release, cannot be ascertained from the results of the present experiment. The higher plasma insulin level in glucose than in lipid-infused rats (Table I) suggests that insulin may have played a role since insulin is known to inhibit muscle proteolysis (48). However, in the face of diminished proteolysis, there was no significant difference between plasma insulin levels of starved and lipid-infused rats (Table I). Therefore, other factors besides insulin are probably involved. There is evidence that both glucose and fatty acids, independent of insulin, influence amino acid metabolism (49–51). For example, an acute increase in plasma free fatty acid concentration, without a change in insulin level, was accompanied by decreases in whole body rates of leucine flux and oxidation (51).

The fact that muscle proteolysis and BCKA dehydrogenase activity were both higher in lipid than glucose-infused rats suggests a relation between these two biochemical processes. We and others have previously shown that BCKA dehydrogenase exists in both active (dephosphorylated) and inactive (phosphorylated) forms (52). Furthermore, a number of metabolic and hormonal factors have been shown to activate BCKA dehydrogenase (6). Among these factors leucine appears to play a prominent role (6). Leucine inhibits BCKA dehydrogenase kinase, resulting in activation of BCKA dehydrogenase (53). In our experiment the greater muscle concentration of leucine could, therefore, account for greater leucine oxidation in lipid than in glucose-infused rats.

Another contribution of the present experiment is the finding of nitrogen sparing by exogenous lipid during nitrogen deprivation (Fig. 1). The failure of previous studies (54), including our own (11), to show that ingestion of lipid reduces loss of nitrogen during fasting can be explained by the fact that the amount of lipid given to subjects was not sufficiently large to meet the daily need for energy. The reason included difficulties with consuming a diet that consists entirely of lipid and contains no carbohydrate or protein. These difficulties were circumvented in the present experiment by using the intravenous route for administration of lipid. It is pertinent to note that previous studies have shown maintenance of nitrogen balance during total parenteral nutrition when patients received adequate amounts of amino acids with a large portion of glucose being replaced by lipid (55).

The sparing of body nitrogen during nitrogen deprivation could be achieved by attenuation of (*a*) hepatic gluconeogenesis, (*b*) renal ammoniagenesis, and (*c*) amino acid oxidation. All these mechanisms appeared to be involved in glucose-induced sparing of body nitrogen. Hepatic gluconeogenesis, as assessed by intracellular hepatic concentration of alanine (Fig. 3) and amino acid oxidation, as assessed by whole body rate of leucine oxidation (Fig. 5), were both lower in glucose-infused than in starved rats. The enhancement of renal ammoniagenesis during fasting is a consequence of acidosis resulting from ketosis (56). Since glucose infusion abolished ketosis (Table II), it is quite likely that renal ammoniagenesis was also attenuated by glucose. In contrast to glucose, lipid-induced sparing of body nitrogen appeared to involve only one of the above three mechanisms: namely, a reduction in amino acid oxidation (Fig. 5). Lipid emulsion, such as the one used in the present experiment, contains glycerol. Glycerol is a substrate

for gluconeogenesis and may be responsible for the effect of lipid emulsion on nitrogen excretion (57). However, the amount of glycerol was probably not large enough to account for the nitrogen sparing of the lipid infusion. This impression is supported by similar hepatic intracellular concentrations of alanine (Fig. 3) during lipid infusion and starvation. Since the present study was not designed to identify the fraction of lipid emulsion responsible for protein sparing, additional studies will be needed for a firm conclusion regarding this question.

In conclusion, using a new approach, we have investigated the effect of lipid and glucose infusions on metabolism of body proteins and BCAA. The results provide new insight into mechanism of nitrogen sparing by caloric sources. It appears that lipid is not as effective as glucose in inhibiting muscle proteolysis. The reason for greater muscle proteolysis includes a greater need for amino acids as substrates for hepatic gluconeogenesis, and renal ammoniagenesis during lipid than glucose infusion. The greater muscle proteolysis also increases BCAA concentration, which activates BCKA dehydrogenase, resulting in increased catabolism of BCAA.

Acknowledgments

References

1. Adibi, S. A. 1980. Roles of branched-chain amino acids in metabolic regulations. *J. Lab. Clin. Med.* 95:475–484.

2. Adibi, S. A. 1984. Nutritional, physiological, and clinical significance of branched-chain amino acids. *In* Branched-Chain Amino Acids and Keto Acids in Health and Disease. S. A. Adibi, W. Fekl, U. Langenbeck, and P. Schauder, editors. S. Karger, Basel. 1–14.

3. Walser, M. 1984. Therapeutic aspects of branched-chain amino and keto acids. *Clin. Sci.* 66:1–75.

4. Adibi, S. A. 1976. Metabolism of branched-chain amino acids in altered nutrition. *Metab. Clin. Exp.* 25:1287–1302.

5. Harper, A. E., R. H. Miller, and K. P. Block. 1984. Branched-chain amino acid metabolism. *Annu. Rev. Nutr.* 4:409–454.

6. Paul, H. S., and S. A. Adibi. 1984. Regulation of branched-chain amino acid catabolism. *In* Branched-Chain Amino Acid and Keto Acids in Health and Disease. S. A. Adibi, W. Fekl, U. Langenbeck, and P. Schauder, editors. S. Karger, Basel. 182–219.

7. Wohlheuter, R. M., and A. E. Harper. 1970. Coinduction of branched-chain α-ketoacid dehydrogenase activities. *J. Biol. Chem.* 245:2391–2401.

8. Laurent, B. C., L. L. Moldower, V. R. Young, B. R. Bistrian, and G. L. Blackburn. 1984. Whole body leucine and muscle protein kinetics in rats fed varying protein intake. *Am. J. Physiol.* 246:E444–451.

9. Motil, K. J., D. E. Matthews, D. M. Bier, J. F. Burke, H. N. Munro, and V. R. Young. 1981. Whole body leucine and lysine metabolism: response to dietary protein intake in young men. *Am. J. Physiol.* 240:E712–E721.

10. Block, K. F., R. P. Aftring, W. B. Mehord, and M. G. Buse. 1987. Modulation of rat skeletal muscle branched-chain α-keto acid dehydrogenase in vivo. Effects of dietary protein and meal consumption. *J. Clin. Invest.* 79:1349–1358.

11. Vazquez, J. A., E. L. Morse, and S. A. Adibi. 1985. Effect of dietary fat, carbohydrate, and protein on branched-chain amino acid catabolism during caloric restriction. *J. Clin. Invest.* 76:737–743.

12. Nair, K. S., P. W. Woolf, S. L. Welle, and D. E. Matthews. 1987. Leucine, glucose, and energy metabolism after 3 days of fasting in healthy human subjects. *Am. J. Clin. Nutr.* 46:557–562.

13. Paul, H. S., and S. A. Adibi. 1978. Leucine oxidation in diabetes and starvation. Effects of ketone bodies on branched-chain amino acid oxidation in vitro. *Metab. Clin. Exp.* 27:185–200.

14. May, M. E., V. J. Mancusi, R. P. Aftring, and M. G. Buse. 1980. Effects of diabetes on oxidative decarboxylation of branched-chain keto acids. *Am. J. Physiol.* 239:E215–E222.

15. Tessari, P., R. Nosadini, R. Trevisan, S. V. Kreutzenberg, S. Inchiostro, E. Dunar, G. Biolo, M. C. Marescotti, A. Tiengo, and G. Crepaldi. 1986. Defective suppression by insulin of leucine-carbon appearance and oxidation in type I, insulin-dependent diabetes mellitus. Evidence for insulin resistance involving glucose and amino acid metabolism. *J. Clin. Invest.* 77:1797–1804.

16. Nair, K. S., G. C. Ford, and D. Halliday. 1987. Effect of intravenous insulin treatment on in vivo whole body leucine kinetics and oxygen consumption in insulin-deprived Type I diabetic patients. *Metab. Clin. Exp.* 36:491–495.

17. Munro, H. N. 1964. General aspects of the regulation of protein metabolism by diet and by hormones. *In* Mammalian Protein Metabolism. Vol. 1. H. N. Munro and J. B. Allison, editors. Academic Press, New York. 381–481.

18. Nutrient Requirements of Laboratory Animals. 1972. Second revised edition. National Academy of Sciences, Washington, DC. 56–93.

19. Vazquez, J. A., H. S. Paul, and S. A. Adibi. 1986. Relation between plasma and tissue parameters of leucine metabolism in fed and starved rats. *Am. J. Physiol.* 250:E615–621.

20. Paul, H. S., and S. A. Adibi. 1980. Leucine oxidation and protein turnover in clofibrate-induced muscle protein degradation in rats. *J. Clin. Invest.* 65:1285–1293.

21. Hutson, S. M., T. C. Cree, and A. E. Harper. 1978. Regulation of leucine and α-ketoisocaproate metabolism in skeletal muscle. *J. Biol. Chem.* 253:8126–8133.

22. Chappell, J. B., and S. V. Perry. 1954. Biochemical and osmotic properties of skeletal muscle mitochondria. *Nature (Lond.).* 173:1094–1095.

23. Shinnick, F. L., and A. E. Harper. 1977. Effects of branched-chain amino acid oxidation by isolated rat tissue preparations. *Biochim. Biophys. Acta.* 437:477–486.

24. Wagenmakers, A. J. M., J. T. G. Schepens, J. A. M. Veldhuizen, and J. H. Veerkamp. 1984. The activity state of the branched-chain 2-oxo acid dehydrogenase complex in rat tissues. *Biochem. J.* 220:273–281.

25. Paul, H. S., and S. A. Adibi. 1982. Role of ATP in the regulation of branched-chain α-keto acid dehydrogenase activity in liver and muscle mitochondria of fed, fasted, and diabetic rats. *J. Biol. Chem.* 257:4875–4881.

26. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

27. Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymic determination of D-(−)β-hydroxybutyric acid and acetoacetic acid in blood. *Biochem. J.* 82:90–96.

28. Cederblad, G., O. Finnstrom, and J. Martensson. 1982. Urinary excretion of carnitine and its derivatives in newborns. *Biochem. Med.* 27:260–265.

29. Bergmeyer, H. U., and E. Berndt. 1974. D-glucose. Determination with glucose oxidase and peroxidase. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press Inc., New York. 1205–1212.

30. Wahlefeld, A. W. 1974. Triglycerides. Determination after enzymatic hydrolysis. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press Inc., New York. 1831–1835.

31. Wassner, S. J., J. L. Schlitzer, and J. B. Li. 1980. A rapid, sensitive method for the determination of 3-methylhistidine levels in urine and plasma using high-pressure liquid chromatography. *Anal. Biochem.* 104:284–289.

32. Rudiger, H. W., U. Langenbeck, and H. W. Goedde. 1972. A simplified method for the preparation of ^{14}C-labeled branched-chain 2-oxo acids. *Biochem. J.* 126:445–446.

33. Nallathambi, S. A., A. M. Goorin, and S. A. Adibi. 1972. Hepatic and skeletal muscle transport of cycloleucine during starvation. *Am. J. Physiol.* 223:13–19.

34. Glantz, S. A. 1981. Primer of biostatistics. McGraw Hill, Inc., New York. 87–90.

35. Paul, H. S., and S. A. Adibi. 1978. Effect of carnitine on branched-chain amino acid oxidation by liver and skeletal muscle. *Am. J. Physiol.* 234:E494–E499.

36. May, M. E., R. P. Aftring, and M. G. Buse. 1980. Mechanism of the stimulation of branched-chain oxoacid oxidation in liver by carnitine. *J. Biol. Chem.* 255:8394–8397.

37. Bremer, J. 1983. Carnitine—metabolism and functions. *Physiol. Rev.* 63:1420–1480.

38. Young, V. R., and H. N. Munro. 1978. N-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. *Fed. Proc.* 37:2291–2300.

39. Fulks, R. M., J. B. Li, and A. L. Goldberg. 1975. Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. *J. Biol. Chem.* 250:290–298.

40. Felig, P. 1975. Amino acid metabolism in man. *Annu. Rev. Biochem.* 44:933–955.

41. Adibi, S. A. 1971. Interrelationships between level of amino acids in plasma and tissues during starvation. *Am. J. Physiol.* 221:829–838.

42. Adibi, S. A., T. A. Modesto, E. L. Morse, and P. M. Amin. 1973. Amino acid levels in plasma, liver, and skeletal muscle during protein deprivation. *Am. J. Physiol.* 225:408–414.

43. Elia, M., G. Neale, and G. Livesey. 1985. Alanine and glutamine release from the human forearm: effects of glucose administration. *Clin. Sci.* 69:123–133.

44. Guroff, G., and S. Udenfriend. 1960. The uptake of tyrosine by isolated rat diaphragm. *J. Biol. Chem.* 235:3518–3522.

45. Bates, P. C., and D. J. Millward. 1983. Myofibrillar protein turnover. *Biochem. J.* 214:587–592.

46. Lowell, B. B., and M. N. Goodman. 1987. Protein sparing in skeletal muscle during prolonged starvation. Dependence on lipid fuel availability. *Diabetes.* 36:14–19.

47. Lowell, B. B., N. B. Ruderman, and M. N. Goodman. 1986. Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. *Biochem. J.* 234:237–240.

48. Jefferson, L. S., J. B. Li, and S. R. Rannels. 1977. Regulation by insulin of amino acid release and protein turnover in the perfused rat hemicorpus. *J. Biol. Chem.* 252:1476–1483.

49. Adibi, S. A., E. L. Morse, and P. M. Amin. 1975. Role of insulin and glucose in the induction of hyperaminoacidemia in man. Studies in normal, juvenile diabetic and insulinoma patients. *J. Lab. Clin. Med.* 86:395–409.

50. Wolfe, R. R., J. H. F. Shaw, F. Jahoor, D. N. Herndon, and M. H. Wolfe. 1986. Response to glucose infusion in humans. Role of changes in insulin concentration. *Am. J. Physiol.* 250:E306–E311.

51. Tessari, P., S. L. Nissen, J. M. Miles, and M. W. Haymond. 1986. Inverse relationship of leucine flux and oxidation to free fatty acid availability in vivo. *J. Clin. Invest.* 77:575–581.

52. Harris, R. A., and R. Paxton. 1985. Regulation of branched-chain α-ketoacid dehydrogenase complex by phosphorylation-dephosphorylation. *Fed. Proc.* 44:305–315.

53. Paxton, R., and R. A. Harris. 1984. Regulation of branched-chain α-ketoacid dehydrogenase kinase. *Arch. Biochem. Biophys.* 231:48–57.

54. Munro, H. N. 1951. Carbohydrate and fat as factors in protein utilization and metabolism. *Physiol. Rev.* 31:449–488.

55. Jeejeebhoy, K. N., G. H. Anderson, A. F. Nakhooda, G. R. Greensberg, I. Sanderson, and E. B. Marliss. 1976. Metabolic studies in total parenteral nutrition with lipid in man. Comparison with glucose. *J. Clin. Invest.* 57:125–136.

56. Cahill, G. F. 1970. Starvation in man. *N. Engl. J. Med.* 282:668–675.

57. Brennan, M. F., G. F. Fitzpatrick, K. H. Cohen, and F. D. Moore. 1975. Glycerol: Major contribution to the short term protein sparing effect of fat emulsions in normal man. *Ann. Surg.* 182:386–394.