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Research Article

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Effect of Dietary Fat, Carbohydrate, and Protein on Branched-chain Amino Acid Catabolism during Caloric Restriction

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Abstract

To assess the effect of each dietary caloric source on the catabolism of branched-chain amino acids, we investigated the rate of leucine oxidation before and after obese volunteers consumed one of the following diets for one week: (*a*) starvation, (*b*) 300 or 500 cal of fat/d, (*c*) 300 or 500 cal of carbohydrate/d, (*d*) 300 or 500 cal of protein/d, (*e*) a mixture of carbohydrate (300 cal/d) and fat (200 cal/d), or (*f*) a mixture of carbohydrate (300 cal/d) and protein (200 cal/d).

Starvation significantly increased the rate of leucine oxidation (1.4±0.11 vs. 1.8±0.16 mmol/h, $P < 0.01$). The same occurred with the fat and protein diets. In sharp contrast, the 500-cal carbohydrate diet significantly decreased the rate of leucine oxidation (1.3±0.13 vs. 0.6±0.09 mmol/h, $P < 0.01$). The same occurred when a portion of the carbohydrate diet was isocalorically replaced with either fat or protein. The cumulative nitrogen excretion during the fat diet and starvation was not significantly different. As compared with the fat diets, the carbohydrate diets on the average reduced the urinary nitrogen excretion by 12 g/wk. Nitrogen balance was positive during the consumption of the 500-cal protein diet, but negative during the consumption of carbohydrate-protein diet. The fat diets, like the protein diets and starvation, greatly increased plasma leucine (119±13 vs. 222±15 μM, $P < 0.01$) and β-hydroxybutyrate (0.12±0.02 vs. 4.08±0.43 mM, $P < 0.01$) concentrations, and significantly decreased plasma glucose (96±4 vs. 66±3 mg/dl, $P < 0.01$) and insulin (18±4 vs. 9±1 μU/ml, $P < 0.05$) concentrations. These changes did not occur, or were greatly attenuated, when subjects consumed carbohydrate alone or in combination with fat or protein.

We conclude that (*a*) during brief caloric restriction, dietary lipid and protein, unlike carbohydrate, do not diminish the catabolism of branched-chain amino acids and (*b*) the decrease in branched-chain amino acid oxidation is associated with protein sparing.

Introduction

Branched-chain amino acids are major constituents of body (1) and dietary (2) proteins and comprise 46% of the dietary requirement for essential amino acids for man (3). Furthermore, they may have important roles in metabolic regulation (4, 5). For example, leucine may stimulate synthesis and inhibit

degradation of skeletal muscle protein (6–8). Therefore, sparing branched-chain amino acids from catabolism may make a significant contribution to the promotion of protein synthesis and/or the conservation of body proteins. Indeed, there is evidence that the stimulation of branched-chain amino acid oxidation is associated with increased muscle proteolysis (9).

In the present experiments we have investigated the effects of hypocaloric amounts of carbohydrate, fat, and protein consumed individually and in combination on parameters of leucine metabolism and protein nutrition in groups of obese, female volunteers. A week of dietary caloric restriction has been used as the clinical model for testing the effects of these diets. The design of these experiments is based on the following considerations: (*a*) the greatest loss of body nitrogen in starvation occurs during the first week (10); (*b*) there is substantial evidence that starvation increases muscle oxidation of branched-chain amino acids (11, 12); and (*c*) the alteration in protein metabolism in response to dietary caloric restriction appears to be similar in obese and nonobese human subjects (10, 13).

Methods

Experimental protocol. The studies of leucine metabolism were performed in 63 obese, female volunteers who were admitted to the Clinical Research Unit of the University of Pittsburgh School of Medicine. Each patient gave informed consent before the study. For the initial 3–4 d, subjects were given a diet that simulated their intake at home, usually exceeding 3,000 cal/d. During the control period, after an overnight fast, peripheral venous blood was obtained for determination of concentrations of β-hydroxybutyrate, glucose, insulin, and amino acids, and then the rates of leucine oxidation and turnover were investigated. After the completion of these studies, the subjects were assigned to a diet containing either 0 cal, only fat (300 or 500 cal), only carbohydrate (300 or 500 cal), a mixture of carbohydrate (300 cal) and fat (200 cal), only protein (300 or 500 cal), or a mixture of carbohydrate (300 cal) and protein (200 cal). The sources of fat, carbohydrate, and protein were soybean oil, vegetables and fruits, and water-packed tuna, respectively. In addition, all patients received multivitamins daily. After 1 wk of treatment with each of the above diets, the studies performed during the control period were repeated. In addition, all urine and stool excreted during the week of treatment was collected and pooled for nitrogen analysis. The mean age, height, and body weight of all groups were comparable (Table I); statistically there was no significant difference in regard to any of these characteristics. Furthermore, there was no significant difference in the rates of leucine oxidation and turnover and plasma concentrations of β-hydroxybutyrate, glucose, insulin, and amino acids among the groups before dietary treatments (see Tables III–V).

The rates of valine oxidation and turnover during the control period and after 1 wk of starvation were determined in an additional group of five obese subjects. The group was comparable to the group used for the investigation of the effect of starvation on parameters of leucine metabolism.

Parameters of leucine metabolism. The rates of turnover and oxidation of [^{14}C]leucine were investigated by a technique requiring

Table I. Characteristics of Groups

Dietary treatment	No. of subjects	Age	Height	Weight
		yr	cm	kg
Starvation	7	37.9±4.8	164.0±1.3	105.8±5.5
Fat diet				
300 cal	7	32.6±3.1	163.5±2.8	113.5±13.2
500 cal	7	39.1±4.3	160.7±2.7	122.9±7.3
Carbohydrate diet				
300 cal	4	34.9±3.0	159.5±2.3	124.0±15.2
500 cal	7	35.0±3.3	160.9±2.5	117.7±11.8
Protein diet				
300 cal	8	37.6±5.0	163.9±4.7	134.9±17.4
500 cal	6	38.3±3.3	171.4±2.6	139.5±14.9
Carbohydrate–fat diet	6	33.0±1.7	159.0±1.6	127.8±13.1
Carbohydrate–protein diet	6	33.4±3.0	164.8±3.5	138.6±20.9

All subjects were women. The data are presented as means±SEM.

intravenous infusion of a trace amount of this amino acid at a constant rate. Details of this technique have been previously published (14). In brief, after an overnight fast, a bolus dose of 5 μCi of [1-^{14}C]leucine (Amersham Corp., Arlington Heights, IL; 58 mCi/mmol) was administered through an indwelling catheter into a peripheral vein. Immediately after this priming dose, a continuous infusion of leucine was begun. [1-^{14}C]leucine dissolved in physiologic saline was delivered over a period of 3 h at a constant rate of 0.21 ml/min (0.045 μCi/min). For determination of specific activity of CO_2 in breath, the patients blew through a tube into scintillation counting vials containing a CO_2-trapping mixture. The CO_2-trapping mixture consisted of 4 ml of hydroxide of hyamine-10× (Rohm & Haas Co., Philadelphia, PA), absolute ethanol (1:1, vol/vol), and thymolphthalein indicator. The solution was calibrated to change color from blue to clear when 2 mmol of CO_2 had been collected. After addition of 10 ml of Liquifluor-toluene scintillation counting fluid (New England Nuclear, Boston, MA), samples were assayed for radioactivity in a scintillation counter.

Peripheral venous blood and expired air were collected at 135, 150, 165, and 180 min after the start of the infusion. Previous studies in fed and starved obese subjects showed that the specific activities of leucine in plasma and CO_2 in expired air had reached near-constant values at these time intervals (14). The fact that the specific activity of leucine in plasma and CO_2 in expired air had reached plateau in each subject of the present experiment was confirmed by close agreement among the four values; coefficient of variation was 5% or less of the mean specific activity.

The rate of CO_2 production was measured in each experiment utilizing an automatic system that allows breath-to-breath measurement of expired air volume and CO_2 concentration (Beckman Metabolic Measurement Cart, Sensormedics, Anaheim, CA). To assure complete collection of expired air, patients were furnished with a rubber mouthpiece and a nose clip. The accuracy of expired air volume and CO_2 concentration measurement with this system is ±2% and ±0.1%, respectively.

Rates of turnover and oxidation of leucine were calculated by the following formulas: $Q = I/SA_{pl}$, where Q = turnover rate (millimoles per hour), I = infusion rate of tracer (disintegrations per minute/hour), and SA_{pl} = plasma specific activity of tracer during steady state (disintegrations per minute/millimole); $Ox = (SA_b \times PR)/(SA_{pl} \times FR)$, where Ox = oxidation rate (millimoles per hour), SA_b = specific activity of CO_2 in expired air at steady state, PR = CO_2 production rate (millimoles per hour), SA_{pl} = plasma specific activity of tracer at steady state (disintegrations per minute/millimole), and FR = fraction of CO_2 recovered during infusion of [^{14}C]bicarbonate.

In calculating the above rates the following three factors were considered:

(a) Specific activity. The plasma specific activity of ketoisocaproate (KIC)[1] instead of leucine has been suggested for calculating the rate of oxidation of leucine (15). However, there is no evidence that specific activity of KIC in plasma has any relevance to the specific activity of KIC in tissues. In contrast, we and others have found that during a constant infusion when the specific activity of leucine reaches a constant value in plasma, it also reaches a constant value in tissue intracellular compartment (16, 17). Furthermore, at plateau, intracellular specific activities closely approximate the specific activity of leucine in plasma in both the fed and starved state. A previous study (15) has shown that during infusion of [^{13}C]leucine over a wide range of dietary intake the ratio of KIC to leucine specific activity in plasma remains constant at ~0.77. Therefore, using specific activity of KIC instead of leucine would not alter the findings of the present experiment. It would similarly increase rates of leucine oxidation before and after each dietary treatment.

(b) Weight. For the following reasons the rates of leucine oxidation and turnover are not expressed per kilogram of body weight: each subject served as her own control; the amount of weight loss during each treatment when compared with the initial body weight was rather small; and there was uncertainty as to the proportion of the decrease in body weight which was due to body fluid and tissue.

(c) $^{14}CO_2$ recovery. To assess the effect of starvation on the recovery of $^{14}CO_2$, we investigated the rate of $^{14}CO_2$ production in expired air during a primed continuous infusion of NaH$^{14}CO_3$. The rate of $^{14}CO_2$ production was investigated before and after 1 wk of starvation in three obese volunteers. The infusion solution was prepared by adding NaH$^{14}CO_3$ to 8.4% NaHCO$_3$ solution (pH 7.0–8.5). The subjects received a priming dose of 3 μCi over 1 min before undergoing a continuous infusion with the bicarbonate solution at a rate of 0.15 ml/min (0.045 μCi/min). The period of continuous infusion was 3 h. The breath samples for determination of CO_2 specific activity were collected at 135, 150, 165, and 180 min after the start of infusion. The specific activity of CO_2 in expired air rose immediately and reached steady state by 120 min. The rate of $^{14}CO_2$ recovery during the infusion of [^{14}C]bicarbonate was calculated by the following formula: $R = (SA_b \times PR \times 100)/I$, where R = percent recovery of $^{14}CO_2$, and others are as defined above.

The mean specific activity of CO_2 in the expired air, CO_2 production rate, and %CO_2 recovery before and after starvation are shown in Table II. The recovery of $^{14}CO_2$ in expired air was 60% in the control period and remained the same after 1 wk of starvation. Although we did not investigate $^{14}CO_2$ recovery after each hypocaloric diet, it is unlikely that partial starvation would alter $^{14}CO_2$ recovery in that total starvation, the most severe caloric treatment in our experiment, was without effect. We, therefore, corrected all rates of leucine oxidation by the same $^{14}CO_2$ recovery factor (60%).

Parameters of valine metabolism. The rates of [^{14}C]valine oxidation and turnover were investigated by the same methods used to study parameters of leucine metabolism. The only difference was the substitution of [1-^{14}C]valine (Amersham Corp., 57 mCi/mmol) for [1-^{14}C]leucine.

Methods of analysis. Plasma concentrations of β-hydroxybutyrate, amino acids, glucose, and insulin were determined by enzymatic (18), ion-exchange chromatography (13), hexokinase, and radioimmunoassay (Cambridge Nuclear Radiopharmaceutical Corp., Billerica, MA) methods, respectively. Total nitrogen in the urine and feces was determined by a micro-Kjeldahl technique.

The concentrations of radioactively labeled valine and leucine were determined by modification of the method of Hutson et al. (19). Briefly, 4 ml of a 6% sulfosalicylic acid extract (3:1, vol/vol) was

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1. *Abbreviations used in this paper:* BCKA, branched-chain α-ketoacid; KIC, ketoisocaproate.

Table II. Effect of Diet on $^{14}CO_2$ Recovery

Starvation	CO₂ specific activity	CO₂ production rate	CO₂ recovery
	dpm/mmol	mmol/min	% infused
Before	7,068±300	8.23±0.12	60±3
After	8,171±393	7.02±0.63	60±2

The data are presented as means±SEM in three subjects.

applied to a 4 × 0.9-cm column of Dowex 50W-X8 cation exchange resin (Dow Chemical Co., Indianapolis, IN). The column was eluted with 10 ml 0.01 N HCl to remove ketoacids. Subsequently, amino acids were eluted with 6 ml of 4 N NH₄OH; the sample was evaporated to dryness. The residue was dissolved in 10 ml of Liquifluor scintillation cocktail and was assayed for radioactivity in a Packard Tricarb scintillation counter (Packard Instrument Co., Downers Grove, IL).

Statistics. Data from the same and different groups were evaluated statistically by the paired *t* test and analysis of variance, respectively (20). The relationship of oxidation rate and leucine, β-hydroxybutyrate, glucose and insulin concentrations was evaluated by the correlation coefficient (20). Two-tailed tests were used in all cases. All data are presented as mean±standard error of the mean.

Results

Parameters of branched-chain amino acid metabolism. The rates of leucine oxidation of each subject before and after 1 wk of starvation and consumption of diets that consisted of 500 cal of fat, carbohydrate, or protein are detailed in Fig. 1. The mean±standard error of the mean of these rates are shown in Table III. The rate of leucine oxidation increased in each subject receiving the starvation, fat, and protein diets and decreased in each subject consuming the carbohydrate diet. These changes were all statistically significant (Table III). The average increase in the rate of leucine oxidation during consumption of the 500-cal fat diet was 57% and the average decrease during consumption of the 500-cal carbohydrate diet was 38%.

To assess the quantitative importance of these diets, we investigated the effect of consuming diets consisting of 300 cal
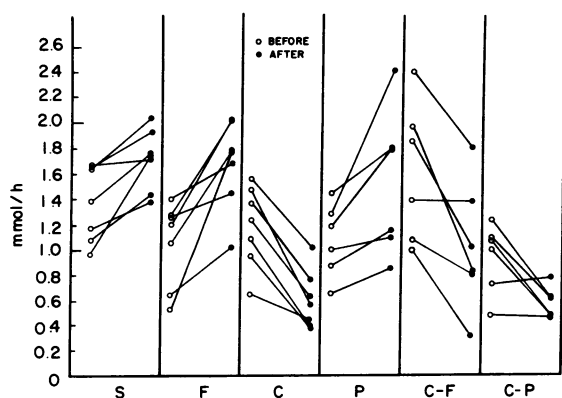
Table III. Parameters of Leucine Metabolism

Dietary treatment	Oxidation rate	Turnover rate
	mmol/h	mmol/h
Starvation		
Before	1.4±0.11	8.3±0.5
After	1.8±0.16§	6.4±0.4‡
Fat diet		
300 cal		
Before	1.6±0.22	9.9±0.8
After	2.0±0.24‡	6.8±0.7§
500 cal		
Before	1.1±0.14	10.0±0.9
After	1.8±0.14§	7.3±0.7*
Carbohydrate diet		
300 cal		
Before	1.6±0.14	8.6±0.5
After	1.1±0.22	7.4±0.6*
500 cal		
Before	1.3±0.13	9.2±0.8
After	0.6±0.09§	6.8±0.4§
Protein diet		
300 cal		
Before	1.2±0.12	7.8±0.8
After	1.5±0.20*	6.7±0.6
500 cal		
Before	1.1±0.12	8.3±0.6
After	1.6±0.25*	7.3±0.6
Carbohydrate-fat diet		
Before	1.7±0.24	7.9±0.8
After	1.1±0.22‡	5.7±0.7‡
Carbohydrate-protein diet		
Before	1.0±0.12	8.8±0.6
After	0.6±0.05*	7.5±0.8

Values given as means±SEM.
*, ‡, § P values < 0.05, 0.02, and 0.01, respectively, as evaluated by the paired *t* test. Duration of treatment with each diet was 1 week.

Figure 1. The rates of leucine oxidation before and after each subject received the starvation (S), 500-cal fat (F), 500-cal carbohydrate (C), 500-cal protein (P), 300-cal carbohydrate and 200-cal fat (C-F), and 300-cal carbohydrate and 200-cal protein (C-P) diets. The statistical analysis of data is shown in Table III.

of fat, carbohydrate, or protein. These data are summarized in Table III. The increase in leucine oxidation with the 300-cal protein diet was similar to increases with 500-cal diets (29 vs. 29%), and the decrease in the rate of leucine oxidation with the 300-cal carbohydrate diet was similar to the decrease by the 500-cal diet (40 vs. 38%). On the other hand, the increase in leucine oxidation was greater with the 500-cal than with the 300-cal fat diet (57 vs. 20%).

We then investigated whether dietary carbohydrate would reduce the oxidation of leucine if fat or protein were added to the diet. The rate of leucine oxidation was determined before and 1 wk after subjects consumed a diet containing 300 cal as carbohydrate and 200 cal of fat or protein. Except for one subject, consumption of the carbohydrate-fat and carbohydrate-protein diet resulted in a decrease in the rate of leucine oxidation (Fig. 1). These decreases were statistically significant (Table III).

The rates of leucine turnover of each subject before and after 1 wk of starvation and the 500-cal hypocaloric diets are

detailed in Fig. 2. The mean±standard error of the mean of these rates as well as those before and after the 300-cal hypocaloric diets are summarized in Table III. All dietary treatments reduced the rate of leucine turnover. However, changes with the protein-containing diets were not significant, while reductions with the other diets were (Table III). The magnitudes of reduction in leucine turnover rate with starvation (23%), the 500-cal fat diet (27%), and the 500-cal carbohydrate diet (26%) were approximately similar.

To investigate whether metabolism of other branched-chain amino acids is similarly affected by dietary caloric restriction, we determined the rates (millimoles per hour) of valine oxidation and turnover in five subjects before and after 1 wk of starvation. Starvation significantly increased the rate of oxidation (0.77±0.3 vs. 1.00±0.19 [mean±SEM], $P < 0.05$) and significantly reduced the rate of turnover (6.12±0.85 vs. 5.37±0.83, $P < 0.02$).

Parameters of protein nutrition. The cumulative urinary excretion of nitrogen during each diet is shown in Table IV. Statistical analysis of the data showed that nitrogen excretion during the 300- and 500-cal carbohydrate and the carbohydrate-fat diets were all significantly smaller than the nitrogen excretion during starvation and the 300- and 500-cal fat diets. Furthermore, there was no significant difference between nitrogen excretion during starvation and the fat diets. Although nitrogen excretion was smaller during the 500-cal than during 300-cal carbohydrate diet, the difference was not statistically significant. As compared with the fat diets, the carbohydrate diets on the average reduced the urinary nitrogen excretion by 12 g/wk.

As might be expected, nitrogen excretion during consumption of the protein-containing diets was considerably greater than when protein was not included in the diet (Table IV). Therefore, to account for the dietary intake we calculated the cumulative nitrogen balance [(dietary intake) − (urinary and fecal losses)] when the diet contained protein. The data showed that nitrogen balance was near neutral when subjects consumed the 300-cal protein diet, positive during the 500-cal protein diet, but negative during the carbohydrate-protein diet. Among
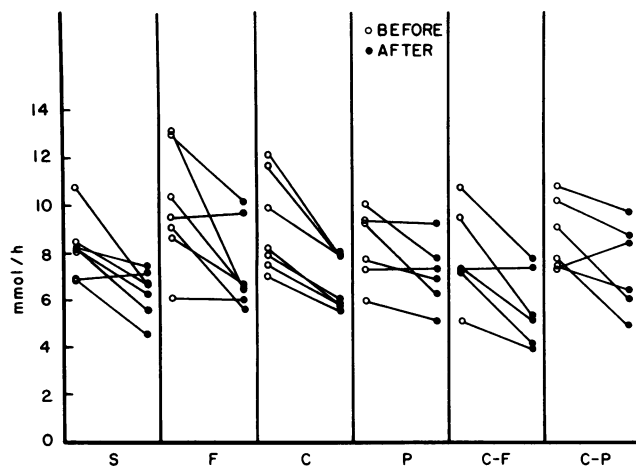

Figure 2. The rates of leucine turnover before and after each subject received the starvation (S), 500-cal fat (F), 500-cal carbohydrate (C), 500-cal protein (P), 300-cal carbohydrate and 200-cal fat (C-F), and 300-cal carbohydrate and 200-cal protein (C-P) diets. The statistical analysis of the data is shown in Table III.

Table IV. Cumulative Nitrogen Excretion and Balance

Dietary treatment	Nitrogen intake	Urinary excretion	Nitrogen balance
	gN/wk	*gN/wk*	*gN/wk*
Starvation	0	58.5±3.7	−61.4±3.7
Fat diet			
300 cal	0	58.1±3.3	−61.1±3.3
500 cal	0	52.4±3.2	−55.4±3.2
Carbohydrate diet			
300 cal	0	46.6±2.7*	−49.5±2.7*
500 cal	0	40.3±2.9*	−43.2±2.9*
Carbohydrate-fat diet	0	46.6±2.0*	−49.6±2.0*
Protein diet			
300 cal	76.8	75.0±5.4*	−1.1±5.4§
500 cal	128.1	118.1±9.9§	+7.1±9.9§
Carbohydrate-protein diet	51.0	72.8±4.0*	−24.7±4.0§

Values given as means±SEM.
*, § P values < 0.05, 0.01, respectively, as compared to values on the starvation diet.

the nonprotein-containing diets, the cumulative nitrogen balance was least negative with the 500-cal carbohydrate diet.

As in previous studies with brief starvation (13, 21, 22), the changes in plasma aminogram with all diets were confined to branched-chain amino acids and alanine. Therefore, only the concentrations of alanine and leucine (representative branched-chain amino acid) are shown in Table V. There was a near-twofold increase in plasma leucine concentration with starvation and 300- and 500-cal fat and protein diets. In contrast, consumption of the 300-cal carbohydrate diet resulted in a modest increase in plasma leucine concentration. When the daily carbohydrate intake was increased to 500 cal, there was no significant change in leucine concentration. This inhibitory effect on plasma branched-chain amino acid concentration persisted even when fat or protein was combined with carbohydrate. Unlike plasma leucine concentration, plasma alanine concentration was significantly reduced by all diets except the carbohydrate-protein diet.

Metabolic profile. In view of previously reported effects of ketone bodies, glucose and insulin on branched-chain amino acid oxidation (12), we also investigated plasma concentrations of these substrates and hormone before and after starvation and each of the hypocaloric diets (Table VI). The plasma concentration of β-hydroxybutyrate was increased over 40-fold by starvation and the fat diets, and nearly 20-fold by the protein diets. In comparison, there were far smaller increases in the plasma concentration of β-hydroxybutyrate with the carbohydrate diets. This inhibitory effect on plasma ketone body concentration persisted even when fat or protein was mixed with carbohydrate.

The fat, carbohydrate, and protein diets, like starvation, significantly reduced the plasma glucose concentration. However, the decrease in plasma glucose concentration was greater during the 500-cal fat diet (31%) than during the 500-cal carbohydrate (16%) or protein (17%) diet. The decrease in plasma glucose concentration with the carbohydrate-fat and carbohydrate-protein diets was not statistically significant.

Table V. Plasma Amino Acid Concentrations

Dietary treatment	Leucine	Alanine
	μM	*μM*
Starvation		
Before	127±9	296±42
After	209±12§	189±14*
Fat diet		
300 cal		
Before	122±13	357±42
After	240±15§	199±16*
500 cal		
Before	119±13	312±38
After	222±15§	204±12‡
Carbohydrate diet		
300 cal		
Before	111±6	308±24
After	148±9§	195±17§
500 cal		
Before	144±7	365±26
After	131±8	257±18§
Protein diet		
300 cal		
Before	121±8	372±21
After	223±8§	251±23§
500 cal		
Before	111±9	384±26
After	196±14§	246±10§
Carbohydrate-fat diet		
Before	150±10	365±26
After	153±19	285±25§
Carbohydrate-protein diet		
Before	118±7	363±22
After	125±11	315±47

Values are given as means±SEM.
*, ‡, § P values < 0.05, 0.02, and 0.01, respectively, as evaluated by the paired *t* test. Duration of treatment with each diet was 1 wk.

The fat and protein diets, like starvation, significantly reduced plasma insulin level. The decrease in insulin level was more pronounced during the fat than protein diets (53–50% vs. 35–39%). The plasma insulin level also fell when the 300-cal carbohydrate diet was consumed, but remained unchanged when carbohydrate intake was increased to 500 cal per day. The decrease in insulin level was statistically significant with the carbohydrate–protein diet, but not with the carbohydrate–fat diet.

It is pertinent to mention that, although we have presented data on plasma concentrations of amino acids, fuels, and insulin on the 7th d of treatment, essentially the same results were obtained from analysis of plasma obtained on earlier days.

Statistical correlations. Statistical correlations between changes in the rate of leucine oxidation and changes in plasma concentrations of leucine, β-hydroxybutyrate, glucose, and insulin are shown in Fig. 3. The relation was direct as far as changes in plasma concentration of leucine (r = 0.937, P

Table VI. Plasma Ketone Body,
Glucose, and Insulin Concentrations

Dietary treatment	β-Hydroxybutyrate	Glucose	Insulin
	mM	*mg/dl*	*μU/ml*
Starvation			
Before	0.09±0.02	97±3	19±3
After	3.46±0.25§	73±4§	10±1‡
Fat diet			
300 cal			
Before	0.08±0.02	87±4	15±3
After	3.19±0.15§	60±3§	7±1§
500 cal			
Before	0.12±0.02	96±4	18±4
After	4.08±0.43§	66±3§	9±1*
Carbohydrate diet			
300 cal			
Before	0.05±0.02	97±6	20±3
After	0.62±0.14§	76±5§	12±1*
500 cal			
Before	0.10±0.03	87±3	17±2
After	0.38±0.08*	73±3§	17±3
Protein diet			
300 cal			
Before	0.10±0.01	87±3	23±3
After	2.06±0.25§	59±3§	15±2§
500 cal			
Before	0.09±0.01	97±4	23±2
After	1.48±0.36§	80±3§	14±2§
Carbohydrate-fat diet			
Before	0.13±0.07	90±5	21±4
After	0.87±0.17§	88±7	18±4
Carbohydrate-protein diet			
Before	0.09±0.02	99±5	18±3
After	0.56±0.07§	93±4	11±3§

Values are given as means±SEM.
*, ‡, § P values of <0.05, 0.02, and 0.01, respectively, as evaluated by the paired *t* test. Duration of treatment with each diet was 1 wk.

< 0.001) and β-hydroxybutyrate (r = 0.866, P < 0.001) were concerned, but inverse with respect to plasma concentrations of glucose (r = −0.787, P < 0.001) and insulin (r = −0.754, P < 0.001).

Discussion

The results of the present experiments show that there is a sharp contrast between the effect of carbohydrate and that of fat and protein on branched-chain amino acid oxidation during brief caloric restriction. The rate of leucine oxidation was significantly reduced in subjects receiving the 300- and 500-cal carbohydrate diets, whereas there was a significant increase in oxidation rate when subjects consumed the same amount of calories in the form of fat or protein. In view of the fact that the same enzyme complex is responsible for committing all three branched-chain amino acids to oxidative degradation (11) and starvation similarly affected metabolism
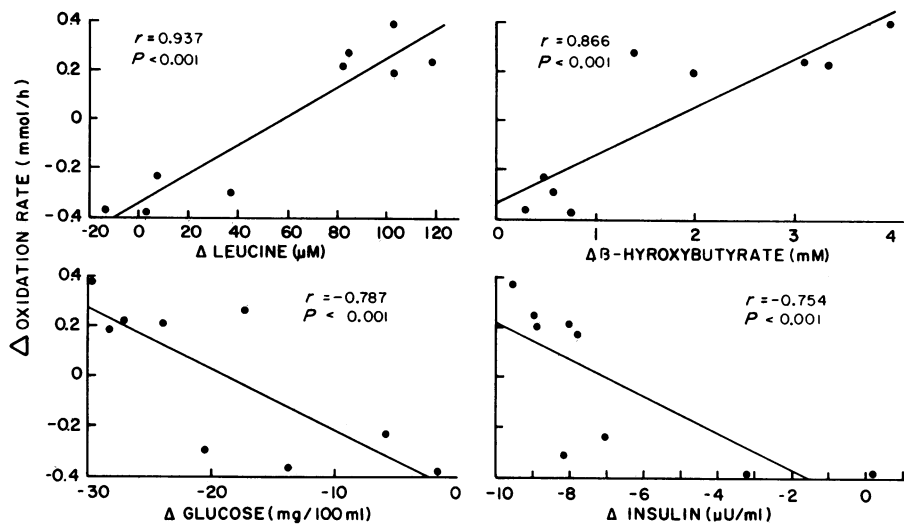
Figure 3. Statistical correlation between changes in the rate of leucine oxidation and changes in plasma concentration of either leucine, β-hydroxybutyrate, glucose, or insulin.

of leucine and valine, we considered it unnecessary to investigate the effects of various hypocaloric diets on oxidation of either valine or isoleucine.

Although the biochemical mechanism of alteration in leucine catabolism was not investigated, the data suggest that the different effects of carbohydrate versus fat and protein on leucine oxidation may possibly be related to their varied effects on protein metabolism. For example, plasma leucine concentration was greatly increased during the consumption of the 500-cal fat and protein diet whereas it was not significantly affected with the 500-cal carbohydrate diet (Table V). A consistent finding of the present experiment was that, whenever there was a large (65% or higher) increase in plasma leucine concentration, there was invariably a significant increase in the rate of leucine oxidation. In fact, statistically there was a direct relationship between changes in oxidation rates and changes in plasma concentration of leucine (Fig. 3).

The rate of leucine oxidation was studied as the rate of α-decarboxylation of this amino acid. This reaction is catalyzed by branched-chain α-ketoacid (BCKA) dehydrogenase, which normally exists in inactive (phosphorylated) and active (dephosphorylated) forms. Previous studies in rats have shown that caloric deficiency, caused by dietary deprivation (starvation) or impaired utilization (diabetes), results in a greater conversion of BCKA dehydrogenase from inactive to active form, accounting for the increased branched-chain amino acid oxidation by rat tissues in these conditions (23). It appears well established that leucine activates BCKA dehydrogenase (12). For example, oral administration of leucine in rats is followed by prompt activation of BCKA dehydrogenase and enhancement of valine oxidation (24). The mechanism of enzyme activation appears to be inhibition of phosphorylation of BCKA dehydrogenase by leucine (25, 26).

Undoubtedly, other factors (12) besides leucine are involved in regulation of branched-chain amino acid oxidation. This is suggested by the decrease in leucine oxidation despite near normal plasma leucine concentration during consumption of the carbohydrate diet. Furthermore, statistically there was a direct relationship between changes in oxidation rates and changes in plasma ketone body concentration and an inverse relationship between changes in oxidation rates and changes in plasma concentration of either glucose or insulin (Fig. 3).

There is evidence that in the skeletal muscle, the principal site for branched-chain amino acid catabolism (11), ketone bodies stimulate, and glucose and insulin inhibit, leucine oxidation (19, 27, 28). Therefore, it appears that changes in plasma concentrations of leucine, ketone bodies, glucose, and insulin are all associated with alterations in leucine oxidation. From in vivo studies, such as the present experiment, it is difficult to ascertain whether the effect is a direct or indirect one and to assign quantitative importance to each factor.

In contrast to the rate of oxidation, the rate of leucine turnover was similarly affected by the fat and carbohydrate diets. In other words, neither diet prevented the decrease in leucine turnover that occurs during caloric restriction. The rate of turnover is a measure of the amount of leucine that moves in and out of plasma per unit time. The rate of inflow is determined by the rate of body protein breakdown and the rate of outflow is determined by the rates of protein incorporation and oxidation of leucine. Under the condition of a plateau in the specific activity of leucine in plasma, such as in the present experiments, the rates of inflow and outflow become equal and are represented by the rate of turnover. Therefore, knowing the rates of turnover and oxidation, the rates of synthesis and breakdown of body proteins can be calculated. However, due to uncertainty regarding the extent of recycling of leucine within tissue proteins, the calculation of rates of protein synthesis and breakdown may not be entirely valid. Nevertheless, it seems reasonable to speculate that the change in plasma leucine turnover reflects a change in tissue protein turnover. If this speculation is valid, the data then indicate that maintenance of tissue protein turnover during caloric restriction is more dependent on dietary protein than either carbohydrate or fat.

In conclusion, our data show that dietary carbohydrate spares body protein during brief caloric restriction whereas dietary fat has no such effect. This observation is supported by the differences in nitrogen excretion, leucine oxidation, and plasma aminogram. Based on these parameters, protein sparing is near maximum when the daily intake of carbohydrate is as little as 300 cal per day, because increasing the intake to 500 cal per day only modestly increased the protein sparing effect. Although subjects receiving the 500-cal protein diet were in positive nitrogen balance, they had alterations in plasma

aminogram and branched-chain amino acid oxidation, which were similar to those in subjects receiving no calories. A discrepancy between parameters of protein nutrition was also observed in subjects receiving the carbohydrate–protein diet. These subjects had a negative nitrogen balance even though their catabolism of branched-chain amino acids was reduced and their plasma aminogram was maintained near normal. Finally, the present data may have application in the design of hypocaloric diets for treating obesity. The data show that substitution of a portion of carbohydrate calories by either fat or protein does not compromise the effect of carbohydrate on conservation of body proteins and branched-chain amino acids, respectively.

Acknowledgments

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