Modulation of Rat Skeletal Muscle Branched-Chain α-Keto Acid Dehydrogenase In Vivo

Effects of Dietary Protein and Meal Consumption

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Abstract

The effects of dietary protein on the activity of skeletal muscle branched-chain α-keto acid dehydrogenase (BCKAD) were investigated. BCKAD is rate-limiting for branched-chain amino acid (BCAA) catabolism by muscle; its activity is modulated by phosphorylation–dephosphorylation. In rats fed an adequate protein (25% casein) diet, BCKAD was ~ 2% active postabsorptively and increased to 10% or 16% active after a 25% or 50% protein meal, respectively. Prolonged feeding of a 50% protein diet increased postabsorptive BCKAD activity to 7% with further increases to 40% active postprandially. On a low protein (9% casein) diet BCKAD remained ~ 2% active regardless of meal-feeding. Dose-dependent activation of BCKAD by intravenous leucine in postabsorptive rats was blunted by a low protein diet. We conclude that excesses of dietary protein enhance the capacity of skeletal muscle to oxidize BCAA, muscle conserves BCAA when protein intake is inadequate, and skeletal muscle may play an important role in whole-body BCAA homeostasis.

Introduction

Chronic consumption of high protein diets results in induction of rate-limiting degrading enzymes for most essential amino acids (1, 2). Hence, the concentrations of most amino acids do not increase markedly in body fluids after long-term high protein feeding and may actually decrease as more protein is consumed (3). When dietary protein intake is restricted to less than the requirement for growth and maintenance these enzymes decrease (1, 2), resulting in conservation of essential amino acids. The catabolic system for the branched-chain amino acids (BCAA)[1]—leucine, isoleucine, and valine—appears to be an exception to this homeostatic scheme for essential amino acids in that (*a*) their concentrations in blood and tissues rise in direct proportion to the protein content of the diet (3–5), and (*b*) BCAA aminotransferase, the initial enzyme in BCAA catabolism, is relatively refractory to changes in dietary protein (6). This apparent lack of adaptation has prompted some investigators to conclude that the body is unable to maintain the free body pools of BCAA by increasing oxidation during times of surfeit or by decreasing oxidation during times of deficiency. However, studies in man and rat have shown that BCAA oxidation in vivo is increased markedly after high protein feeding (5, 7, 8) and is severely depressed after consumption of low protein diets (5, 7). The mechanisms responsible for these alterations in BCAA oxidation are incompletely understood. Recent studies suggest that fluctuations in BCAA oxidation with dietary manipulations cannot be explained based solely on the mass action principle (9). Control of BCAA metabolism by dietary protein is of particular interest, as branched-chain amino and keto acids are being used in the treatment of hepatic encephalopathy and renal insufficiency, conditions in which low protein diets are usually indicated (10, 11).

The first committed reaction in the metabolism of BCAA is catalyzed by branched-chain α-keto acid dehydrogenase (BCKAD), a multienzyme complex located on the inner surface of the inner mitochondrial membrane of most cells (12, 13). BCKAD catalyzes the irreversible oxidative decarboxylation of branched-chain α-keto acids (BCKA)—α-ketoisocaproate (ketoleucine), α-keto-β-methylvalerate (ketoisoleucine), and α-ketoisovalerate (ketovaline)—that are formed from their respective BCAA through the action of BCAA aminotransferase. Both BCKAD and BCAA aminotransferase are distributed ubiquitously but nonuniformly throughout the body. BCKAD activity is highest in liver, kidney, and heart, but very low in skeletal muscle (12, 13). In contrast, BCAA aminotransferase activity is highest in skeletal muscle but very low in liver (12, 13). BCKAD is believed to limit the rate of BCAA catabolism in vivo (14).

BCKAD is unusual among amino acid–degrading enzymes in that it is regulated by a phosphorylation (inactivation)–dephosphorylation (activation) mechanism similar to that which exists for the pyruvate dehydrogenase complex (12, 15). As with pyruvate dehydrogenase, a protein phosphatase and kinase are believed to be associated with BCKAD in vivo. Alteration of the phosphorylation state of BCKAD permits rapid changes in enzyme activity that are not possible via an induction-repression mechanism (13). The physiological significance of BCKAD modulation by phosphorylation is currently an active area of study (16).

In normally fed rats, BCKAD from liver is essentially fully active (i.e., dephosphorylated) (13, 17–19). In contrast, < 20% of skeletal muscle BCKAD is in the active form (12, 13, 18, 20, 21). As skeletal muscle comprises ~ 40% of the rat's body weight (22), changes in the phosphorylation state of muscle BCKAD

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1. *Abbreviations used in this paper:* BCAA, branched-chain amino acid; BCKA, branched-chain α-keto acid; BCKAD, branched-chain α-keto acid dehydrogenase.

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could have profound effects on BCAA metabolism in vivo. However, efforts to detect changes in the activity state of muscle BCKAD in response to physiological manipulations have been largely unsuccessful (13, 20, 23). In view of the high BCAA aminotransferase and low BCKAD activity of muscles, it was proposed that muscle may primarily deaminate BCAA and release BCKA into the circulation for eventual oxidation by liver where BCKAD is found in abundance (12, 13, 24). Uncertainty about the role of muscle in BCAA metabolism has stemmed in part from the difficulty in measuring activities of BCKAD from solubilized muscle preparations (20, 25–27). Therefore, intact muscle mitochondria were used in most studies to assay BCKAD activity (13, 18, 23, 26). Problems associated with substrate transport, quantitation of precursor-specific radioactivity, and possible activation of the enzyme during mitochondrial isolation or incubation complicate interpretation of such studies. Recently, we described an assay for measuring BCKAD in solubilized rat muscle tissue and showed that the enzyme was activated in vivo after intravenous leucine administration (21). We have used this assay in the present study and found that muscle BCKAD is activated rapidly in vivo after consumption of meals containing adequate-to-high levels of protein, and that the activation state of the enzyme is responsive both acutely and chronically to the protein content of the diet.

Methods

Animals. Male Wistar (WI) BR rats (Charles River Laboratories, Wilmington, MA), initially weighing 140–160 g, were used in all studies. Rats were kept in a room maintained at 24°C with a reverse 12-h-light–12-h-dark cycle. The dark period was from 3:00 a.m. to 3:00 p.m. Rats were offered their daily allotment of food within the last 6 h of the dark cycle (9:00 a.m.–3:00 p.m.). This meal-feeding regimen minimized variations in enzyme and metabolite measurements due to fluctuations in food intake (3). Water was supplied ad libitum.

Diets and treatments. All diets were specially prepared in pellet form by United States Biochemical Corp., Cleveland, OH, and contained in percentage by weight: Rogers and Harper's mineral mix, 5 (28); AIN 76 vitamin mix, 1; corn oil, 5; choline chloride, 0.2; vitamin-free casein, 0, 9, 25, or 50; and equal amounts of glucose monohydrate (A. E. Staley Manufacturing Co., Oak Brook, IL) and cornstarch (American Maize, Hammond, IN) to make up 100%. The 9% casein diet was supplemented with methionine, 0.3% (wt/wt) (29). Rats were acclimated to the 25% casein diet ad libitum for 2 d, then trained to consume the 25% casein diet during the last 6 h of the dark cycle for 2 wk (meal-feeding). After this training period, both chronic and acute effects of dietary protein on BCAA metabolism were studied. In the chronic study, rats were meal-fed the 0, 9, 25, or 50% casein diets for 10 d. Body weights were measured each day. On the 10th day, rats were killed before or 3 h after presentation of the meal. Previous studies have shown that circulating levels of BCAA are greatest 3 h postprandially in meal-fed rats (29). In the acute studies, rats previously fed the 25% casein diet for 2 wk were offered a single meal containing 9, 25, or 50% casein. Rats failed to consume a single meal of the 0% casein diet, hence this group was omitted. Rats were killed before and 3, 6, and 12 h after presentation of the meal.

Previous studies (3, 29, 30) have shown that during the first few days of offering an adequate protein diet for 6–8 h/d (meal-feeding), food consumption is less than that of ad libitum–fed rats. However, food intake increases gradually and after 1 wk, meal-fed and ad libitum–fed rats consume equal amounts of food. Rats maintained chronically on casein diets similar to those used here consume equal amounts of food except for those given a protein-free diet, which consume 43% less (30). In the present study, food intake was measured in the acute experiments and was not significantly different between rats fed 9, 25, or 50% casein diets (14±1 g/meal).

In a separate experiment, the effects of leucine infusions on muscle BCKAD activity were investigated in rats fed 25 or 9% casein diets ad libitum for 2 wk. Rats were anesthetized with methoxyflurane (Metofane) after an overnight fast (21) and L-leucine was infused via the tail vein at doses of 0, 10, 25, 50, and 100 μmol/100 g body weight. Muscles were excised 15 min after injections.

BCKAD assay. Our method for determining BCKAD activity in muscle has been described in detail previously (21, 31). Briefly, the method involved freeze-clamping whole muscle in situ. BCKAD was extracted from powdered frozen muscle in the presence of protease, phosphatase, and kinase inhibitors and then precipitated with polyethylene glycol. Basal (in vivo) BCKAD activities were determined immediately after redissolving the precipitated proteins, and total (fully active) enzyme activities were measured after preincubation of the extract at 37°C with 15 mM Mg^{2+} before assay. In these studies BCKAD activity was determined by $^{14}CO_2$ release from 0.1 mM α-keto[1-^{14}C]isovalerate at 37°C. Percentage of BCKAD in the active form was calculated as the ratio of basal to total BCKAD activities.

Amino acids, protein, and DNA. Heparinized plasma was deproteinized by adding ice cold sulfosalicylic acid (0.1 vol of a 50% wt/vol solution). After standing 20 min on ice, samples were centrifuged at 30,000 *g* for 10 min. Muscle samples were deproteinized by adding 4 vol of ice cold 5.9% wt/vol sulfosalicylic acid to powdered muscle samples. Muscles were then homogenized with a Polytron tissue homogenizer and left on ice for 20 min. The mixture was then centrifuged at 30,000 *g* at 4°C for 10 min. Plasma and muscle BCAA concentrations in deproteinized samples were measured using reversed-phase high performance liquid chromatography separation of the *o*-phthalaldehyde derivatives and fluorescent detection (32). BCAA concentrations (μmol/g) were expressed per milliliter of intracellular water assuming that muscle contains 80% water: 60% intracellular and 20% extracellular, the latter at equilibrium with plasma (33).

Muscle protein concentrations were determined according to the method of Lowry (34) with bovine serum albumin as the standard. Muscle DNA concentrations were measured using a modification of the Burton assay (35) with herring sperm DNA as the standard.

Statistical analyses of differences between groups were performed by Student's *t* test or by the Peritz multiple range F test (36) and were

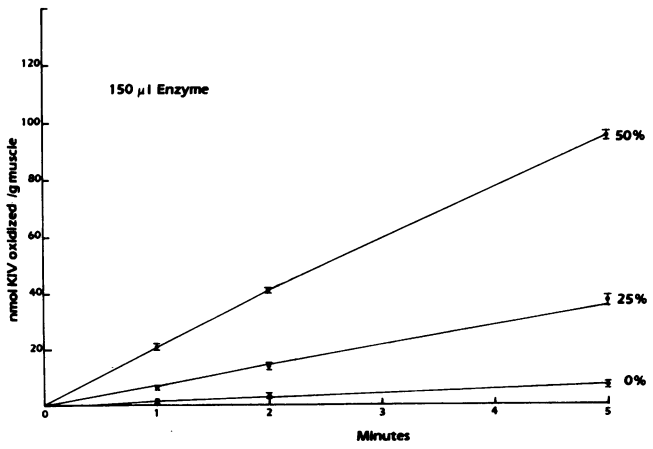

Figure 1. Linearity of basal muscle BCKAD from rats fed differing levels of protein. Rats were meal-fed diets containing 0, 25, or 50% casein for ~ 2 wk. Muscles were frozen in situ after 3 h of feeding. Basal BCKAD activity was measured for 1, 2, or 5 min using 150 μl of enzyme extract, as described in Methods. Values represent means±SEM for enzyme activities from 4 rats per diet group. Identical values were obtained when 100 μl of enzyme extract was used (data not shown).

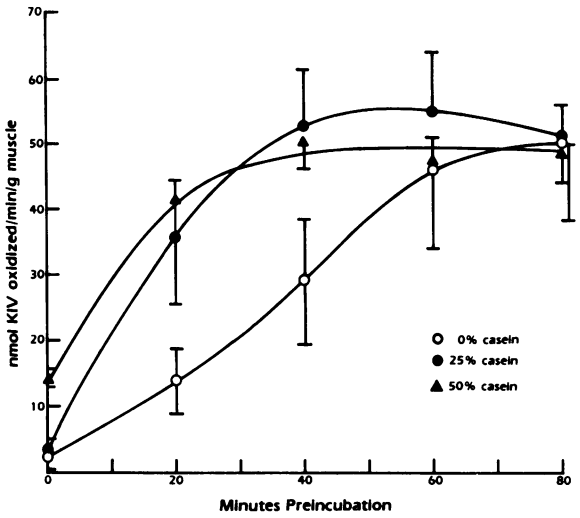

Figure 2. Effect of preincubation time on activation of muscle BCKAD isolated from rats fed differing levels of protein. Rats were treated as described in Fig. 1. Total BCKAD activity was measured as described in Methods. Enzyme activities were measured after 20, 40, 60, or 80 min preincubation with 15 mM Mg^{2+}. Values represent means±SEM for enzyme activities from 3–4 rats per diet group.

considered significant if $P < 0.05$. Values for percent active complex were subjected to the inverse sine transformation before statistical analysis as percentages are distributed binomially rather than normally (37). This transformation is especially recommended for values in the range of most of the present observations (0–20%). Regression analysis was performed by the linear least squares method and slopes compared by Student's t test (37). All values shown are means±SEM.

Results

BCKAD assay. As others have shown diet-mediated changes in BCKAD activities from liver, kidney, and heart (12, 13, 17, 19, 20), we first sought to optimize the assay for BCKAD isolated from muscles of rats fed differing levels of protein. Rats were meal-fed 0, 25 (normal), or 50% (high) casein diets for ~ 2 wk. Muscles were excised 3 h after presentation of the meal. As shown in Fig. 1, basal (in vivo) BCKAD activity increased as the protein content of the diet increased. The assay for basal BCKAD activity was linear up to 5 min in all diet groups, whether 100 or 150 μl of muscle extract was used. To ensure that no more than 10% of α-ketoisovalerate was utilized during the reaction (38), basal activities from 0 and 9% casein groups were routinely assayed for 5 min with 150 μl of extract. Basal BCKAD activities from 25 and 50% casein groups were assayed for 2–5 min with 100 μl of extract. Total BCKAD activities were linear up to 3 min in all diet groups; therefore, total activities were routinely assayed over 1 min (21, 31).

Recently, Randle and associates have shown that BCKAD from liver of rats fed low protein diets is refractory to dephosphorylation (39). To investigate whether dietary protein might affect the rate of muscle BCKAD activation, total BCKAD activities were measured 20, 40, 60, and 80 min after preincubation at 37°C in a buffer containing 15 mM Mg^{2+}. The time required to attain maximal BCKAD activities in muscle extracts from rats fed diets containing 0, 25, or 50% casein are shown in Fig. 2. Maximal activities for rats fed the 25 or 50% casein diets were obtained after 40 min of preincubation. In contrast, maximal BCKAD activities in muscles of rats fed the 0% casein diet were not obtained until 60 min of preincubation. This lag phase of muscle BCKAD activation was also observed in rats fed the 9% casein diet (data not shown). Fully activated (total) enzyme activities (nmol/min per g tissue) were not significantly different among the groups.

Chronic effects of dietary protein. Rats fed the protein-free diet failed to thrive and lost ~ 40 g of weight during the 10-d study (Table I). Addition of 9, 25, or 50% casein to the diet resulted in growth rates of 4–6 g/d. Rats fed the 9% casein diet attained 60% of the weight gain observed in rats fed the 25% casein diet. Continued growth on the 9% casein diet was most probably due to its supplementation with methionine, the first limiting amino acid in casein; hence this diet constituted a protein restricted rather than an essential amino acid deficient diet. The changes in growth due to dietary protein were accompanied by alterations in the protein and DNA concentrations in skeletal muscle (Table I). The protein/DNA ratio, a measure of cell size, was significantly depressed when protein was omitted from the

Table I. Effects of Dietary Protein on Total Muscle BCKAD Activities Expressed on a Wet Weight, Protein, or DNA Basis

				Total BCKAD activity		
Casein	Body weight change	DNA	Protein	Tissue	DNA	Protein
%	*g/10 d*	*μg/g*	*mg/g*	*nmol/min/g*	*nmol/min/mg*	*nmol/min/g*
0	−41±2*	537±16*	174±7*	64±5*	120±10*	368±23*‡
9	+40±4‡	448±15‡	188±12*‡	63±6*	140±9*	332±14*‡
25	+64±2§	466±13‡	222±11‡	63±5*	135±14*	304±35*
50	+59±2§	432±16‡	200±10*‡	78±4*	185±15‡	406±35‡

Rats were trained to meal-feeding on a 25% casein diet as described in Methods, then changed to diets with varying casein content as shown for a period of 10 d. Starting weights were not significantly different among rats fed the four diets (205±1 g; $n = 64$). DNA, protein, and total BCKAD activities expressed per gram of tissue were unaltered 3 h after feeding. Therefore, postabsorptive and postprandial values for each diet group were pooled. Numbers represent means±SEM for $n = 13$ (0% casein), 15 (9 and 50% casein), or 21 (25% casein) rats/group. DNA, protein, and total BCKAD activities were measured as described in Methods. Values in vertical columns with different superscript symbols are significantly different ($P < 0.05$).

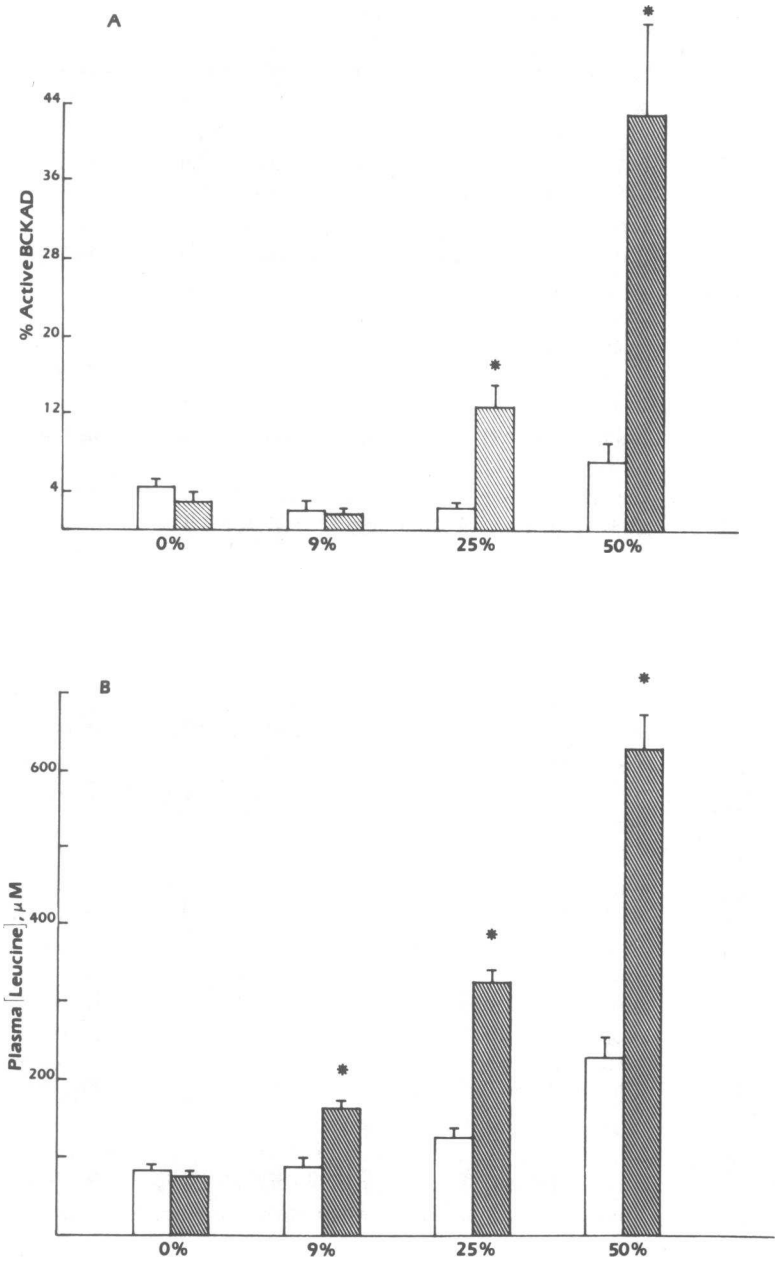

Figure 3. Chronic effects of dietary protein on the percentage of active muscle BCKAD and plasma concentration of leucine. Rats were meal-fed diets containing 0, 9, 25, or 50% casein for 10 d. On the 10th day, rats were killed before (*open bars*) or 3 h after (*hatched bars*) presentation of the meal. All postprandial rats had food in their stomachs at the time of sacrifice. Muscle BCKAD activity was measured as described in Methods and the percent active complex (*A*) computed as basal (in vivo) activity divided by total (fully active) activity × 100. Total BCKAD activities for each diet group are given in Table I. Plasma leucine concentrations (*B*) were measured as described in Methods. Before meal values represent means±SEM for $n = 5$ (0, 9, 50% casein) or 11 (25% casein) rats per group. After-meal values represent means±SEM for 8–10 rats per group. $^*P < 0.01$ compared with before meal values.

diet (g protein/g DNA = 336±16, 418±16, 458±18, and 464±17 for rats fed 0, 9, 25, or 50% casein respectively).

The effects of consuming 0, 9, 25, or 50% casein diets for 10 d on the percentage of active BCKAD in muscle are shown in Fig. 3 *A*. Measurements were made before (postabsorptive) or 3 h after (postprandial) presentation of the meal. All postprandial rats had food in their stomachs at sacrifice. Compared with postabsorptive values, consumption of the 0 or 9% casein diets had no significant effect on the activity state of the complex. In contrast, the activity state of BCKAD increased markedly (approximately sevenfold) after consumption of the 25 or 50% casein diets. The postabsorptive BCKAD activity was identical in rats fed 9 or 25% casein; however the postprandial activity was 80% lower in the former group. Consumption of the diet containing 50% casein resulted in greater than threefold increases in both postabsorptive and postprandial BCKAD activities when compared with the group fed the normal protein diet (25% casein). The postabsorptive BCKAD activity in rats fed 50% casein was significantly higher than the activities in the 9 and 25% casein-fed rats. Interestingly, consumption of a protein-free diet resulted in significantly higher postabsorptive activities than observed in the 9 and 25% casein groups.

The effects of dietary protein on total BCKAD activity (an estimate of enzyme content) are shown in Table I. Total BCKAD activity was not significantly different in rats fed the 25, 9, or 0% casein diets. In contrast, consumption of high levels of protein resulted in increases in total BCKAD activity when expressed per milligram DNA or per gram protein (Table I). Hence, the apparent amount of BCKAD complex per muscle cell increased ~ 35% after feeding a high protein diet for 2 wk.

We have recently demonstrated that infusions of leucine result in activation of rat muscle BCKAD (21). Therefore, plasma

BCAA concentrations were measured in the present study. As shown in Fig. 3 *B*, the concentration of plasma leucine increased in direct proportion to the protein content of the diet. In general, the increases in plasma leucine paralleled the changes in the activation state of the complex ($r = 0.781$; $n = 62$; $P < 0.001$). Similar relationships were observed between the activity state of BCKAD and the plasma concentrations of isoleucine or valine ($r = 0.783$ for both; $n = 62$; $P < 0.001$). However, a causal relationship between these amino acids and the activity of the complex is unlikely as we have shown previously that infusions of equimolar amounts of valine or isoleucine fail to activate BCKAD (21). The intracellular concentrations of leucine were measured in muscles of some rats before and after consuming the four diets (Table II). Intracellular leucine concentrations were significantly correlated ($r = 0.498$; $n = 40$; $P < 0.01$) with the activity of muscle BCKAD. The intracellular concentrations of leucine increased postprandially in rats fed 9, 25, or 50% casein, but not in rats fed the protein-free diet. The postabsorptive intracellular leucine concentrations were similar among the groups fed 0, 9, or 25% casein diets, but were increased in the group fed the 50% casein diet. The postprandial intracellular leucine concentrations were significantly higher in the groups fed 25 and 50% casein than in those fed 0% casein. Meal consumption had no significant effect on the ratio of leucine concentrations between muscle and plasma in any of the diet groups. The highest muscle/plasma ratios were observed in the group fed the protein-free diet. Postprandially this ratio was significantly higher in the 0% casein group than in any of the groups fed protein containing meals.

Acute effects of dietary protein. To examine the effects of acute protein consumption on muscle BCKAD, rats previously fed a 25% casein diet for 2 wk were offered a single meal of 9, 25, or 50% casein. No differences in food intake were observed between the three groups. As shown in Fig. 4 *A*, consumption of the 9% casein meal had no effect on muscle BCKAD activity 3, 6, or 12 h after feeding. In contrast, consumption of the 25 and 50% casein meals resulted in 6- and 11-fold increases in the percentage of active complex 3 h after presentation of the meal (postabsorptive, 1.6±0.1% active; postprandial, 9.4±2.0 and 17.2±3.6% active, respectively). By 6 h, the proportion of BCKAD in the active form in rats offered the 25% casein meal returned towards baseline and changed little after 12 h. The percentage of active complex in rats offered the 50% casein meal decreased in a linear fashion between 3 and 12 h after presentation of the meal. By 12 h, BCKAD activity in the high protein group was still 50% greater than baseline.

The effect of consuming single meals of varying protein content on the plasma concentrations of leucine is illustrated in Fig. 4 *B*. A strong correlation ($r = 0.852$; $n = 45$; $P < 0.001$) was observed between the percentage of active BCKAD and the concentration of leucine in plasma. Significant correlations were also observed between the activity of BCKAD and the plasma concentrations of isoleucine ($r = 0.854$; $n = 45$; $P < 0.001$) and valine ($r = 0.906$; $n = 45$; $P < 0.001$).

The relationship between the percentage of active BCKAD and circulating leucine in both the chronic and acute studies is shown in Fig. 5. Linear regression analysis between percent active complex and plasma leucine concentration resulted in a significant direct correlation between these values ($r = 0.788$, $n = 107$, $P < 0.001$). A better linear least squares fit was obtained by semilog transformation of the data (i.e., log percent active complex vs. plasma leucine concentration) which gave a correlation coefficient of 0.848 ($P < 0.001$). The solid line in Fig. 5 represents the best fit line for the semilog transformation.

Leucine infusion studies. In rats fed a 9% casein diet for 10 d, consumption of the meal resulted in an approximate doubling in plasma and intracellular leucine without a change in the activity of the complex (compare Fig. 3 *A* and *B* and Table II). Also, the enzyme from rats fed protein-free or low-protein diets required a longer period of incubation to attain maximal activity (Fig. 2). To study the apparent refractoriness of the enzyme from low protein-fed rats to activation, rats previously fed adequate (25% casein) or low (9% casein) protein diets for ~ 2 wk were infused with varying doses of leucine (Fig. 6). BCKAD activity was closely correlated with the dose of leucine infused ($r = 0.92$ for each diet group; $P < 0.001$) and leucine infusions at doses of 25 μmol/100 g body weight or greater resulted in activation of muscle BCKAD in both diet groups (Fig. 6 *A*). However, the leucine-mediated activation of muscle BCKAD from low protein-fed rats was less than that observed in rats fed adequate amounts of protein ($P < 0.001$). Plasma BCAA concentrations were essentially identical in the two groups (Fig. 6 *B* and *C*).

Discussion

The body pools of most amino acids are enlarged in animals immediately after they have consumed a high protein diet (1–3). Continued consumption of amino acids in quantities greatly in excess of requirements would result in death if no homeostatic controls were present. The relatively high Michaelis constants of amino acid–degrading enzymes enable the rate of oxidation of surplus amino acids to increase initially as the result of increased substrate concentrations (1). However, if a high influx of amino acids continues, the organism synthesizes more of many of the rate-limiting amino acid–degrading enzymes found in liver. Prominent among these inductions are serine-threonine

Table II. Effects of Dietary Protein on Muscle Intracellular Leucine Concentrations and Intracellular Muscle/Plasma Leucine Ratios before or after the Meal

	Intracellular leucine concentration*		Muscle/plasma leucine	
Casein	Before	After	Before	After
%	*nmol/ml*	*nmol/ml*	*nmol/ml*	*nmol/ml*
0	134±24‡	106±16‡	1.61±0.26‡	1.64±0.35‡
9	94±10‡	162±8‡§	1.25±0.31‡	1.02±0.13
25	128±24‡	300±17§	1.00±0.12	0.95±0.03
50	244±53	371±52§	1.02±0.14	0.70±0.07

Casein diets were fed for 10 d and values listed are from before or three hours after presentation of the meal. Plasma and muscle leucine concentrations were determined as described in Methods. Values are means±SEM for 5 rats per group.

* Intracellular concentrations of leucine were calculated according to the formula: intracellular [leucine] = nmol leucine/g muscle − (0.2)(nmol leucine/ml plasma)/0.6.

‡ Significantly different ($P < 0.05$) from values in column without ‡.

§ $P < 0.05$ compared with before-meal values.

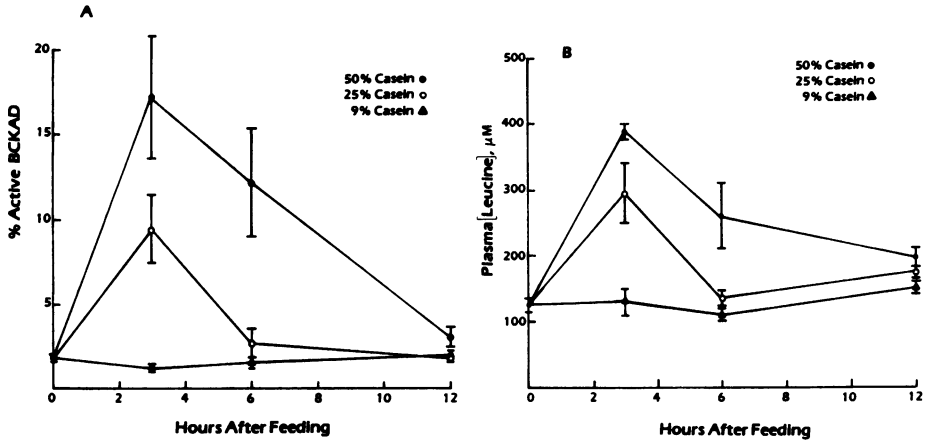

Figure 4. Effects of single meals of varying protein content on muscle BCKAD and plasma concentration of leucine. Rats were meal-fed a diet containing adequate amounts of protein (25% casein) for ~ 2 wk, then killed before or 3, 6, or 12 h after presentation of meals containing 9, 25, or 50% casein. Food was withdrawn 3 h after presentation. All postprandial rats had food in their stomachs after 3 h and no difference in food intake was observed (14±1 g consumed; n = 15 rats). Muscle BCKAD activity (*A*) and plasma leucine concentrations (*B*) were measured as described in Methods. Values represent means±SEM for 4–5 rats per group.

dehydratase, histidase, tryptophan pyrrolase, tyrosine-α-ketoglutarate aminotransferase, glutamate-oxaloacetate and glutamate-pyruvate aminotransferases, and all the urea cycle enzymes (reviewed in 1, 2, 40, 41). Hence, excesses of amino acids are catabolized and in the process, essential precursors for gluconeogenesis, i.e., pyruvate and tricarboxylic acid cycle intermediates, are formed. A similar control mechanism for BCAA is believed to be absent (1). Reports of BCAA aminotransferase induction by diet are inconsistent and the observed effects are relatively small compared to other amino acid–degrading enzymes (1, 13). Yet, BCAA oxidation in vivo is increased markedly after high-protein feeding (5, 7, 8). In rats adapted to a high-protein diet, the protein content of the body does not increase, and BCAA excretion in urine and feces is negligible (2). Based on these considerations, Harper and coworkers (13, 30) calculated that when rats are switched from a 20 to 50% protein diet, 12,000 μmol BCAA are available for degradation, whereas the increase of BCAA in bodily fluids is only ~ 100 μmol. Hence, extensive oxidation of BCAA must be occurring. In the present study, a small increase in total BCKAD activity per milligram DNA was observed in rats after feeding a high-protein diet for 2 wk. This may represent stimulation of enzyme synthesis and/or decreased degradation of the complex. However, the major effect of protein intake was exerted at the level of enzyme activation.

BCKAD activity is thought to be the major regulator of BCAA oxidation in vivo (14). As liver BCKAD is fully active in normally fed rats (13, 17–19), activation of extrahepatic BCKAD would be expected to result in enhanced oxidation of BCAA in vivo. In the present study, skeletal muscle BCKAD was only 1–2% active during the postabsorptive period in rats fed 25% casein (Figs. 3 *A* and 4 *A*). Increasing the casein content of the diet from 25 to 50% for 10 d resulted in a greater than three-fold increase in the percentage of active muscle BCKAD measured either before or 3 h after feeding (Fig. 3 *A*). Furthermore, compared with postabsorptive values, consumption of the 25 or 50% casein diet resulted in a greater than seven-fold activation of muscle BCKAD. The proportion of BCKAD in the active form rose from 2% in postabsorptive rats fed adequate amounts of protein to > 40% in rats 3 h after consuming a high-protein diet. That a single normal or high-protein meal could also activate muscle BCKAD is shown in Fig. 4 *A;* the duration and magnitude of activation was proportional to the protein content of the diet.

Considering all available data, we observed a strong correlation between circulating leucine and the activity of muscle BCKAD. Inspection of the points in Fig. 5 suggests that a threshold phenomenon exists at leucine concentrations < 0.2 mM with a fairly steep slope for activation of muscle BCKAD at leucine concentrations > 0.2 mM. At low circulating leucine concentrations, liver may therefore assume greater importance in BCAA homeostasis, but as circulating BCAA and muscle BCKAD activity increase the latter may become the predominant tissue in BCAA metabolism. The correlation between plasma leucine and muscle BCKAD activity (21) is most simply explained by inhibition of BCKAD kinase by α-ketoisocaproate (12), the transamination product of leucine. In studies of rats it was found that the concentrations of BCKA in muscle paralleled those of the BCAA in rats fed low, normal, or high-protein diets (42), and plasma α-KIC was found to increase in man after a protein meal (43). It is clear however that this simple explanation for the relationship between muscle BKCAD and plasma leucine is inadequate. Despite similar circulating leucine concentrations,

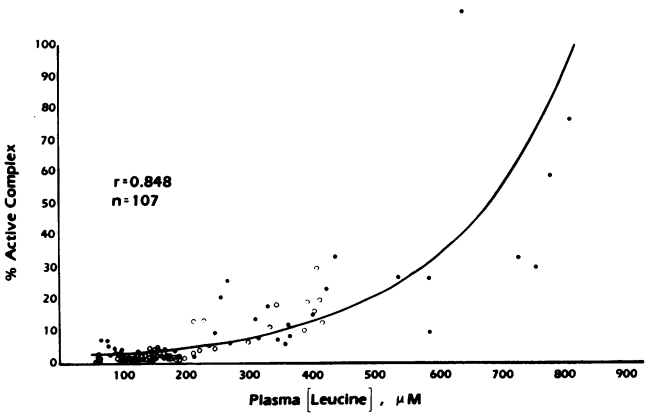

Figure 5. Relationship of skeletal muscle BCKAD activity to the concentration of circulating leucine. Values from the chronic (*closed circles*) and acute (*open circles*) protein studies were taken from Figs. 3 and 4 respectively. The curve shown was calculated from the best linear least squares fit, which was obtained after semilog transformation of the data (i.e., log % active complex vs. plasma leucine) and yielded a linear correlation coefficient of 0.848 ($P < 0.001$; n = 107). Analysis of the nontransformed data by linear regression yielded a correlation coefficient of 0.788 ($P < 0.001$; n = 107).

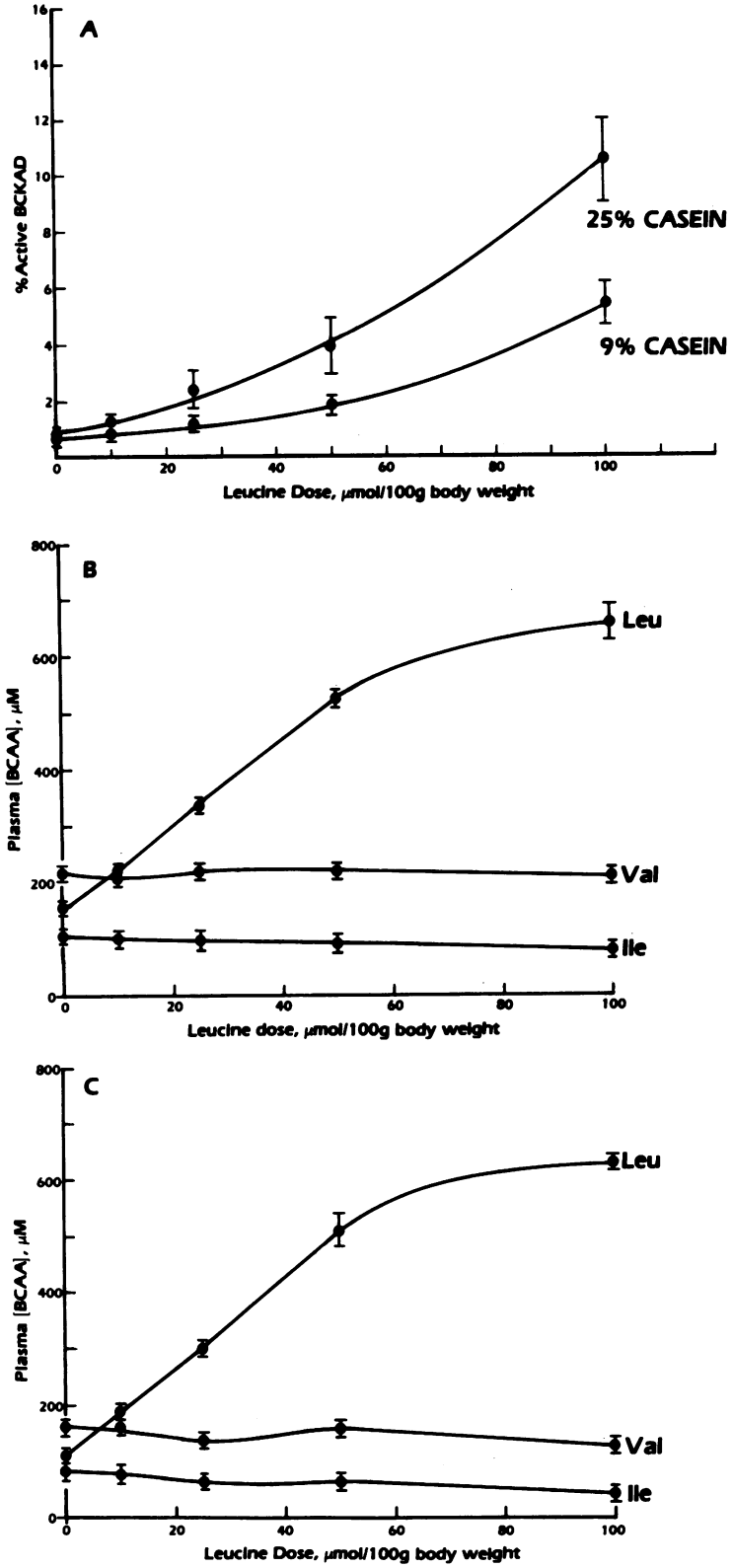

Figure 6. Leucine-mediated activation of muscle BCKAD isolated from rats fed adequate or low protein diets. Rats were fed a 9 or 25% casein diet ad libitum for ~ 2 wk. After an overnight fast, rats were infused with saline or 10, 25, 50, or 100 μmol L-leucine per 100 g body weight via the tail vein. Rats were killed 15 min after infusion and the percentage of BCKAD in the active state (*A*) and plasma BCAA concentrations (*B*, 25% casein, and *C*, 9% casein) were measured as described in Methods. Values represent means±SEM for 4 rats per group. BCKAD activation in response to leucine was significantly depressed in rats fed the low-protein diet ($P < 0.01$ by t test after injection of 50 or 100 μmol leucine/100 g; $P < 0.001$, by comparison of the slopes of the linear regression lines for leucine dose vs. BCKAD activity).

muscle BCKAD in rats fed a 9% protein diet activated less than BCKAD from rats fed a 25% protein diet (Fig. 6). Futhermore, meal-induced activation of BCKAD was greater than that observed after leucine infusions achieving similar plasma leucine concentrations (compare Figs. 4 and 6). The two studies are not directly comparable however because the duration of hyperleucinemia before sacrifice was much longer in the former experiments. It is also noteworthy that the postprandial activation of BCKAD by a high-protein meal (40% active) was ~ 2.5 times greater in rats maintained on a high-protein diet than in those maintained on a normal diet although plasma leucine was only 50% higher in the former group after consumption of the protein

meal (compare Figs. 3 and 4). It seems unlikely that meal-induced insulin release played a role in the postprandial activation of BCKAD, as treating postabsorptive rats with large doses of insulin and glucose did not activate the complex in muscle (31). Furthermore, in perfused hindlimbs of postabsorptive rats, insulin added to the perfusate decreased flux through BCKAD (44).

Despite a marked activation of muscle BCKAD in rats fed a high protein diet, plasma and tissue concentrations of BCAA remained elevated (Figs. 3 and 4, Table II). The BCAA aminotransferase reaction is readily reversible and indeed 80–90% of leucine transaminated to α-ketoisocaproate is reaminated to leucine in humans in vivo (14). High levels of tissue glutamate in animals fed high-protein diets would favor maintenance of elevated BCAA by restraining their transamination and favoring reamination of BCKA (13, 45). The physiological advantage of maintaining enlarged BCAA pools during high-protein feeding is unknown. Elevated BCAA concentrations in blood could act as a protective buffer, competing with other large neutral amino acids (e.g., tyrosine, tryptophan, and phenylalanine) for transport across the blood-brain barrier (3, 46), thus preventing increased synthesis of certain neurotransmitters (47, 48). The toxic effects of elevated brain aromatic amino acids are well documented (49). BCAA have been used clinically to reduce the levels of brain aromatic amino acids in hepatic encephalopathy (10) and phenylketonuria (50).

Consumption of protein-deficient diets results in inactivation of liver BCKAD (17, 19, 20, 39). Recently, increased BCKAD kinase activity was reported in livers and hearts of rats fed a protein-free diet (51). The decrease in the percentage of active complex in livers of rats fed low-protein diets presumably represents a mechanism by which essential BCAA are conserved during periods of protein depletion. Indeed, BCAA oxidation is severely depressed in man and rat after low-protein feeding (5, 7, 52–55). In the present study, switching from an adequate to a low-protein diet for 10 d had no significant effect on muscle BCKAD activity in the postabsorptive state (Fig. 3 *A*). This is not surprising as most of the complex was already in an inactive form in postabsorptive rats fed the adequate protein diet (i.e., 2% active). However, the following observations were of interest: (*a*) muscle BCKAD from rats fed protein-free or low-protein diets required a longer period of preincubation with Mg^{2+} to attain maximal activity than BCKAD from rats fed adequate protein diets (Fig. 2), and (*b*) plasma leucine concentrations increased approximately two-fold after consumption of the low-protein diet, but muscle BCKAD activity remained unchanged (Fig. 3). To further investigate these differences, varying amounts of leucine were infused into rats fed adequate or low-protein diets. Despite similar circulating leucine concentrations, the complex from rats fed the low-protein diet was less responsive to leucine activation than the enzyme isolated from rats given adequate amounts of protein (Fig. 6). The refractoriness of muscle BCKAD to activation in rats fed low-protein diets may be of physiological significance. An activation-resistant complex in muscle from low-protein–fed rats, along with an inactivated complex in liver, would ensure efficient conservation of BCAA during periods of protein depletion. The mechanism of this resistance is not clear. In view of the findings of Espinal et al. (51), increased skeletal muscle BCKAD kinase activity in response to protein depletion is an attractive hypothesis.

We have no explanation for the increased basal BCKAD activity observed in postabsorptive rats maintained on the protein-free diet (Fig. 3). These animals were in a protein-catabolic state, but intracellular leucine concentrations were not significantly different from the values in the 9 and 25% casein groups (Table II). The apparent increase in the muscle/plasma leucine ratio in this group (Table II) may be a reflection of accelerated net degradation of muscle protein. α-Ketoisocaproate is compartmentalized in muscle cells (56); whether intramitochondrial α-ketoisocaproate is increased in muscles of protein-starved rats or if other mechanisms lead to the relatively modest activation of the complex in the postabsorptive state is not known.

Our studies were carried out in rats trained to consume their daily caloric requirements during a relatively short period of time. Because rats are predominantly nocturnal "nibblers" rather than "meal eaters," the postprandial activation of BCKAD may be less marked when normal feeding patterns are maintained. In rats fed a normal diet ad libitum the plasma leucine concentration cycles between a nadir of 170 μM at 6:00 p.m. and a peak of 275 μM at 6:00 a.m. (57). Man however is a meal eater; following a protein meal most of the absorbed amino acids (except for the BCAA) are taken up by the liver, while a large fraction of BCAA is taken up by muscle (58). Because BCAA concentrations in muscle generally parallel plasma concentrations (reviewed in 13) and the latter increase two- to three-fold after a protein meal in man (7, 43, 58), muscle BCKAD may be activated and likely contributes to the postprandial increase in BCAA oxidation (7). Our studies in rats may have practical implications for nutritional therapy in some patients. Distribution or presentation of dietary protein in a manner to avoid large increases in circulating BCAA, or slowing the rate and extending the duration of amino acid infusions, may prevent or decrease activation of muscle BCKAD and spare BCAA for protein synthesis.

Our data support an active role for skeletal muscle in the disposal and conservation of BCAA. Muscle releases BCKA to the blood stream for eventual uptake and oxidation by the liver (13, 24). In addition, during periods of protein surfeit, the capacity to oxidize BCAA carbon increases markedly in skeletal muscle, whereas during periods of protein inadequacy, skeletal muscle, along with liver, adapts to conserve essential BCAA.

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