Dietary Protein Level Regulates Expression of the Mitochondrial Branched-Chain Aminotransferase in Rats¹,²

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ABSTRACT The first step in the degradation of branched-chain amino acids (BCAA) is transamination catalyzed by the branched-chain aminotransferase (BCAT), which is located in extrahepatic tissues. Studies of the effect of dietary protein on BCAT activity have given contradictory results. Therefore, we established the levels of BCAT activity and mitochondrial BCAT (BCATm) mRNA expression in different organs and tissues of rats. We then determined the effect of different levels of dietary protein in well-nourished rats, the effect of feeding a 0.5% casein diet for 5 wk (protein-malnourished rats) and nutritional rehabilitation of these rats with different levels of dietary protein on BCAT activity and BCATm mRNA expression in selected tissues. Finally, the response of tissue BCAT activity and BCATm mRNA levels in rats fed a 10% casein diet and injected with glucagon (4 d) or hydrocortisone (7 d) was determined. The highest concentration of BCATm mRNA was found in stomach, followed by kidney, heart, muscle, brain, skin and lung. Levels were found in intestine, and no BCATm mRNA was detectable in liver. Although BCAT activity was significantly higher in muscle, kidney and brain from rats adapted to consume a 50% casein diet for 7 h/d for 10 d than in rats fed 6, 18 or 35% casein diets, only muscle had significantly higher levels of BCATm mRNA. In protein-malnourished rats, BCAT activity and BCATm mRNA expression in kidney, muscle and heart were not different from those of rats with free access to an 18% casein diet. Nutritional rehabilitation of the protein-malnourished rats with 50% casein for 21 d significantly increased the BCAT activity and BCATm mRNA expression in muscle. Neither hydrocortisone nor glucagon injection affected BCAT activity or BCATm mRNA concentrations in rat kidney, muscle or heart. We conclude that the nutritional regulation of BCATm is extrahepatic, tissue specific and may involve transcriptional and post-translational mechanisms. J. Nutr. 128: 1368–1375, 1998.

KEY WORDS: • branched-chain aminotransferase • hormones • malnutrition • protein • rats

The first step in the degradation of the branched-chain amino acids (BCAA),³ leucine, valine and isoleucine, is transamination of BCAA catalyzed by the branched-chain aminotransferase (BCAT; EC 2.6.1.42). This enzyme can accept an amino group from any of the three branched-chain amino acids and transfer it to α-ketoglutarate or any of the three corresponding branched-chain α-keto acids. The branched-chain α-keto acids can be reaminated in the mitochondria to their respective amino acids (Hutson et al. 1988, Torres et al. 1993) or irreversibly decarboxylated by the branched-chain α-keto acid dehydrogenase enzyme complex. The decarboxylation products are isovaleryl-CoA, α-methylbutyryl-CoA and isobutyryl-CoA for valine, isoleucine and leucine, respectively.

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⁴ Abbreviations used: BCAA, branched-chain amino acids; BCAT, branched-chain aminotransferase; BCATm, mitochondrial branched-chain aminotransferase; BCATc, cytosolic branched-chain aminotransferase; KIC, α-ketoisocaprate; MOPS, 3-4-morpholino)propanesulfonic acid.

The BCAT isoenzymes, which are separate gene products (Bledsoe et al. 1997, Hutson et al. 1995), are specific for the three branched-chain amino acids (Hall et al. 1993, Ichihara and Koyama 1966, Taylor and Jenkins 1966) and are distributed in the cytosol (BCATc) or the mitochondria (BCATm) (Hutson 1988) with the highest specific activity in pancreas, followed by stomach (Ichihara 1975), lactating mammary gland (Cappuccino et al. 1978, De Santiago et al. 1998), heart, kidney, brain and skeletal muscle (Hutson 1988, Shinnick and Harper 1976). Adult rat liver does not contain either BCAT isoenzyme (Hall et al. 1993, Hutson et al. 1992).

The unique feature of the metabolism of BCAA in animals is its tissue specificity; most indispensable amino acids are degraded in the liver, whereas BCAA are metabolized extensively in extrahepatic tissues, where they are transported across the plasma membrane by a large neutral amino acid carrier system in almost all tissues including heart (Torres et al. 1995, Tovar et al. 1992), muscle (Tovar et al. 1991) and brain (Tovar et al. 1988). This characteristic pattern of BCAA catabolism is due to the specific tissue distribution of BCAT and the branched-chain α-keto acid dehydrogenase enzyme complex (Hutson et al. 1978, Shinnick and Harper 1976).
The high Kᵋ values reported for BCAT (0.4–4.91 mmol/L) (Davoodi et al. 1998, Hall et al. 1993, Ichihara et al. 1973, Taylor and Jenkins 1966) ensure that rates of catabolism of amino acids are low when tissue amino acid concentrations are low and high only when amino acid concentrations are elevated (Harper 1984b). BCATₘ, which is expressed in most tissues, is important in body nitrogen conservation due to its capacity to reaminate the branched-chain α-keto acids to form BCAA, thereby sparing nitrogen (Harper et al. 1984). Reported changes in BCAT activity in response to dietary and hormonal treatments of animals have been small and inconsistent (Harper et al. 1984, Ichihara et al. 1967, Mimura et al. 1968, Wohlhueter and Harper 1970). This study was conducted to clarify the effect of the protein content of the diet in well-nourished and protein-undernourished rats, and the effects of glucagon and hydrocortisone on the BCAT activity and mRNA concentration of the mitochondrial BCATₘ iso-enzyme.

MATERIALS AND METHODS

Animals and collection of tissues. Male Wistar rats (70–90 g) were obtained from the Instituto Nacional de Nutrición (Mexico, D.F.). At the end of each study, rats were anesthetized with 30 mg/kg sodium pentobarbital before decapitation. Tissue samples used for RNA preparations were removed rapidly and frozen immediately in liquid nitrogen. These frozen tissue samples were stored at −80°C for <3 wk before isolation of total RNA. Tissue samples used for enzyme assays were removed and processed immediately. All experimental procedures using laboratory animals were approved by the Committee on Animal Research of the Instituto Nacional de Nutrición.

Diets. The composition of the 0, 6, 10, standard 18, 35 and 50% casein diets is shown in Table 1. The 6% casein diet was supplemented with 2 g/kg l-methionine and 4 g/kg l-threonine, at the expense of carbohydrate, to improve the nutritional quality of the dietary protein. With the exception of Experiment 2, all rats were allowed free access to the diets. All rats had free access to water throughout the study.

Experiment 1. Male Wistar rats (80–90 g) were allowed free access to an 18% casein diet for 1 wk. Brain, heart, kidney, muscle, liver, lung, skin, stomach and intestine were removed, and a sample of each tissue was frozen immediately in liquid nitrogen for the determination of BCATₘ mRNA. A separate sample was used to measure BCAT activity.

Table 1

<table>
<thead>
<tr>
<th>Percentage of casein</th>
<th>0</th>
<th>6</th>
<th>10</th>
<th>18</th>
<th>35</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>0</td>
<td>60</td>
<td>100</td>
<td>180</td>
<td>350</td>
<td>500</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>445</td>
<td>412</td>
<td>395</td>
<td>355</td>
<td>270</td>
<td>195</td>
</tr>
<tr>
<td>Cellulose</td>
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<td>50</td>
<td>50</td>
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<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Tekland Test diets, Madison, WI.
2 Rogers-Harper, Teklad Test diets, Madison, WI (Rogers and Harper 1965).
3 Vitamin mix, Tekland 40060 (mg/kg diet): α-tocopheryl acetate, 242.
4 Sigma Chemical, St. Louis, MO.

Experiment 2. Sixty male Wistar rats weighing 70–90 g were housed in individual stainless steel cages in air-conditioned rooms. The rooms were lighted from 0700 to 1900 h and dark from 1900 to 0700 h. Rats had free access to an 18% casein powdered diet for 1 d. Then, rats were divided into five groups of 12 rats each and fed a 0% (protein-free), 6% (low protein), 18% (control), 35% (high protein) or 50% (high protein) casein diet. The five groups of rats were allowed free access to these diets for 4 d. To synchronize food intake, the rats were then trained to consume the diets in a restricted period of 7 h (0900–1600 h) for 10 d (i.e., 7 h feeding, 17 h food-deprivation). On d 10, three rats from each group were killed 3 and 6 h after they started eating their meal and after 5 and 17 h of food deprivation. Samples of liver, kidney, skeletal muscle, heart and brain were taken for the determination of BCATₘ mRNA levels and BCAT activity.

Experiment 3. Twenty male Wistar rats weighing 80–90 g were allowed free access to a diet containing 0.5% casein for 5 wk (see Philbrick and Hill 1974). A pair-fed group (n = 5) was fed the same quantity of diet consumed by the group of malnourished rats, but the diet contained 18% casein. On d 36, five rats from the undernourished group and the pair-fed rats were killed. The remaining undernourished rats were divided into three groups of five rats each that were reha bi litated with diets containing 6, 18 or 50% casein for 21 d. All three groups of rats were killed on d 21. Samples of kidney, muscle and heart were obtained for measurement of BCAT activity and BCATₘ mRNA concentrations.

Experiment 4: hormone treatment. Forty-eight male Wistar rats weighing between 75 and 90 g were fed a 10% casein diet for 7 d. The next day, rats were randomly divided into four groups of 12 rats each and subjected to one of the following treatments: 1) the hydrocortisone control group was injected subcutaneously with propylene glycol (100 μL/100 g body weight) twice a day for 7 d; 2) the hydrocortisone group was injected subcutaneously with hydrocortisone (1 mg/100 g body weight) twice a day for 7 d as described previously (Lee and Harper 1971, Lee et al. 1972, Morris et al. 1973); 3) the glucagon control group was injected intraperitoneally with saline (200 μL/100 g body weight) three times a day for 4 d; 4) the glucagon group was injected intraperitoneally with glucagon (0.2 mg/100 g body weight) three times a day for 4 d (Lee and Harper 1971, Lee et al. 1972, Morris et al. 1973). Groups 1 and 2 were killed at 0800 h on d 8, and groups 3 and 4 were killed at the same time on d 5. Kidney, muscle and heart were obtained for measurement of BCAT activity and BCATₘ mRNA concentration.

Tissue preparation and BCAT assay. Tissues were removed, blotted and weighed. One gram of each tissue was used. Livers were homogenized in glass tissue grinders with a teflon pestle driven by an electric mixer.
electric drill. Skeletal muscle, kidney, heart and brain were homogenized with a polytron homogenizer (Kinematica, AG, Luzerne, Switzerland) at the minimum setting. Before homogenization, all tissues were minced in 4 mL of mannitol-sucrose-EDTA medium containing 0.225 mol/L mannitol, 0.075 mol/L sucrose, 0.10 mmol/L EDTA and 5 mmol/L 3-(N-morpholino)propanesulfonic acid (MOPS), pH 7.0, containing 5 mmol/L benzamidine, 1 mmol/L disopropyl fluorophosphate, 1 mmol/L EDTA, 1 mmol/L EGTA, 10 mg/L leupeptin, 5 mmol/L dithiothreitol and 10 mL/L Triton X-100. Tissue homogenates were centrifuged at 30,000 × g for 1 h at 4°C to obtain the supernatant fraction. The tissue extracts were kept at −20°C for 2 wk without loss of BCAT activity. Protein was determined by the biuret reaction in the presence of 2.5 mg/L sodium deoxycholate, with crystalline bovine albumin as standard.

BCAT activity was assayed in the extracts according to the method of Hutson et al. (1988) in small test tubes (10 × 45 mm) with a side arm (Wohlhueter and Harper 1970). Activity was measured at 37°C in 50 mmol/L potassium phosphate buffer, pH 7.8, which contained 50 mmol/L pyridoxal phosphate and 4 g/L 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). The sample was introduced in a volume of 50 μL, and the reaction was initiated by the addition of the α-keto-[1-14C]isocaprate (KIC)/isoleucine substrate mixture with a final concentration in the assay of 1.0 mmol/L [1-14C]KIC and 12 mmol/L isoleucine. After 5 min, the reaction was stopped by the addition of 500 μL of 2 mol/L sodium acetate, pH 3.4. The remaining [1-14C]KIC was removed by chemical decarboxylation by adding 250 μL of 30% hydrogen peroxide. The [14C]-labeled isoleucine formed was quantified by addition of 250 μL of the reaction mixture to 10 mL of BCS liquid scintillation cocktail (Amersham, UK), and samples were counted in a Wallac scintillation counter (Turku, Finland). Each assay was conducted in duplicate. A unit of activity was defined as 1 μmol [1-14C]isoleucine formed/min at 37°C.

RNA preparation and Northern blot analysis. Total RNA was isolated from tissues according to Chomczynski and Sacchi (1987). For Northern analysis, 20 μg of RNA was subjected to electrophoresis in a 1.5% agarose gel containing 37% formaldehyde, transferred onto a nylon membrane filter (Hybond-N+, Amersham) and crosslinked with a UV crosslinker (Amersham). The probe was a 900-bp Pst1-cloned fragment of the rat BCATm cDNA (Bledsoe et al. 1997). It was labeled with [α-32P]dCTP (110 TBq/mmol, Amersham) using the rediprime DNA labeling system (Amersham). Filters were prehybridized with rapid-hyb buffer (Amersham) at 65°C for 45 min, and then hybridized with the labeled probe for 2.5 h at 65°C. Membranes were washed once with 2X SSC (1X SSC = 0.15 mol/L sodium chloride and 15 mmol/L sodium citrate)/0.1% SDS at room temperature for 20 min and then washed twice with 0.1X SSC/0.1% SDS at 65°C for 15 min each. An Instant Imagean electroautoradiography system (Packard Instrument, Meriden, CT) was used to produce digitized images for quantitation of radioactivity in the bands. Membranes were also exposed to Extascan film (Kodak, Guadalajara, Mexico) at −70°C with an intensifying screen.

Chemicals and reagents. [1-14C]Leucine and the nucleotide [α-32P]dCTP were obtained from Dupont NEN (Boston, MA). The radioactive KIC was synthesized from [1-14C]leucine as described by Rüdiger et al. (1972). Hydrocortisone and glucagon were obtained from Sigma Chemical (St. Louis, MO) and Eli Lilly (Indianapolis, IN), respectively. All other reagents were from commercial sources and were at least reagent grade.

Statistics. Differences among treatments were tested by one-way ANOVA and Fisher’s protected least significant difference test (Stat View Version 4.02, Abacus Concepts, Berkeley, CA). Analysis of statistical differences in BCAT activity between dietary treatments and over time was done by two-way ANOVA. The effects of glucagon or hydrocortisone was analyzed by Student’s t test. Differences were considered significant at P < 0.05.

RESULTS

Experiment 1: BCATm mRNA and activity levels in rat tissues. BCAT activity and BCATm mRNA concentration were measured in selected organs and tissues of rats 6 h after they consumed the standard 18% casein diet. Among the tissues assayed for enzyme activity, BCAT specific activity (μmol/mg protein) was highest in stomach, intermediate in heart, brain, kidney, skin and muscle and low in lung and intestine (Fig. 1). BCAT activity has not been measured previously in skin. Interestingly, the specific activity of BCAT in this tissue was similar to values for skeletal muscle. Apparent BCAT activity was almost undetectable in liver. These results are in agreement with previous studies (Hall et al. 1993, Ichihara 1985). Although not strictly parallel, BCATm mRNA expression showed a pattern similar to that observed for BCAT activity (Fig. 1, Panel A). In agreement with the activity results, liver BCATm mRNA was undetectable by Northern analysis. The BCAT activity in brain is due to the expression of both the cytosolic and mitochondrial isoforms of the enzyme (Hall et al. 1993, Ichihara 1985) (see also Fig. 3).

Experiment 2: effect of dietary protein concentration on weight gain, BCAT activity and BCATm mRNA levels in rat tissues. In this study, rats were divided into five groups that were allowed free access to diets containing 0, 6, 18 (control), 35 or 50% casein for 4 d. Food intake was then synchronized by restricting access to the diet to a period of 7 h. One day after the rats began receiving their respective diets on a restricted feeding schedule, they had lost weight. Rats fed the 18, 35 or 50% casein diets began to gain weight after 2 d of consuming their corresponding diet on the restricted feeding schedule. Rats fed a 6% casein diet began to gain weight after 4 d of being fed on the restricted feeding schedule, whereas rats fed the 0% casein diet lost weight continuously during the 10-d treatment period. At the end of the treatment period, the rates of weight gain were 5.6 ± 0.3, 5.9 ± 0.4 and 5.5 ± 0.3 g/d for the groups fed 18, 35 and 50% casein, respectively, and rates were not different among these groups. Rats fed the 6% casein diet had a significantly slower rate of weight gain (1.3 ± 0.09 g/d) than rats fed the 18% control diet. Rats fed the protein-free diet were losing weight at a rate of −1.4 ± 0.08 g/d. The effect of meal-feeding and food deprivation on BCAT

FIGURE 1 Comparison of branched-chain aminotransferase (BCAT) activity and mitochondrial branched-chain aminotransferase (BCATm) mRNA levels in selected rat organs and tissues. Tissues were removed from rats fed a standard 18% casein diet 6 h after the initiation of feeding. Panel A: Northern blot analysis. Total RNA (20 μg) from each rat tissue was subjected to electrophoresis, blotted and hybridized with the uniformly [32P]labeled BCATm partial cDNA probe as described in Materials and Methods. Panel B: ethidium bromide staining of the gels used for Northern blot hybridization in Panel A. BCAT activity in μmol/mg protein and in μg/g tissue appears below each lane. A unit of activity is defined as 1 μmol leucine formed/min at 37°C. Values represent means ± SEM, n = 6 rats in each group.
activity and BCATm mRNA levels in heart, liver, kidney, skeletal muscle and brain was determined during a 24-h period in rats adapted to consume their diet for 7 h (0900–1600 h). Tissues were removed from rats in each dietary group (0, 6, 18, 35 and 50% casein) at 3 and 6 h after receiving their respective diet, and after 5 and 17 h of food deprivation. The results from rats fed the 18% control diet are illustrated in Figure 2. As shown in Figure 2A, BCAT activity did not change significantly during the day in all tissues studied, indicating that there is no diurnal variation of BCAT activity. The same pattern was also observed in rats fed 0, 6, 35 or 50% casein diets (data not shown). BCATm mRNA concentrations in the tissues of rats fed an 18% casein diet (Fig. 2B), as well as in rats fed lower or higher protein concentrations (data not shown), also showed no significant variation throughout the day.

To determine whether dietary protein affected activity and isoantigen mRNA levels, BCAT activity (Table 2) and BCATm and BCATc mRNA levels (Fig. 3) were compared in the same tissues (liver, skeletal muscle, brain, kidney and heart) from rats killed 6 h after receiving their diets. There were significant effects of dietary protein concentration on skeletal muscle, brain and kidney BCAT activity. Skeletal muscle from rats fed the low protein 6% casein diet had lower BCAT activity than the other dietary groups, whereas in rats fed the 50% casein diet, activity was significantly higher than in the other groups. Skeletal muscle BCAT activity in rats fed the 0, 18 and 35% casein diets was similar. In the kidney, BCAT activity was higher in rats fed either a 35 or 50% casein diet than in rats fed lower levels of casein. Brains from rats fed the 50% casein diet had significantly higher BCAT activity than the other groups. BCAT activity in heart was not affected by dietary protein concentration. These results indicate that a high protein diet resulted in higher levels of BCAT activity in all tissues except heart with increases ranging from 15 to 48%. Significant differences in apparent transaminase activity among some dietary groups were found in liver, but there was no consistent effect of dietary protein on this activity.

Northern blots of BCATm mRNA from kidney, heart, muscle and brain of rats fed different concentrations of protein are shown in Figure 3. BCATm mRNA expression was not detected in livers of any of the rats (data not shown). There were no significant differences in the expression of BCATm mRNA in heart, kidney and brain of rats fed different concentrations of dietary protein. In agreement with previous reports (Hall et al. 1993, Hutson et al 1995), both BCATc and BCATm mRNAs were expressed in brain; however, neither message was influenced by dietary protein concentration. Muscle BCATm mRNA expression was greater in rats fed a protein-free diet or a 50% casein diet than in those fed 6, 18 or 35% casein.

Experiment 3: effect of protein rehabilitation in malnourished rats on body weight, BCAT activity and BCATm mRNA levels in rat tissues. After 5 wk of consuming a diet containing 0.5% casein, rats did not gain weight but had actually lost 39% of their initial body weight (Table 3). Rats pair-fed an 18% casein diet did not lose weight; however, their weight had increased by only 11 g (12.5% increase) at the end of 5 wk. Nevertheless, the pair-fed group weighed significantly more than the 0.5% casein group (P < 0.05). In comparison, animals that had free access to the standard 18% casein diet gained 216 g during the same period. Thus growth, as measured by weight gain, was stunted in both the protein-malnourished (0.5% casein) and the pair-fed group. After 21 d of rehabilitation therapy with 6, 18 or 50% casein diets, rats fed a 6% casein diet had gained weight (16% increase). Rats rehabilitated with diets containing 18 or 50% casein had increased their body weights by 213 and 245%, respectively, during the same period of time (Table 3).

BCAT activity in kidney, muscle and heart of protein-malnourished rats (0.5% casein diet) did not differ from rats with free access to an 18% casein diet or to the pair fed group (Table 4). Compared with protein-malnourished rats, there was no effect on BCAT activity in any of the tissues in rats that underwent protein rehabilitation for 21 d with a 6 or 18% casein diet, whereas rehabilitation with a 50% casein diet resulted in significantly higher BCAT activity (81%) in muscle (Table 4). BCATm mRNA levels in kidney and heart were similar in all experimental groups (data not shown). Consistent with the higher levels of BCAT activity in skeletal muscle of rats rehabilitated with 50% casein, skeletal muscle BCATm mRNA levels were higher in this group than in the malnour-
lished, pair-fed, control 18% and 6 or 18% rehabilitated groups (Fig. 4).

**Experiment 4: hormonal treatments.** In comparison with the group injected with the vehicle solution, treatment of rats with hydrocortisone or glucagon for several days did not affect BCAT activity significantly in muscle, heart or kidney. For example, in skeletal muscle, values for control and hydrocortisone-injected rats were 9.1 ± 0.8 and 9.3 ± 1.0 mU/mg protein, respectively, whereas the control and glucagon-injected rat muscle BCAT activities were 8.1 ± 0.7 and 9.3 ± 0.5 mU/mg protein, respectively. There were also no differences in the BCATm mRNA levels in these tissues (data not shown).

### DISCUSSION

The BCAA comprise ~20% of the total amino acids of tissue protein and ~35% of the indispensable amino acids in muscle. They also make up ~40% of the indispensable amino acid requirement of young rapidly growing animals including humans (Harper et al. 1983). Therefore, catabolism of BCAA must be regulated to maintain adequate levels of BCAA to meet the requirements for growth in younger animals and maintenance in adult animals. Most nutritional studies have focused on the effect of diet and hormones on the mitochondrial branched-α-keto acid dehydrogenase, the second enzyme in the catabolic pathway. The activity state of this enzyme in liver is increased by the ingestion of a high protein diet (Croywell et al. 1988, Miller et al. 1988, Wohlhueter and Harper 1970, Zhao et al. 1994).

In this study, we have examined the effect of diet on levels of BCAT activity and expression of the mitochondrial BCATm isoenzyme in rat tissues. A BCATm message of 1.7 kb, corresponding to the mitochondrial form of BCAT (Bledsoe et al. 1997), was observed in all tissues except liver. These results confirm and extend previous reports (Bledsoe et al. 1997, Hutson et al. 1992). The highest BCATm mRNA concentration was found in stomach, which also had the highest BCAT activity. The distribution of BCATm mRNA observed in most of the tissues was in agreement with the published pattern of BCAT activity (see Fig. 1; Cappuccino et al. 1978, Goto et al. 1977, Hall et al. 1993, Hutson 1988, Ichihara 1975). We have demonstrated for the first time the presence of BCATm in skin and lung tissue. The current model of BCAA metabolism predicts that there is extensive transamination of BCAA in skeletal muscle and oxidation of the α-keto acid products in liver (Harper et al. 1984, Hutson et al. 1978). In this model, BCAA are thought to play a critical role in body nitrogen metabolism. Our results suggest that BCAA transamination may serve additional functions in other tissues such as skin; for example, it has been reported that BCAA are precursors of branched-long-chain fatty acids found in rat skin surface lipid (Oku et al. 1994). The extent to which BCAA are oxidized in tissues such as skin or lung remains to be determined.

We did not observe significant oscillations in rat tissue BCAT activity during the day regardless of the concentration of the protein in the diet. BCATm mRNA levels were also unaffected by dietary protein concentration in all tissues ex-
Heart 17.2

TABLE 3

Effect on body weight of feeding rats a 0.5% casein diet followed by rehabilitation with diets containing 6, 18 or 50% casein

<table>
<thead>
<tr>
<th>Protein rehabilitation (21 d) % casein</th>
<th>Free access4</th>
<th>6</th>
<th>18</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnourished2</td>
<td>Pair-fed3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td></td>
<td></td>
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<tr>
<td>Before treatment</td>
<td>87.0 ± 0.6a</td>
<td>88.3 ± 1.2a</td>
<td>82.8 ± 1.1b</td>
<td>52.7 ± 0.8b</td>
</tr>
<tr>
<td>After treatment</td>
<td>53.7 ± 0.6b</td>
<td>77.2 ± 2.6b</td>
<td>301.3 ± 3.9a</td>
<td>61.2 ± 4.0a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 20 for the malnourished group, n = 5 for the pair-fed group, n = 5 for the free-access group and n = 5 for each of the rehabilitation groups. Values in a column with different letter superscripts are significantly different, P < 0.05.
2 Rats were fed a 0.5% casein diet for 5 wk.
3 Rats fed the same quantity of food (18% casein diet) consumed by the malnourished group for 5 wk.
4 Rats fed an 18% casein diet for 5 wk.

BCAT activity in muscle, although prolonged starvation up to 5 d did not produce a further increase in BCAT activity (Adibi et al. 1973). In our study, BCAT activity, as well as BCATm mRNA concentrations, in muscle of malnourished rats was similar to that of rats with free access to the 18% casein diet. These results are in agreement with a previous study that indicated that BCAT activity is unaffected by protein malnutrition (Reeds 1974). However, rehabilitation of malnourished rats with a 50% casein diet did increase BCAT activity and BCATm mRNA expression in muscle. Studies with other hepatic or serum aminotransferases have shown that their activities are increased during malnutrition (Heard et al. 1977, Osifo and Bolodeoku 1982), suggesting that control of the expression of these enzymes is different from that of BCAT, which is an extrahaepatic enzyme.

Contradictory results have been reported in studies that have examined only BCAT activity in kidney and muscle of rats fed high protein diets (Chan and Walser 1978, Ichihara et al. 1967, Mimura et al. 1968, Wohlhueter and Harper 1970). In skeletal muscle, a high protein intake resulted in a modest increase in BCAT activity in rats in the study by Mimura et al. (1968), and no change in rats fed diets containing between 0 and 18% casein in the study by Chan and Walser (1978). We measured BCATm expression and BCAT activity in this study and showed clearly (Table 4, Fig. 3) that high protein intake regulated expression and activity exclusively in muscle. These results suggest that the effect of dietary protein is tissue specific because the increments in BCAT activity in kidney and brain produced by the consumption of a high protein diet were unrelated to changes in BCATm mRNA levels.

It has been shown that rats starved for 1 d had increased BCAT activity, although prolonged starvation up to 5 d did not produce a further increase in BCAT activity (Adibi et al. 1973). In our study, BCAT activity, as well as BCATm mRNA concentrations, in muscle of malnourished rats was similar to that of rats with free access to the 18% casein diet. These results are in agreement with a previous study that indicated that BCAT activity is unaffected by protein malnutrition (Reeds 1974). However, rehabilitation of malnourished rats with a 50% casein diet did increase BCAT activity and BCATm mRNA expression in muscle. Studies with other hepatic or serum aminotransferases have shown that their activities are increased during malnutrition (Heard et al. 1977, Osifo and Bolodeoku 1982), suggesting that control of the expression of these enzymes is different from that of BCAT, which is an extrahaepatic enzyme.

High protein diets produce an increase in the activities of liver amino acid–degrading enzymes to catabolize the excess amino acids (Harper 1984a), and the change in activity of most of these enzymes is associated with an increase in the rate of transcription of their genes (Ogawa et al. 1991, Torres et al. 1998). In this study, the increase in muscle BCATm mRNA concentration due to the consumption of a high protein diet could be explained by an increase in the rate of transcription of the BCAT gene, or by a decrease in the rate of degradation of its mRNA.

Several of the hepatic amino acid–degrading enzymes are induced by hormones such as glucagon or glucocorticoids. Previous reports have shown that hydrocortisone injection pro-

TABLE 4

Branched-chain aminotransferase (BCAT) activity in tissues from protein-malnourished rats and rats rehabilitated with diets containing 6, 18 or 50% casein

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Malnourished2</th>
<th>Pair-fed3</th>
<th>Free access4</th>
<th>Protein rehabilitation (21 d) % casein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Muscle</td>
<td>8.3 ± 0.6bc</td>
<td>9.9 ± 0.8b</td>
<td>9.8 ± 0.8b</td>
<td>7.4 ± 0.4c</td>
</tr>
<tr>
<td>Kidney</td>
<td>11.8 ± 0.5</td>
<td>13.1 ± 0.3</td>
<td>12.0 ± 1.0</td>
<td>13.3 ± 0.3</td>
</tr>
<tr>
<td>Heart</td>
<td>17.2 ± 0.9</td>
<td>19.8 ± 0.8</td>
<td>19.2 ± 0.6</td>
<td>17.7 ± 1.4</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5. Values in a row having different superscripts are significantly different (P < 0.05).
2 Rats fed 0.5% casein diet for 5 wk.
3 Rats fed same quantity of food (18% casein) consumed by the malnourished group for 5 wk.
4 Rats fed 18% casein diet for 5 wk.
5 A unit of enzyme activity is defined as 1 μmol leucine formed/min at 37°C.
FIGURE 4 Effect of protein rehabilitation of malnourished rats (PM) with diets containing 6, 18 or 50% casein on skeletal muscle BCATm mRNA levels. Malnourished rats had free access to a 0.5% casein diet for 5 wk (PM). Pair-fed (PF) rats were fed the same quantity of an 18% casein diet as consumed by the PM group. Rats (FA) had free access to an 18% casein diet. Rats were rehabilitated with 6, 18 or 50% casein diets for 21 d. Values are means ± SEM, n = 5; values are expressed relative to the FA group, which was arbitrarily set at 100% Bars with different letter superscripts are significantly different, P < 0.05.

LITERATURE CITED


