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BRANCHED-CHAIN AMINO ACID METABOLISM

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CONTENTS

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INTRODUCTION

The branched-chain amino acids (BCAA)—leucine, isoleucine, and valine—are three of nine amino acids that cannot be synthesized by animals and are therefore essential nutrients that must be obtained from foods. They are required specifically for the synthesis of proteins, not as precursors of unique biologically active molecules. The BCAA comprise about 35% of the indispensable amino acids in muscle proteins and about 40% of the amino acids required preformed by mammals. As the BCAA make up almost 50% of the indispensable amino acids in the food supply, deficiencies of them do not occur naturally (92). Nutritional investigations of the BCAA have therefore focused primarily on the effects of excessive intakes of the individual BCAA and on the metabolic relationships among them, rather than on problems relating to inadequate intakes.

Investigations of the metabolism of the BCAA have burgeoned in recent years for several reasons. Prominent among these are: some unique features of the catabolism of the BCAA have implications for improving understanding of interorgan relationships in nitrogen and carbon metabolism; the initial enzymes for catabolism of the BCAA are regulated differently from other amino acid–degrading enzymes; one or more of the BCAA appear to exert specific regulatory effects on tissue protein degradation and synthesis that may be of clinical significance; and BCAA, through competition with other amino acids in blood, appear to play a role in controlling brain amino acid concentrations and thereby in the synthesis of amino acid–derived neurotransmitters.

This review is restricted for the most part to discussion of the metabolic and nutritional interrelationships of the BCAA. Some aspects of the subject and observations on other physiologically important effects of BCAA have been covered in other reviews. The reader is referred to these for discussions of other papers that are not cited or discussed in this article because of space limitations. Knowledge of genetic defects of BCAA metabolism has been reviewed in detail (221); an extensive compilation of current research on various aspects of BCAA metabolism has been published (234), as have reviews specifically on BCAA aminotransferases (108) and branched-chain α-ketoacid (BCKA) dehydrogenase (191). Several authors have discussed various aspects of amino acid and interorgan relationships in the metabolism of the BCAA (60, 78, 94, 131, 213). Observations on the effects of excessive intakes of BCAA (88, 92) and the nutritional and physiological factors that affect BCAA metabolism (4) have been summarized. Differing views on the role of leucine as an etiological factor in the development of pellegra have been presented (81, 138, 139). Overviews of the clinical and pharmacological significance of the BCAA are available (5, 10), and more detailed reviews on specific aspects of this subject include the role of leucine in insulin release (57), BCAA in hepatic disease (16,

22), and BCAA and BCKA in renal disease and urea cycle defects (231, 232). Recent publications on the effects of BCAA on tissue protein degradation (186) and synthesis (149) bear on the question of potential nitrogen-sparing effects of BCAA in patients with trauma and sepsis (33, 67, 121). Observations on the effects of competition among plasma amino acids for uptake into brain and the implication of this, especially in relation to the role of BCAA, for neurotransmitter synthesis have been reviewed (62, 241); the bearing of observations of this type on the control of feeding behavior is being debated (93, 128, 129).

METABOLISM OF BCAA AND BCKA

The catabolic pathways of the BCAA have several features in common. The initial step for each is transamination, a readily reversible reaction that yields the corresponding BCKA. Each BCKA then undergoes an irreversible oxidative decarboxylation, the product of which is the acyl-CoA derivative with one less carbon. Thereafter, the pathways resemble those for fatty acid oxidation and lead to end products that can enter the tricarboxylic acid cycle. The end products of isoleucine catabolism are propionyl-CoA and acetyl-CoA (Figure 1); hence it is both glucogenic and ketogenic. Leucine yields acetoacetate and acetyl-CoA; it is therefore ketogenic. Valine yields succinyl-CoA; it is therefore glucogenic.

BCAA Aminotransferase

DISTRIBUTION AND CHARACTERISTICS BCAA aminotransferase (BCAAT, EC 2.6.1.42), the catalyst for transamination of the BCAA, is widely distributed among tissues. The enzyme was purified and characterized from pig heart in 1966 (109, 224). It is a pyridoxal phosphate–dependent enzyme and accepts all three BCAA as substrates. Among the tissues assayed for the enzyme, activity per gram of wet tissue was highest in heart and kidney, intermediate in skeletal muscle, and lowest in liver (109). Subsequently stomach, pancreas, and lactating mammary gland were found to have higher BCAAT activity than kidney (32, 113).

BCAAT occurs in both the cytosol and the mitochondria. The proportions in the two fractions differ from organ to organ, ranging from 75% cytosolic in brain to 22% cytosolic in kidney (116). Detergent treatment of muscle mitochondria increased BCAAT activity (165), so mitochondrial values obtained may be low. The Michaelis constants (K_m) of the cytosolic and mitochondrial enzymes from rat brain, kidney, skeletal muscle, and mammary gland for BCAA differ little (116). Differences in the properties of the cytosolic and mitochondrial enzymes from rat liver, however, indicated that they were different proteins (9).

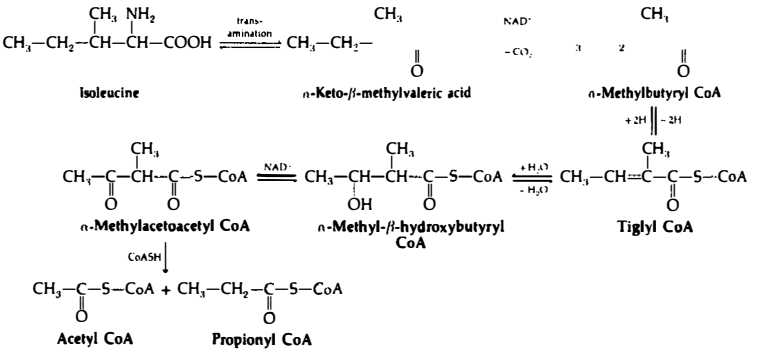

Figure 1 Conversion of isoleucine to propionyl-CoA and acetyl-CoA.

Ichihara and associates (113) have identified three different BCAATs in various mammalian tissues. Enzyme I, which accepts all three BCAA as substrates, is widely distributed and is the predominant form in both cytosol and mitochondria of most organs and tissues. Enzyme II, which is specific for leucine, has been found only in cytosol of rat liver (9). Enzyme III was found in pig and rat brain. It is the predominant form in brain, placenta, and ovary. Another aminotransferase that accepts either leucine or methionine as substrate was isolated from rat liver mitochondria (114), and it has been designated leucine (methionine) aminotransferase. In a survey of human tissues (82), enzymes I and III were found widely distributed, with enzyme I predominating in tissues other than brain. Enzyme II was not detected in human liver.

The K_m's of BCAAT I and III from rat tissues range from about 0.4 to 0.8 mM for leucine and isoleucine and from about 1.2 to 2.5 mM for valine (113, 116). The values are 2–6 times the concentrations of leucine and isoleucine found in tissues and about 4–10 times the concentration of valine found in tissues. The K_m of the leucine-specific enzyme II of rat liver for leucine is about 25 mM (113). The main amino-acceptor in the BCAAT reaction is considered to be α-ketoglutarate, for which the K_m appears to range between 0.1 and 1.0 mM, depending on the tissue (113). The BCKA, however, are excellent amino-acceptors for the various BCAA, as are α-keto-γ-methiobutyrate and α-ketobutyrate (46, 223); very little activity is observed with pyruvate and none with oxaloacetate (46). Glutamate, methionine, and α-aminobutyrate are effective amino donors for each of the BCKA, but other amino acids, including glutamine, apparently do not donate their amino groups to the BCKA. The K_m's for the BCKA as amino-acceptors for BCAA range from 0.03 to 0.11 mM, about one-tenth to one-third of the K_m for α-ketoglutarate.

REGULATION No unique mechanisms for regulation of BCAAT have been identified. The rate of transamination should thus depend primarily on the concentrations of enzyme and substrates. The K_m's of the major aminotransferase for the BCAA exceed 0.4 mM and are thus two- to fourfold higher than tissue BCAA concentrations, so the rate of transamination in vivo should respond rapidly to changes in tissue BCAA concentrations (122). Rapid removal of BCKA, the products of transamination, should increase the rate of this reaction. When aminotransferase activity is rate-limiting, addition of a source of BCKA dehydrogenase to the incubation medium increases the rate of transamination (46). This situation should occur in vivo in liver, which has low aminotransferase but high BCKA dehydrogenase activity.

The potential for competition among at least four amino-donors and an equal number of amino-acceptors in the BCAAT reaction is obviously great (46, 224) and could influence the rate of formation of the individual BCKA. With muscle as the source of aminotransferase, with the three BCAA in the incubation medium at physiological concentrations (0.1–0.2 mM), and with α-ketoglutarate as the amino acceptor, transamination proceeded most slowly with valine, as might be anticipated from the high K_m of the aminotransferase for valine, and more rapidly with leucine and isoleucine, for which the K_m's are lower (46). Less α-ketoisovalerate thus accumulated in the medium than the other two BCKA, as was observed when the isolated hindquarter was perfused with a complete mixture of amino acids (103). Also, because the K_m of BCAAT for α-ketoglutarate is 0.3–0.4 mM above usual tissue concentrations, the supply of amino-acceptor could become limiting for transamination in vivo, especially if concentrations of BCAA were high. Most tissues contain high concentrations of glutamate, so reamination of BCKA might be expected under such conditions (124). Evidence that reamination of the BCKA occurs in vivo has been obtained in studies in which nitrogen and the carbon skeletons of BCAA were labelled with stable isotopes (73, 142).

The BCKA formed in the BCAAT reaction are the substrates for the irreversible oxidative decarboxylation, the second step in the pathway of BCAA degradation. Because BCKA concentrations in most tissues rarely exceed the K_m of the BCKA dehydrogenase, rates of degradation of BCAA in vivo are probably controlled largely through changes in the concentrations of the substrates for the BCAAT reaction (95).

BCKA Dehydrogenase

DISTRIBUTION AND CHARACTERISTICS Branched-chain α-ketoacid dehydrogenase (BCKAD, EC 1.2.4.4) is a multienzyme complex located on the inner surface of the inner mitochondrial membrane. BCKAD catalyzes the oxidative decarboxylation of α-ketoisocaproate (ketoleucine, KIC), α-keto-β-methylvalerate (ketoisoleucine, KMV), and α-ketoisovalerate (ketovaline,

KIV), to form isovaleryl-CoA, 3-methylbutyryl-CoA, and isobutyryl-CoA, respectively. In both structure and function, BCKAD resembles the pyruvate dehydrogenase complex (PDH, EC 1.2.4.1), which catalyzes the conversion of pyruvate to acetyl-CoA (192). Like BCAAT, BCKAD is distributed ubiquitously and nonuniformly throughout the body (Table 1). BCKAD activity is highest in liver, intermediate in kidney and heart, and comparatively low in muscle, adipose tissue, and brain (191).

Early attempts to purify BCKAD met with difficulty (191), but in 1978 it was purified 280-fold to apparent homogeneity from bovine kidney mitochondria (184). Since then, Paxton & Harris (180) have purified BCKAD from rabbit liver 1400-fold. The BCKAD complex has a relative molecular weight (M_r) greater than 2×10^6 and is composed of three separate catalytic subunits held together by noncovalent interactions: (*a*) branched-chain α-ketoacid decarboxylase (E_{1b}) arranged in an $\alpha_2\beta_2$ substructure with thiamin pyrophosphate as a prosthetic group; (*b*) dihydrolipoyl transacylase (E_{2b}) with lipoate as a prosthetic group; and (*c*) dihydrolipoyl dehydrogenase (E_{3b}) with flavin adenine dinucleotide as a prosthetic group. The M_r's of the subunits as derived from sodium dodecylsulfate-polyacrylamide gel electrophoresis are 47,000 for $E_{1b\alpha}$; 38,000 for $E_{1b\beta}$; 51,000 for E_{2b}; and 55,000 for E_{3b} (180). In addition to the covalently bound prosthetic groups listed above, BCKAD requires coenzyme A (CoA) and nicotinimide adenine dinucleotide (NAD^+) as cofactors for oxidation of BCKA. The complex also requires Ca^{2+} and Mg^{2+} for optimal activity (115, 174). Dihydrolipoyl dehydrogenase is only loosely bound to the dihydrolipoyl transacylase core and is readily lost during isolation, which may help to explain the difficulties encountered in the early attempts to purify BCKAD.

All three BCKA serve as substrates for the isolated BCKAD. Earlier work had suggested that there were two separate BCKAD in bovine liver—one specific for KIC and KMV and one specific for KIV (21, 41). However, recent

Table 1 Activities of BCAAT and BCKAD in various rat tissues[a]

Tissue	Disrupted preparations		Oxidation of [1-^{14}C] BCAA using isolated hepatocytes or tissue perfusions[b] (nmol/min/g)
	BCAAT (nmol/min/g)	BCKAD (nmol/min/g)	
Liver	95–116	500–650	1.5–9.1
Skeletal muscle	450–1070	25–50	0.2–7.5
Heart	1210–3750	210–330	2.5–10.2
Kidney	1050–2300	300–470	0.8–7.5

[a]See text for discussion and references.
[b]Ranges of oxidation rates are given for substrate concentrations between 0.1 and 0.5 mM.

work with purified rabbit liver BCKAD indicates that the enzyme accepts all three BCKA as substrates with K_m's for KIC, KMV, and KIV of 15, 14, and 28 μM respectively (180). The purified enzyme also exhibits classical competitive inhibition kinetics with all pairs of BCKA. The pH optima and heat inactivation curves of BCKAD for all of these substrates are similar (48). Maple syrup urine disease, an inborn error of metabolism characterized by low or absent BCKAD activity, results in elevations in all three BCAA and BCKA (221). Also, BCKAD activity in liver from rats fed 0–30% casein increased incrementally whether KIC, KMV, or KIV was used as the substrate (240). Taken together, these observations indicate that there is only one mitochondrial BCKAD. Whether the differential responses of BCKA oxidation to Ca^{2+} (8), NAD^+ (51), hormones (29, 219), adenine nucleotides (8), and carnitine and β-hydroxybutyrate (173) that have been reported are artifactual (i.e. transport phenomena) remains to be established.

In addition to the mitochondrial BCKAD, a cytosolic KIC-preferring oxygenase has been isolated from rat liver and kidney (197). The oxygenase from rat liver has been purified to near homogeneity by Bieber and associates (198). Rat liver KIC oxygenase is a monomer of $M_r \cong 50{,}000$ and catalyzes the oxidative decarboxylation and hydroxylation of KIC to form β-hydroxyisovalerate. Unlike the mitochondrial BCKAD, the cytosolic oxygenase does not require CoA and NAD^+. However, Fe^{2+} is essential for activity. The K_m and maximal velocity (V_{max}) values for KIC are 0.32 mM and 130 nmol/min/mg protein respectively (198). This is markedly different from the kinetic values for mitochondrial BCKAD reported by Paxton & Harris (180), who found a K_m of 15 μM and a V_{max} of 1.99 μmol/min/mg protein. KMV and KIV do not serve as substrates for the cytosolic oxygenase, but α-keto-γ-methiolbutyrate (ketomethionine) is actively decarboxylated to form a product unidentified at this time. Together with the specific leucine (methionine) aminotransferase in rat liver mitochondria, KIC-oxygenase provides an alternate pathway for leucine catabolism. It has been estimted that about 15% of the KIC oxidizing capacity of rat liver is due to this oxidase (145).

REGULATION The activity of BCKAD, unlike that of BCAAT, is highly regulated. This is accomplished through a reversible phosphorylation (inactivation)-dephosphorylation (activation) mechanism similar to that for PDH. Kinetic studies indicated that BCKAD is associated with two regulatory proteins, a tightly bound protein kinase and a loosely bound phosphatase (191). More recently, Paxton & Harris (180) used sodium dodecylsulfate-polyacrylamide gel electrophoresis with purified rabbit liver BCKAD to detect a band that has an M_r identical to the PDH kinase. Whether this band actually represents the BCKAD kinase remains to be determined.

In 1972, Johnson & Connelly (115) showed that ATP could inhibit the

activity of partially purified BCKAD from bovine liver. Subsequently, ATP-mediated inhibition of rat heart (171), skeletal muscle (164, 165), and kidney (125) BCKAD were reported. The BCKAD complex was shown by Parker & Randle (170, 172) to exist in active and inactive forms. In 1981, Hughes & Halestrap (102) demonstrated phosphorylation of the M_r=48,000 protein ($E_{1b\alpha}$) of BCKAD from heart, liver, and kidney. There have since been other reports of ^{32}P incorporation into the $E_{1b\alpha}$ subunit of BCKAD from liver (174, 180), kidney (126, 163), and perfused heart (30). Correlations between the degree of phosphorylation and the level of BCKAD activity have been observed (191). Certain serine residues in the $E_{1b\alpha}$ protein are phosphorylated; recently, Lau et al (127) and Cook et al (42) showed that there are at least three phosphorylation sites on the $E_{1b\alpha}$ subunit of bovine kidney BCKAD. The $E_{1\alpha}$ subunit of PDH also has three sites of phosphorylation (192). Cook et al (42) reported that phosphorylation at one site results in total inactivation of BCKAD. In contrast, Lau et al (127) reported that total inactivation of BCKAD requires phosphorylation at more than one site.

Various attempts have been made to measure the activation state of BCKAD. The methods involve full activation of the enzyme (i.e. near-complete dephosphorylation); the ratio of basal to fully active enzyme is termed the activation state. To attain fully activated complex, preincubation with buffer (18, 177) or treatment with nonspecific phosphatases (77) have been used.

Among amino acid–degrading enzymes, only BCKAD and phenylalanine hydroxylase are regulated by phosphorylation-dephosphorylation mechanisms. Alteration of the phosphorylation state permits rapid modulation of enzyme activity. The inactivation-activation systems for the two enzymes are distinctly different: phosphorylation activates phenylalanine hydroxylase but inactivates BCKAD. Also, phenylalanine hydroxylase is phosphorylated by a cAMP-dependent process, but BCKAD is not (87). Most other amino acid–degrading enzymes show adaptive responses (induction) in conditions that increase the demand for gluconeogenesis. These responses, which result in changes in enzyme contents, occur slowly and act as a course control for enzyme activity.

In addition to control by phosphorylation, BCKAD activity is modulated by end-product inhibition. Products of the BCKAD reaction, NADH and branched-chain acyl-CoA derivatives, are competitive inhibitors of the complex (170, 184). The ratios of NADH to NAD^+ and acyl-CoA to CoA are believed to control the flux of BCKA through the BCKAD (24, 43, 44, 144, 167, 173, 227, 238). Stimulation of BCAA oxidation by carnitine is believed to be due to the efflux of acylcarnitines from the mitochondria (144).

Recently, there have been reports of BCKAD regulation by unique protein factors. Paul & Adibi (178, 179) have described a cytosolic macromolecular skeletal muscle factor that activates hepatic BCKAD, presumably by stimulat-

ing the BCKAD phosphatase. The physiological significance of this muscle factor is questionable, however, as barriers exist for transport of proteins across membranes. Randle and coworkers (58, 191) reported that high-speed supernatant fractions prepared from rat liver or kidney mitochondria activate phosphorylated ox-kidney BCKAD without causing dephosphorylation. In addition to the control mechanisms described above, this mitochondrial "activator protein" may play a role in regulating BCKAD activity in vivo.

Maximum activity values for BCKAD in different tissues are listed in Table 1. Owing to the variation in V_{max} values reported by different investigators, values are given as ranges. A plausible explanation for this variation is that differing degrees of phosphorylation may occur during tissue preparation. Variability may also be caused by differences in the compositions of the assay media used by different investigators, e.g. in the contents of CoA, NAD^+, thiamin pyrophosphate, lipoate, Mg^{2+}, inorganic phosphate, and EDTA. Also, BCKAD is located within the mitochondrion, so transport of substrate and cofactors across the outer and inner mitochondrial membrane is required. To exclude the process of transport and attain true V_{max} values, two methods have been employed: (*a*) bursting mitochondrial membranes by freeze-thawing, sonication, or use of detergents; and (*b*) swelling mitochondrial membranes by including 2–3 mM calcium in the medium. We have used calcium to swell mitochondrial membranes of liver and successfully bypass the transport barrier (18, 240). Any perturbation of the mitochondrial membrane of skeletal muscle (detergents, freeze-thawing, or calcium) results in loss of activity (165, 177, 227; P. H. Crowell and A. E. Harper, unpublished results). Therefore, true V_{max} activities may not yet be available for some tissues, especially muscle.

METABOLISM OF BCAA AND BCKA IN DIFFERENT ORGANS AND TISSUES

In the early 1960s, it was shown that BCAA, unlike other indispensible amino acids, are oxidized extensively by peripheral tissues (152). In hepatectomized dogs, the concentrations of most indispensible amino acids rose steadily, but BCAA concentrations did not (148). These and similar observations in rat studies (101), measurements of amino acid uptake across organs (19), and studies of the distribution of BCAAT (110, 205) have demonstrated that liver has less capacity than peripheral tissues to degrade BCAA. Nonetheless, perfused liver was found by Miller (152) to be 30–50% as effective as the eviscerated carcass in oxidizing [U–^{14}C]leucine added to the medium. Also, Noda & Ichihara (161) reported that ligation of the blood vessels to the liver depressed the amount of $^{14}CO_2$ expired by rats injected with [U–^{14}C]leucine by about 40%, while $^{14}CO_2$ expiration in those injected with [U–^{14}C]lysine or

[1-^{14}C]tyrosine was almost completely suppressed. Blackshear et al (17) observed dramatic increases in plasma amino acid concentrations 30 min after removal of the liver from the circulatory system of the rat; however, BCAA concentrations rose less than did those of many other amino acids. These results indicate that despite the importance of peripheral tissues, liver participates actively in BCAA degradation.

In view of the differences in the activities of BCAAT and BCKAD among organs, rates of degradation of both BCAA and BCKA may be expected to vary from organ to organ. In efforts to establish the relative contributions of different organs and tissues to overall BCAA metabolism, the degrading capabilities of both disrupted preparations (tissue homogenates, isolated mitochondria, and purified enzymes) and intact preparations (slices, organ perfusions, and isolated cells) have been investigated. In vitro assays on disrupted tissue preparations should represent maximum activities because they are not ordinarily limited by substrate concentration or, except for assays done with mitochondria, by transport of substrate to the site of enzyme activity. Separate measurements of BCAAT and BCKAD activities under such conditions should permit identification of the more rate-limiting of the initial steps of BCAA degradation in the tissue and, from knowledge of organ size, calculation of the enzymatic capacity of the organ. Most measurements of BCKAD, however, were made before it was known that the enzyme exists in active and inactive forms, so it is unclear how closely they reflect the activity of the enzyme in vivo. In experiments with intact tissue preparations, the ability of a tissue to oxidize BCAA is usually measured without drastic modification of transport systems, enzymes, intracellular relationships, or physiological concentrations of substrates. Preparations of this type from liver, kidney, skeletal muscle, adipose tissue, pancreas, stomach (111), diaphragm, heart (26), skin fibroblasts (47), and brain and nerve tissue (35) degrade BCAA actively. Comparisons between results obtained with disrupted and intact tissue preparations can be made for liver, skeletal muscle, heart, and kidney because for each of these, values have been reported for homogenates, tissue slices, and perfusions.

Enzyme Activities Determined on Disrupted Tissue Preparations

Values for BCAAT and BCKAD shown in Table 1 for disrupted preparations should represent maximum values. In liver, BCKAD activity is high (77, 173, 205, 240), and BCAAT activity is relatively low (32, 205); therefore, BCAAT is more rate-limiting than BCKAD. Conversely, BCKAD is more rate-limiting in skeletal muscle (227, 228) than BCAAT (32). Heart has very high BCAAT activity (32) and substantial BCKAD activity (23, 77, 228). In kidney as in heart, activities of both BCAAT (32) and BCKAD are high (23, 77). Interestingly, BCKA oxidation measured in heart during perfusion with a saturating

concentration of KIC was 550–800 nmol/min/g tissue, while with a saturating concentration of KIV, it was 1000 nmol/min/g tissue (24, 200), values much higher than those reported for disrupted tissue preparations (Table 1). These results suggest that the enzyme became more fully activated during the perfusions (see discussion on isoleucine and valine antagonism).

Rates of Metabolism of BCAA and BCKA in Intact Tissues

Measurements of rates of oxidation of L-[1–^{14}C]leucine or L-[1–^{14}C]valine that use intact tissue preparations with physiological concentrations of amino acids should provide reasonable estimates of the contributions of different tissues to total body BCAA oxidation. Also, from results obtained with the use of different concentrations of BCAA with such preparations, the validity of assumptions about BCAA metabolism based on enzyme measurements can be evaluated.

Liver slices, isolated hepatocytes, and perfused liver of the rat (Table 1) oxidize 0.1–1.0 mM L-[1–^{14}C]leucine at rates of 1.5–20 nmol/min/g tissue (45, 96, 164, 173, 186, 218). Skeletal muscle (hindquarter preparations), with high BCAAT and low BCKAD activities, i.e. the reverse of liver, oxidizes 0.1–0.5 mM L-[1–^{14}C]leucine at rates between 0.2 and 7.5 nmol/min/g tissue (103, 106, 218). Heart (slices and perfused organ), with high BCAAT and BCKAD activities, oxidizes 0.1–0.5 mM L-[1–^{14}C]leucine at rates between 2.5 and 10.2 nmol/min/g tissue (25, 26, 199, 200, 226). Perfused kidney oxidizes 0.2–1.0 mM L-[1–^{14}C]valine at rates between 1.1 and 28 nmol/min/g tissue (R. H. Miller and A. E. Harper, unpublished results). Oxidation rates of all of these intact tissues with physiological concentrations of substrate are similar despite their substantially different enzyme activities. This implies that factors other than enzyme concentrations, such as substrate and amino-acceptor concentrations, acyl-CoA to CoA and NADH to NAD^+ ratios, enzyme activation/inactivation, and transport, must influence flux through the BCAA catabolic pathway within tissues.

Also, with heart (26), skeletal muscle (218, 244), and adipose tissue (70), the rate of oxidation of [1–^{14}C]KIV (ketovaline) usually equals or exceeds that of [1–^{14}C]KIC (ketoleucine) (24, 228), yet the rate of oxidation of L-[1–^{14}C]leucine is two- to sixfold more than that of an equivalent concentration of L-[1–^{14}C]valine. These differences have been attributed to the fact that the K_m of the BCAAT is lower for leucine than for valine, with the result that KIV is produced less rapidly than KIC (see section on BCAA Aminotransferase).

An often-overlooked facet of control of metabolism of amino acids that are degraded in many tissues is the extent of blood flow to the different tissues and organs of the body. In the human adult, kidneys account for 0.5% of body weight and receive 23% of the total cardiac output; the liver, which accounts for 4% of body weight, receives 28% of the total cardiac output. Skeletal muscle

and adipose tissue receive only 16% and 2–3% of the total cardiac output respectively (74). These differential flow rates will influence the quantity of substrate reaching tissues and the rate of removal of metabolic products. Also, as blood flow is regulated, the rate of flow to certain organs can differ considerably in subjects as a result of different nutritional and physiological states, e.g. rate of flow to muscle during rest vs exercise. Noda & Ichihara (161) measured [U–^{14}C]leucine oxidation after ligating the renal arteries of rats and concluded that kidney could account for 20% of the leucine oxidized by the cortisol-treated rat fed a high-protein diet. Ligation of the blood vessels to the liver depressed the amount of leucine oxidized by about 40%. The high blood flow rates to the kidney and liver may account in part for the magnitude of these effects. In addition, release of BCKA from muscle into blood with subsequent uptake by liver may contribute to the importance of liver in whole-body BCAA metabolism (103, 133).

When the proportion of body weight represented by each of the various tissues and organs is taken into consideration, muscle, which makes up 35–40% (74) of total body weight, should contribute substantially to total body BCAA utilization even if its rate of BCAA metabolism is low. Direct measurements indicate that liver, accounting for 4% of body weight, is a more important site of BCAA catabolism than would be assumed on the basis of its low BCAAT activity. Tissues such as heart and kidneys, which each account for 0.5% of body weight, probably contribute less to BCAA metabolism than their high enzyme activities would suggest. Adipose tissue degrades 0.1–1.0 mM L-[1–^{14}C]leucine at rates comparable to those observed for heart and kidney, 1.0–11.0 nmoles/min/g tissue (70, 226). Brain, diaphragm, nerve, and other tissues oxidize BCAA in vitro at rates comparable to those of the tissues discussed above, so all contribute to total BCAA oxidation.

INTERORGAN RELATIONSHIPS IN BCAA AND BCKA METABOLISM

Upon being taken up into the intracellular pool of a tissue, BCAA are used for protein synthesis or undergo transamination to yield BCKA and glutamate. BCAAT is present in both the cytosol and the mitochondria, whereas BCKAD occurs only in mitochondria, so part of the BCKA formed in the transamination reaction may pass from the cytosol into the blood, rather than into the mitochondria, and be transported to other organs. The nitrogen released during catabolism of BCAA in various tissues must be transported to the liver for conversion to urea. Interorgan cooperativity is therefore to be anticipated in the disposal of both the nitrogen and the carbon of the BCAA.

Interorgan Cooperativity in the Metabolism of BCAA Nitrogen

In studies of arterio-venous differences in postabsorptive man, Felig et al (61) observed that BCAA were taken up by muscle and that alanine was released in an amount in excess of its relative abundance in muscle proteins. The splanchnic bed was the major site of removal of alanine (187). On the basis of their observations, Felig et al (61) proposed the existence of a glucose-alanine cycle for the shuttling of nitrogen and gluconeogenic substrate from muscle to liver; Mallette et al (137) proposed the existence of the same cycle based on reciprocal observations in liver. Marliss et al (141) observed that muscle released glutamine as well as alanine. Ruderman & Berger (196) used perfused rat hindquarter, and Odessey et al (166) used rat hemidiaphragm to demonstrate that after addition of BCAA to the media, alanine and glutamine were released in amounts well in excess of their relative abundance in muscle proteins, thus indicating that amino groups from BCAA were being used for synthesis of alanine and glutamine. Release of glutamine from human muscle has been reported frequently (2, 12, 55, 230). With diaphragm from starved rats, addition of valine to the medium stimulated release of alanine in excess of that observed with diaphragm from fed rats (214). Starvation was also shown to enhance the oxidation of leucine by diaphragm (164), which thereby increased the amount of nitrogen available for alanine synthesis.

Felig (60) and Ruderman (195) have discussed in detail synthesis of alanine and glutamine in muscle and amino acid exchange across and between tissues. The splanchnic bed has been shown to extract alanine and glutamine from the circulation, and in subjects in the fed state, BCAA uptake can account for more than 50% of the total peripheral amino acid uptake by leg muscle. Recently, Haymond & Miles (99) infused [^{15}N]leucine into postabsorptive man and found 28% of the leucine nitrogen in alanine. Galim et al (73) found 30–53% of nitrogen from [^{15}N]leucine in alanine in dogs. These in vivo studies have demonstrated conclusively that leucine nitrogen is used in alanine formation. Using ^{15}N labeling, Golden et al (79) have shown that BCAA donate their nitrogen to glutamine in vivo. Also, after infusions of leucine into human subjects, glutamine has been found to rise while alanine falls (2, 12, 55, 83). What determines the ultimate fate of BCAA nitrogen in muscle is not clear.

How the nitrogen from BCAA is transferred to glutamine has received little attention. Because α-ketoglutarate is an effective amino-acceptor for BCAAT, BCAA amino groups should be used efficiently to form glutamate, which in the presence of pyruvate and the glutamate-pyruvate aminotransferase (GPT) can readily pass BCAA amino groups on to form alanine. Golden et al (79), however, found that in human subjects who were administered [^{15}N]valine or leucine, the amide nitrogen of glutamine was more greatly enriched with ^{15}N than the amino group. For this to occur, the amino group of the BCAA first

must be converted to ammonia. This could occur via the glutamate dehydrogenase reaction, but the activity of this enzyme in muscle is very low (237). An alternative possibility is transfer of the amino group of glutamate to oxaloacetate to form aspartate, with the aspartate being used in the purine cycle for regeneration of AMP from inosinic acid. Lowenstein (135) has suggested that this cycle might function catalytically in the conversion of amino groups from amino acids to ammonia. The activity of glutamate-oxalacetate aminotransferase (GOT) in muscle is as much as 30 times that of GPT, which in turn is about 6 times that of BCAAT (122). The proposed pathways for transfer of nitrogen from BCAA to either alanine or glutamine are shown in Figure 2.

In a study of transamination by a muscle preparation in vitro, Cree (46) included each of the BCAA in the medium at 0.4 mM, used pyruvate, α-ketoglutarate, and oxaloacetate (OAA) at 2.4 mM each as amino-acceptors, and monitored formation of alanine, glutamate, and aspartate. Aspartate accumulated steadily throughout the experiment, glutamate accumulated during the initial 10 min, and little alanine accumulated throughout the entire 30 min of incubation. Although only low concentrations of OAA are present in muscle (122), glutamate concentration is 4–10 times that of aspartate; because the equilibrium constant of GOT for aspartate formation is 6.52 (123), aspartate formation might therefore be anticipated. Alanine concentration in muscle exceeds that of glutamate, which might be expected to inhibit alanine formation. In fact, Krebs (122) reported that glutamine production by rat hindquarter preparation increased after alanine was added to the perfusion medium. The rates of formation of aspartate and alanine would be influenced by many factors, particularly by processes that alter the concentrations of any of the compounds participating in these reactions. This complex of reactions might account for the variations observed in the relative rates of release of alanine and glutamine and in the variable degree of labeling of alanine and glutamine nitrogen with BCAA nitrogen. Transfer of amino groups from BCAA via glutamate and aspartate to IMP with their subsequent release from AMP (135) would provide a pathway for conversion of BCAA amino-N to ammonia and hence to the amide-N of glutamine, which is released into blood in vivo along with alanine.

The source of carbon for de novo synthesis of alanine in muscle has been disputed. Pyruvate from glycolysis has been claimed by several groups to be the source of alanine carbon (60, 78, 195); others have proposed that plasma and tissue amino acids provide carbon for alanine synthesis (75, 76, 213). The carbon for glutamine synthesis is considered to come primarily from amino acids (78, 195). Using [U–^{14}C]valine, Hutson & Zapalowski (105) did not find significant amounts of ^{14}C in either alanine or glutamine in the free amino acid pool, but ^{14}C from leucine, which is ketogenic and cannot give rise to net synthesis of glutamate, was readily incorporated into glutamate and glutamine,

presumably via the tricarboxylic acid (TCA) cycle. BCAA apparently do not contribute significantly to either alanine or glutamine carbon-skeleton formation in rat muscle, but α-ketoglutarate from the TCA cycle does. Whatever the carbon source for their formation, alanine and glutamine provide a shuttle for transfer of BCAA nitrogen from muscle to liver for urea formation (137). Glutamine serves as an energy source for the small intestine (239) and as a source of ammonia and a substrate for glucose formation in kidney (100).

Interorgan Cooperativity in the Metabolism of BCAA Carbon

It is clear from tissue analyses that BCAA and BCKA do not accumulate to high concentrations in tissues (104, 133), so any excess must be oxidized or released into the blood. During perfusions of rat hindquarter (103, 106), heart (199), and kidney (153) with media containing concentrations of BCAA in the physiological range, BCKA were released into the perfusate. When fat pads (226), muscle (120), heart (226), kidney (49), diaphragm (205, 228), or brain slices (203, 205) were incubated with physiological concentrations of leucine, KIC accumulated in the media. BCKA are present in blood of rats (104, 133), dogs (3, 148), and humans (2, 118, 189, 202). Also, β-hydroxyisobutyrate, a metabolite of valine degradation, is released during perfusion of rat hindquarter (217) and heart (182). All of these studies indicate that BCKA and some other BCAA metabolites are released into the blood, media, or perfusate by tissues; further, in the rat, blood concentrations exceed those in tissues.

Release of BCKA into the extracellular fluid might be anticipated if the rate of BCAA transamination in a tissue greatly exceeds that of BCKA oxidation. Under these conditions, BCKA would also be expected to accumulate within the cells, but this does not occur even in muscle, where the ratio of aminotransferase to dehydrogenase is the highest of any tissue examined. In fact, the rate of oxidation of leucine by the rat hindquarter increased roughly fivefold when perfusate leucine concentration was increased from 0.1 to 0.5 mM, but BCKA were still released into the medium. This suggests that BCKA may be transported specifically out of the tissue. The discovery that albumin has binding sites for BCKA (132, 134, 160) suggests that albumin binding can serve as a mechanism for concentrating BCKA in plasma and may explain the gradient between blood and tissues (134, 160).

The contributions of different tissues to the plasma BCKA pool are not known. The mass of the tissues, the quantities of BCAAT and BCKAD they contain, and blood flow to the tissues would all influence the contributions. Because of its large mass and high BCAAT activity, skeletal muscle would be expected to provide the bulk of the BCKA in the circulation. In view of the release of BCKA by tissue preparations, accumulation of BCKA in blood, and the high BCKAD activity of liver, Harper and coworkers (94, 95) proposed that

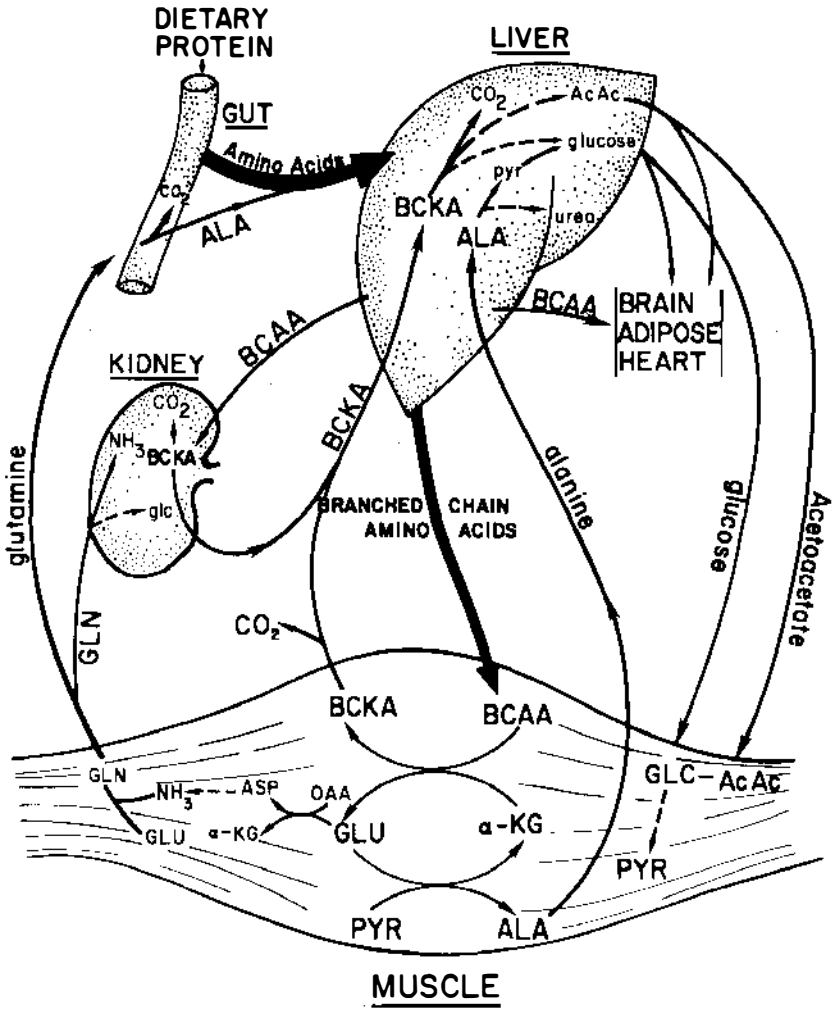

Figure 2 Interorgan cooperativity in the metabolism of BCAA carbon and nitrogen.

interorgan cooperation is involved in the metabolism not only of the nitrogen but also of the carbon of the BCAA.

Evidence of Interorgan Relationships in BCAA and BCKA Metabolism

Evidence supporting the concept of interorgan relationships in BCAA and BCKA metabolism has been accumulating (see Figure 2). Hutson & Harper (104) observed that BCKA concentrations in rat plasma and tissues are responsive to changes in nutritional and metabolic states. Livesey & Lund (133)

determined that liver could extract a quantity of BCKA equivalent to that released by muscle. Several investigators (110, 136, 238) have demonstrated that isolated hepatocytes can readily convert KIC to ketone bodies. Recently, Matthews et al (143) demonstrated a direct linear relationship in humans between increasing plasma concentrations of BCAA and those of their respective keto acids. Pozefsky & Walser (189) demonstrated that 52% of the KIC infused into six men in the postabsorptive state was extracted in a single pass across forearm muscle and that the leucine released could account for 31% of the KIC extracted. Abumrad et al (3) used postabsorptive dogs with implanted venous catheters and demonstrated that after infusion of KIC into the gut, roughly 59% of the absorbed KIC was taken up by the liver; one-third of this was transaminated to leucine. There was also an increase in ketone body production. Over 80% of the KIC extracted by kidneys was released as leucine. The ability of tissues to reaminate BCKA and release the respective amino acids has also been shown in vitro using rat brain, heart, and kidney slices (23), and perfused rat hindquarter (103), liver (233), and kidney (155). This ability of tissues to reaminate BCKA provides the basis for treating renal disease patients with BCKA as a means of reducing the nitrogen load that must be cleared by the kidney (232).

Nissen & Haymond (159) measured radioactivity in transamination products after simultaneous infusion of tracer doses of L-[4,5–^{3}H]leucine and [U–^{14}C]KIC into postabsorptive dogs and demonstrated interconversion of leucine to KIC and vice versa. Their results suggest a two-pool reversible model whereby nearly 80% of the total leucine carbon entering the circulation is converted to KIC, of which 66% is subsequently reaminated. Matthews et al (142) determined the fate of L-[1–^{13}C, ^{15}N]leucine infused into postabsorptive humans and demonstrated that the rate of irreversible loss of leucine (oxidation of KIC) was only 9% of the leucine flux, while reamination of KIC to leucine was 91% of the flux. These values changed slightly when subjects were fed; 21% of the flux was oxidized, and 79% was reaminated. In dogs, ^{15}N from [^{15}N]leucine was found in valine, isoleucine, and alanine in blood (73). These results suggest that cycling of BCAA carbon skeletons via transamination-reamination is much more extensive than irreversible decarboxylation but that this depends on BCAA intake. In other experiments on humans (99, 143, 189), substantial recycling of leucine carbon via transamination-reamination has been observed.

Although considerable evidence indicates that extensive cycling of BCAA and BCKA occurs in rats, dogs, and humans, a question has been raised as to whether skeletal muscle is as important in interorgan relationships in human BCAA metabolism as in the BCAA metabolism of the rat (2, 55). Human muscle contains 60% of the total body BCKA dehydrogenase, while rat muscle contains 10–30% (111, 119, 228); however, this is mainly because the activity

in human liver is low, not because that in muscle is high. Although a greater proportion of BCKA may be oxidized in human than in rat muscle, BCKA are released from human muscle and circulate in the blood.

Results obtained with stable isotopes indicate that cycling of BCAA and BCKA is a substantial component of interorgan relationships in the metabolism of the BCAA. Reamination of BCKA, with transport of both substrate and product among tissues, can serve as a mechanism for conservation of nutritionally indispensable BCAA carbon skeletons.

Estimation of Capacity for BCAA Metabolism In Vivo

The values in Table 1 clearly demonstrate that activity of BCAAT exceeds that of BCKAD in tissues other than liver. If, on the basis of these measurements, we accept that BCKAD is the rate-limiting enzyme for BCAA metabolism in the body as a whole, we can then compare estimates of whole-body capacity for BCAA metabolism based on (*a*) maximum BCKAD activities; (*b*) rates of oxidation of BCAA by intact tissues; and (*c*) rates of oxidation of BCAA in vivo.

Based on estimates of maximum BCKAD activities (Table 1), the BCAA oxidizing capacity of a 100-g rat is 350–810 μmol/hr (191). These are conservative estimates, as the degree of activation of BCKAD under different conditions in vivo remains to be established. Moreover, although all tissues tested to date have BCKAD activity, only muscle, liver, kidney, heart, and brain were included in calculating the potential capacity; the values therefore underestimate the true total oxidizing capacity of the rat.

Results from studies done with intact tissue systems allow for another means of estimating total-body BCAA oxidative capacity. A limitation of the method is its inability to estimate the contributions of all tissues and the influence of blood flow rates on overall BCAA metabolism; the accuracy of the estimates produced is therefore also limited. Nonetheless, if we accept the range of 0.1–0.5 mM leucine as within physiological concentrations and if we accept the oxidation rates reported in Table 1 for liver, muscle, heart, and kidney, and the estimates for tissues for which oxidation measurements are available (e.g. adipose tissue, brain, stomach and intestine), we obtain oxidation rates of 3–26 μmol/hr/100 g body wt. These values are from 0.4 to 8% of the total body capacity estimate based on maximum enzyme activities.

Another consideration in estimating the capacity of intact tissues for BCAA oxidation is interorgan relationships in BCAA metabolism. Assuming that all of the KIC released from muscle at leucine concentrations of 0.1–0.5 mM, reported to be 0.9–15 μmol/hr/100 g body wt (103), is removed from the circulation and oxidized by the liver (133), the estimated rate of whole-body leucine oxidation would increase to 4–51 μmol/hr/100 g body wt.

There have been few studies of BCAA oxidation in vivo. In a study with rats,

the rate of oxidation of L-[1–^{14}C]leucine increased dramatically after the leucine requirement of approximately 0.75% of the diet had been met (89). With leucine intakes of 0–0.6% of the diet, oxidation rates were 2.5–3.3 μmol/hr/100 g body wt; with leucine intakes equivalent to that of a chow-fed rat (20% of protein, 2% of leucine in the diet), the oxidation rate was 83 μmol/hr/100 g body wt. In a study of valine oxidation with rats consuming a low-protein diet containing 1% of valine, the rate of oxidation of L-[1–^{14}C]valine was about 13.3 μmol/hr/100 g body wt (18). The rate of oxidation of BCAA in vivo thus depends upon BCAA intake and ranges from 3–83 μmol/hr/100 g body wt. The agreement between this estimate and that based on measurement of intact tissues of 4–51 μmol/hr/100 g body wt is reasonably satisfactory despite the limitations of the procedure. Estimates of total BCAA oxidation capacity based on maximum BCKAD activities of rat tissues would be valid only if BCAA concentrations were greatly in excess of those ordinarily present in tissues.

Work by Meguid et al (150) allows for similar calculations of in vivo oxidation rates for human subjects. Oxidation rates for L-[1–^{13}C]leucine ranged from 0.7 μmol/hr/100 g body wt at low leucine intakes (4 mg/kg/day) to 3.2 μmol/hr/100 g body wt at high leucine intakes (80 mg/kg/day). Rates of oxidation of L-[1–^{13}C]valine were 0.5 μmol/hr/100 g body wt at low valine intakes (4 mg/kg/day), 0.7 μmol/hr/100 g body wt at approximately the valine requirement (16 mg/kg/day), and 2.1 μmol/hr/100 g body wt at high valine intakes (70 mg/kg/day). So, although rats have 10–100 times the BCKAD activity (119) and 5–10 times the BCAAT activity (111) of humans, BCAA oxidation rates of both humans and rats are low, 0.7 and 3.0 μmol/hr/100 g body wt respectively, when leucine intake is less than the requirement. These low rates of oxidation when leucine intake is low should enable the organism to conserve whatever BCAA are available for protein synthesis.

It appears that rats have a greater oxidative response to increased BCAA intake than humans. In the rat, an intake of 2.7 times the leucine requirement (2.0% dietary leucine) caused the oxidation rate to increase 18-fold over the rate observed when intake equalled the leucine requirement; in humans, a leucine intake of 5.5 times the requirement caused the oxidation rate to increase 3- to 5-fold above the rate when intake equalled the leucine requirement.

EFFECTS OF DIET AND HORMONES ON BCAA METABOLISM

Most of the enzymes of amino acid catabolism that are confined mainly to the liver show increased activity in response to increased protein intake (66). Most of these enzymes are also induced to high activity in animals administered hormones such as glucagon and cortisol that stimulate gluconeogenesis (185). This adaptability of amino acid–degrading enzymes provides a mechanism

whereby the capacity of amino acid–degrading systems can be modulated in response to the changing metabolic demands caused by alterations in nutritional or endocrine states. Although there have been a number of reports on the effects of such treatments on BCAA metabolism and degrading enzymes, there have been few methodical investigations of the subject. This may be because the responses observed generally have not been consistent and have usually been small and were thus considered to be of little physiological significance.

BCAA Aminotransferase

DIET Effects of changes in dietary protein content on the activity of BCAAT in tissues have been neither striking nor consistent. The two- to threefold increase in the activity of liver cytosolic BCAAT II (leucine-specific) in rats fed a high-protein (50% or more) diet seems to be well established (112, 122, 207), but the response appears to be proportional to dietary protein content only over the 25–50% of protein range (207). The general mitochondrial BCAAT I of liver does not appear to increase in activity in response to increasing protein intake (112, 122, 240). The effects of protein deficiency observed have not been consistent; decreases (146), increases (34, 154), and no change (209) have been reported.

The situation is similar for kidney BCAAT; no response to a high-protein intake was reported in two studies (112, 240), but modest increases were reported in other studies of the rat (154) and the chick (59). Similarly, responses in animals fed low-protein and protein-free diets have been inconsistent; no response (7, 209), a modest increase (34), and a decrease (154) have been observed. The only effects of a high protein intake on BCAAT in muscle reported are a modest increase in the rat (154) and no change in the chick (59). Of the several groups who have studied effects of low-protein or protein-free diets on muscle BCAAT, two reported no change (34, 210), two reported increased activity (154, 209), and one reported a decrease (7). Except for the response of BCAAT II of rat liver, changes in BCAAT activity due to diet modification have not exceeded 50% of the control value, well below those observed for most other amino acid–degrading enzymes. In view of the immense capacity of the organism for BCAA transamination, the physiological significance of the BCAAT responses observed is questionable.

Starvation does not appear to alter liver BCAAT (7, 112, 240). Modest increases in kidney BCAAT have been reported (7, 240), and in one study (7) a rise was observed in muscle BCAAT of rats that were not fed for only one day.

BCAAT has also been measured in tissues of rats that consumed diets containing BCKA in amounts that were approximately double the requirement for BCAA (34) and in kidney perfused with 2 mM KIC (155, 156). BCAAT increased about 30% in liver and muscle; the results in kidney were inconsis-

tent, however, as dietary BCKA caused a 30% decrease and perfusion with BCKA caused a 30% increase. In rats fed a high-leucine diet, liver BCAAT was unaffected; kidney activity rose about 35%, but variability was high (240).

HORMONES Hormonal effects on the activity of BCAAT have been studied only sporadically. The activity of the leucine-specific enzyme II of liver increases three- to sixfold in rats injected with 10 mg cortisol/100 g body wt (112, 207). BCAAT I of liver does not seem to be altered in animals treated with cortisol, and cortisol effects observed in kidney have been small (112, 154, 240) except for the observation of Shirai & Ichihara (207), who reported a threefold increase in kidney BCAAT in cortisol-treated rats and a 50% decline after adrenalectomy. A small rise in muscle BCAAT in cortisol-treated rats has also been reported (154). BCAAT increased threefold in kidneys of diabetic rats and returned to the basal level when they were treated with insulin, but the liver enzyme was unaffected (112). In rats treated with growth hormone, activity of BCAAT in liver fell about 25%; in hypophysectomized rats, it increased about 40% in liver and doubled in kidney (207).

BCKA Dehydrogenase

Most investigations of effects of diet and hormones on the activity of BCKAD were done before much was known about activation of this enzyme. Differences in incubation time and in the composition of the assay media can influence the degree of activation of BCKAD. Also, when measurements of BCKAD are made using intact mitochondria, a change in activity may reflect a change in the rate of transport of substrate across the mitochondrial membrane rather than a change in true enzyme activity. Results of assays of BCKAD activity in liver mitochondria in the presence and absence of high concentrations (2.5 mM) of Ca^{2+} illustrate this problem (see section on BCKA Dehydrogenase). In rats fed a high-protein (50% casein) diet, liver BCKAD activity increased; when there was no Ca^{2+} in the assay medium, the increase was between two- and threefold, but when Ca^{2+} was present, it was five- to sixfold (53). Hence, information available on BCKAD activity in animals that are in different physiological states or that have been subjected to various treatments must be viewed with reservation. It is assumed in the discussion below that when measurements have been made under uniform conditions in a single study, meaningful comparisons are obtained.

DIET In the earliest study of effects of dietary protein content (240), liver BCKAD activity in the rat was assayed in the presence of Ca^{2+}; a fourfold increase was observed when the dietary level of casein was increased from 0 to 30%. No further increase was observed when dietary protein content was raised to 80%. Renal BCKAD did not increase significantly in rats fed the highest

dietary level of protein, but because kidney size increased greatly, total kidney BCKAD capacity rose significantly. In chicks fed a high-protein (75% soybean protein) diet, liver BCKAD was elevated fivefold or more over control values; kidney BCKAD, less than twofold; and muscle not at all. The observations on hepatic BCKAD responses in rats fed a low-to-adequate level of protein have been confirmed (98). In this study, no effect on kidney or brain BCKAD was observed when the dietary protein content was increased from 0 to 22%. The full response of mitochondrial BCKAD in liver of rats fed a high-protein diet can be measured only if Ca^{2+} or some other agent that swells the mitochondrial membrane is included in the medium, as indicated above. Dixon (52) examined the response of the cytosolic leucine (methionine) decarboxylase in rats fed 9 or 50% casein diets. The cytoplasmic enzyme, representing about 10–15% of liver leucine decarboxylating activity (145, 197), increased about threefold when rats were fed the 50% casein diet for five days. An unusual observation reported by Hauschildt & Brand (97) is that BCKAD falls to very low activity in liver of rats fed low-nitrogen diets in which amino acids have been substituted for protein.

There have been a number of studies of effects on BCKAD activity, particularly with BCKAD in muscle, of feeding rats protein-deficient or protein-free diets. In most of these the amino acids leucine or valine were used as substrates instead of the α-ketoacids. In the earliest such study, McFarlane & von Holt (147) found depressed BCKAD activity in muscle of protein-depleted (2% casein for four months) rats. Subsequently, Sketcher et al (209, 210) also observed that BCKAD activity was depressed in muscle of protein-depleted rats on the order of 50% below values for rats fed adequate diets. Reeds (193) reported that liver and muscle BCKAD activities, measured with valine as the substrate, were depressed. In assessing BCKAD activity with BCAA as substrate, it is assumed that the supply of substrate for BCKAD is not limited by the rate of transamination (205). In these studies it is not clear whether the actual enzyme concentration or the degree of activation of BCKAD has been altered (77); nevertheless, the lower BCKAD activities observed are in agreement with results from other studies on less severely protein-depleted rats (98, 240).

Results of in vivo studies of metabolism of [1–^{14}C]BCAA done in conjunction with several of these enzyme studies correspond well with the in vitro measurements. In studies in which [1–^{14}C]BCAA were used (193, 209, 210) as the tracers, oxidation of BCAA by protein-depleted rats was depressed. Subsequently, low rates of oxidation of [1–^{14}C]valine by hindlimb preparations from protein-depleted rats were reported (211). Neale & Waterlow (157) used [U–^{14}C]leucine and found no evidence that rats fed protein-free or low-protein diets had the ability to conserve BCAA, which suggested that overall BCAA catabolism was not depressed by an inadequate protein intake. Reeds (193) however, found that oxidation of [1–^{14}C]valine was depressed in vivo when

rats were fed only 2.5% of casein, but that oxidation of [U–^{14}C]valine was not. The results of the studies with [1–^{14}C]BCAA have been consistent with the enzyme measurements. The difficulties encountered in obtaining meaningful measurements of in vivo oxidation rates with [U–^{14}C]BCAA may be the result of pool changes or channeling of carbon into alternate pathways (158, 193).

In an effort to resolve some of these questions, BCKAD activity has been measured in our laboratory in liver and muscle of rats fed diets containing 0–50% of casein for six days (A. E. Harper, S. Soemitro, P. Crowell, and K. P. Block, unpublished data). After six days, BCKAD activity in liver of rats fed the 50% casein diet was about threefold that in liver of rats fed a protein-free diet, but the degree of activation of the enzyme in liver of rats fed the protein-free diet was only about 40%, while it was 75% in liver of rats fed the 50% casein diet. In contrast, BCKAD activity in muscle did not appear to be influenced by protein intake, but in all groups the degree of activation of the enzyme complex was only about 15%, in agreement with observations by Randle et al (191). Although no change in the activation state of muscle BCKAD was detected, any small alteration in the degree of activation would have a marked effect on BCAA metabolism in vivo because of the high percentage of muscle mass in the body. Gillim et al (77) reported that liver from rats fed a low-protein diet contains only one-third as much fully activated BCKAD as liver from rats fed a high-protein diet. They also reported that in vivo only 30% of BCKAD is in the active form when protein intake is low, compared to 100% when protein intake is high (77). Neither the degree of activation nor the fully activated BCKAD in heart was influenced by protein intake (77). Fully activated BCKAD in kidney was about 20% less in rats fed low-protein diets than in those fed high-protein diets, but the degree of activation in those fed low-protein diets was 55%, compared to 70% in those fed high-protein diets (77).

A number of observations indicate that the activity of liver BCKAD increases in rats fed diets that contain large amounts of individual BCAA or BCKA (18, 120, 206, 240). The effect does not seem to be specific for BCAA, as similar responses are observed with other amino acids (240).

The rate of oxidation of leucine in vivo increased when rats were kept without food for one day (151, 209). Diaphragm (164) and isolated hindquarter preparations (106, 244) from starved rats oxidized leucine and valine at increased rates. Oxidation of BCAA by muscle increased between 30% and 50% in rats starved for five days (175), and this was associated with increased mitochondrial proliferation (6). In rats kept without food for 48 hours, fully activated BCKAD in heart was elevated about 50%, but only 15% of the enzyme was in the active form (77). BCKAD activity in liver increased more than twofold when rats fed a low-protein diet were starved for one day, but activity in kidney was unaffected (240). Little effect of starvation was observed

on the fully activated BCKAD or the extent of activation in either liver or kidney (77). These observations with kidney are in accord with those of Wohlhueter (240), but the observations with liver are not. A stimulative effect of starvation was observed in the earlier study (240); however, in that study, rats were fed a low-protein diet in which BCKAD in liver is now known to be only 30% active (77). The lack of effect observed subsequently was in rats fed an adequate diet in which liver BCKAD was already fully activated (77).

HORMONES The activity of BCKAD in diaphragm (27), skeletal muscle, kidney (176), and liver (145) of diabetic rats is elevated. The increased activity in liver appears to be associated with mitochondrial proliferation (145). The rates of both KIC oxidation are elevated in perfused rat hindquarter from diabetic rats (244). The rate is depressed by addition of acetoacetate to the perfusion medium, just as it is in isolated skeletal muscle (176). Paul & Adibi (177) reported that diabetes did not alter the activity of fully activated BCKAD in either liver or skeletal muscle in rats, but in diabetic rats the enzyme was 80% activated compared with only 40% in controls. Fully activated BCKAD activities in kidney and heart of diabetic rats differ little from those of controls; the degree of activation in kidney was 75%, the same for both diabetic and non-diabetic rats, and BCKAD in heart was 25% active in diabetics, compared to 50% for controls (77).

Goodman and associates (80) have observed that growth hormone increases leucine oxidation by adipose tissue preparations from hypophysectomized rats, but when the hormone is administered chronically to hypophysectomized rats, it has little effect. They have also observed that incubation of adipose tissue with insulin activates BCKAD within 60 min and causes a reduction in the K_m (69). In adipose tissue isolated from rats after hypophysectomy and thyroidectomy, but not adrenalectomy, the rate of leucine oxidation was doubled (40). Administration of the appropriate hormones restored the rate to control values. Sullivan et al (219) reported a marked but transient increase in BCKAD in rat liver mitochondria in hypophysectomized rats. The magnitude of the increase was different for each BCAA, both initially and after the activities became stable at new steady states three weeks later. The authors suggested that these observations could be explained by the presence of three decarboxylases in the mitochondria or by the occurrence of unusual allosteric changes in the enzyme.

In perfused rat liver, epinephrine caused a significant but transient depression of BCKA oxidation associated with a reduction of the mitochondrial pyridine nucleotide oxidation-reduction state; the size of the effect depended on the concentration of calcium in the medium (29). Epinephrine stimulated BCAA oxidation by perfused heart from fed or starved rats unless glucose was added to the medium (25). It also stimulated oxidation of BCAA by diaphragm from starved rats, but not from fed rats. Glucagon stimulated BCAA oxidation

by perfused hearts from starved rats, but not from fed rats; it did not affect oxidation of BCAA by diaphragm.

Observed effects of dietary and hormonal treatments on the activities in animals of BCAAT and BCKAD, the initial two enzymes in the pathway for BCAA catabolism, have not been entirely consistent. They do nevertheless reveal that these enzymes are much less responsive to dietary and hormonal manipulations than are most other amino acid–degrading enzymes. The enzymes also differ from most of the rate-limiting enzymes for degradation of other indispensable amino acids in that (*a*) they are not confined to the liver but are distributed widely throughout the body, (*b*) the initial reaction is a readily reversible transamination, and (*c*) the catalyst for the subsequent irreversible oxidative decarboxylation, BCKAD, which is rate-limiting in most tissues, exists in both active and inactive forms. Enzyme adaptation or induction would therefore appear to be much less important in controlling flux through the BCAA catabolic pathway than (*a*) the rate of BCAA transamination, which controls the supply of substrate for BCKAD, (*b*) the rate of transport of BCKA or BCAA into mitochondria, and (*c*) regulation of the degree of activation of BCKAD. There is sparse information about the extent to which dietary and endocrine treatments modify the degree of activation of BCKAD, but the limited evidence available indicates that it can be affected by both the endocrine and nutritional state of the organism and by substrate supply and that the responses are different in different organs.

BLOOD AND TISSUE BCAA CONCENTRATIONS

Diet composition, endocrine state, and various pathologic conditions can influence plasma and tissue BCAA concentrations in mammals. Changes in patients with trauma, injury, sepsis, and cirrhosis have been examined as possible indexes of metabolic state and for their prognostic value. In general, plasma BCAA concentrations are low in cirrhotic, traumatized, and septic patients. It is difficult to identify changes as characteristic of specific conditions because differences in the nutritional and pathologic states of individual patients influence amino acid patterns. This subject has been reviewed (10, 67, 72, 121) and will not be discussed here except for an observation by Fürst et al (72) that appears unique. In sepsis and severe injury, BCAA accumulate in muscle but decline in plasma. This causes plasma and muscle BCAA concentrations to shift in opposite directions rather than in the same direction as is usually observed. These changes suggest that transport of, or the transport system for, large neutral amino acids is impaired under these conditions (121).

Plasma BCAA concentrations in mammals tend to be directly proportional to protein intake. Frame (65) noted in 1958 that in human subjects who had consumed a high-protein meal, plasma amino acid concentrations generally

were elevated but BCAA concentrations were elevated disproportionately. Shortly thereafter, Arroyave, as well as Holt and associates, reported that plasma concentrations of BCAA were unduly diminished in children suffering from protein-calorie malnutrition (see Ref. 235 for review). It was thought that these changes might be of diagnostic value in this condition (235). Since then, many observations in laboratory animals and human subjects (4, 63) have confirmed that BCAA concentrations in plasma decline after consumption of a protein-free or low-protein diet and rise disproportionately in both plasma and muscle after consumption of a high-protein diet (93). Of particular interest is the observation that plasma concentrations of BCAA remain elevated after animals adjust to a high-protein diet. Concentrations of most other amino acids decline toward control values after animals have adjusted to a high-protein diet (11, 93), and this suggests that the capacity for BCAA degradation may be exceeded when protein intake is high. Adaptive responses of most amino acid–degrading enzymes occur when animals are fed a high-protein diet, but BCAA-degrading enzymes respond little. Despite this, calculations of total BCAA-degrading capacity indicate it is unlikely that enzyme activity would become limiting (see section on Estimation of Capacity for BCAA Metabolism In Vivo). The BCAA intake of a 150-g rat increases by about 5000 μmol when the protein content of the diet is increased from 20 to 50%, yet the increase in the body BCAA pool is only about 100 μmol. It therefore appears that some factor other than limited oxidative capacity is responsible for the elevated BCAA pools of rats adapted to high protein intakes. Under conditions of high protein intake and with the ready reversibility of the BCAA aminotransferase reaction (122), high tissue glutamate and BCAA concentrations may result in considerable reamination of BCKA (142) and therefore in elevated steady-state concentrations of BCAA.

Plasma and muscle BCAA concentrations in both human subjects and laboratory animals are also elevated during starvation (4, 72, 104). Prolonging starvation for two weeks results in a subsequent decline of plasma BCAA concentrations toward basal values in human adults (4). Even a short period of fasting reduces circulating insulin concentration and increases glucagon concentration while proteolytic degradation of muscle provides amino acids for gluconeogenesis (188). Insulin addition stimulated uptake and oxidation of leucine by isolated perfused hindquarter from starved rats (106). Insulin infusions depressed plasma BCAA concentrations in dogs starved for 18 hr (1) and in fasted human subjects (140). These observations indicate that changes in the amount of insulin in the circulation are important in regulating both the free pools and the metabolism of BCAA.

The effects of insulin have some bearing on the lack of relationship between changes in BCAA pool size and changes in BCKAD activity in starvation. The observations that increased pool size is accompanied by increased BCKAD

dehydrogenase activity and increased BCAA oxidation in muscle in vitro and in the isolated rat hindquarter seem anomalous. However, the plasma pool might be expected to expand despite the increased capacity for BCAA oxidation if uptake of BCAA by muscle is depressed by low circulating insulin concentration during starvation. As the muscle BCAA pool increases in starved animals, presumably from accumulating amino acids released by muscle proteolysis, an expansion of the plasma pool could readily be accompanied by an elevated rate of BCAA oxidation. Nissen & Haymond (159) used infusions of leucine labeled with either of two different stable isotopes in dogs starved for 96 hours, and found that the oxidation rate decreased between 14 and 96 hours of starvation. This suggests that in prolonged starvation, decreased muscle degradation causes a decrease in pool size, which results in depressed BCAA oxidation and increased conservation.

Elia et al (54) also observed that leucine clearance was prolonged in human subjects after four days of starvation. The plasma pool of BCAA was elevated throughout the four days, which suggested to the authors, as to Sherwin (204), that elevated plasma BCAA pools are associated with depressed BCAA oxidation.

It has been known since 1958 that plasma BCAA pools are elevated in diabetic humans and animals (10, 15, 17, 54). Plasma BCKA pools are also elevated in diabetic humans and animals (104, 201). Pools of BCAA and BCKA in muscle are elevated in diabetic rats (104). Administration of insulin restores the BCAA pools to basal values in human subjects (15, 201), and plasma BCAA concentrations are low in patients with insulinomas (15). A highly significant correlation was observed between blood glucose and BCAA concentrations in studies of these patients, which suggested that, in diabetes as in starvation, there is an inverse relationship between circulating insulin concentrations and blood BCAA concentrations. In diabetic and starved animals, elevated plasma and muscle pools seem incompatible with elevated rates of BCAA oxidation by skeletal muscle (176) and elevated BCKAD activity in liver (145). Control of BCAA entry into muscle by insulin may contribute toward an explanation for this incongruity, as it seems to in the case of starvation. If low circulating insulin concentrations curtail uptake of BCAA by muscle, plasma BCAA concentrations might rise while muscle protein degradation contributes to BCAA accumulation. Increased oxidation might then occur in this tissue without causing depressed plasma BCAA pools.

BCAA ANTAGONISM

Interactions among the BCAA themselves can lead to changes in plasma and tissue BCAA pools. In particular, high intakes of leucine by human subjects or animals depress valine and isoleucine concentrations in blood and muscle.

These responses appear to be an important component of a BCAA antagonism observed in animals.

A leucine-induced BCAA antagonism was first observed in animals in 1954, when addition of 3% of L-leucine to a low-protein (9% casein) diet was found to cause marked growth depression in rats that could be partially overcome by a supplement of isoleucine (91). Subsequently, Benton et al (14) observed that the growth depression caused by addition of 3% leucine to the diet was largely overcome when supplements of both isoleucine and valine were provided. These observations indicated that excess leucine increased the requirement for isoleucine and valine in the rat.

Mutual antagonisms among the BCAA had been described earlier in bacteria (50); because of the structural similarities among the BCAA molecules, it had been attributed to antimetabolite action. Similarly, the effects of excess leucine on the growth of the rat were assumed to be the result of leucine acting as an antimetabolite of isoleucine and valine (90). In contrast to observations with bacteria, however, addition of excess isoleucine or valine to the low-protein diet resulted in only slight depression in growth of the rat (90). Only when the 9% casein diet was modified so that leucine became growth-limiting instead of methionine or threonine could excesses of valine or isoleucine be shown to decrease the utilization of leucine for growth (14). Valine-isoleucine antagonisms have also been demonstrated under appropriate conditions (14). All three BCAA evidently can participate in mutual antagonisms; however, only the leucine-induced BCAA antagonism can be demonstrated without careful manipulation of the amino acid composition of the basal diet. Leucine-induced BCAA antagonisms have been produced in chicks, pigs, and turkey poults (31, 92, 212).

Although supplements of isoleucine and valine to a high-leucine, low-protein diet reverse most of the growth-depressing effects of excess leucine, further additions of phenylalanine, tryptophan, and threonine are required for complete normalization of growth (194). Growth-depressing effects of excess leucine were not observed when rats were fed adequate protein (18% casein) (90); thus, a dietary excess of leucine interacts with amino acids other than isoleucine and valine to produce adverse effects.

Many of the growth-depressing effects of excess leucine can be accounted for by curtailment of food intake. Recently, Austic and associates (31) force-fed chicks and estimated that as much as 70% of the decreased growth rate was due to depression in food intake. In other studies, the depression in growth of rats fed high-leucine diets was alleviated when food intake was stimulated by exposure to cold (13), insulin injections (216), or increased dietary protein (90). Other observations show that supplements of as little as 0.16% of isoleucine and 0.15% of valine overcome the growth-retarding effects of excess leucine (88). These results argue against palatability being a factor in the food intake response and indicate that a high intake of leucine is not toxic per se.

Excess Leucine and Blood Amino Acid Responses

After animals have consumed excessive leucine, plasma and tissue pools of isoleucine and KMV and valine and KIV are depleted. These effects are seen in rats, chicks, pigs, turkey poults, kittens, and humans (83, 92, 220). This reinforces the notion that excess leucine increases the requirements for isoleucine and valine. The depression of plasma isoleucine and valine pools is acute, occurring 10–30 min after intragastric administration of a leucine load (18), whereas food intake depression is not evident for one to three hours (216). The following order-dependent sequence can be proposed for the leucine-induced BCAA antagonism: (*a*) elevation of the total body free pools of leucine and KIC, (*b*) depletion of the total body free pools of isoleucine and KMV and valine and KIV, and (*c*) depression of food intake and growth.

Clark et al (39) measured plasma, liver, and muscle amino acid concentrations 45 min after administering 60 mg of leucine or saline intragastrically to rats previously fed an 18% protein diet. Total free amino acid pool changes were estimated based on the assumptions that plasma and liver each represent 4% of the body weight, that muscle accounts for 40%, and that changes in these tissues are representative of those occuring in others. Administration of leucine resulted in expansion of the total free pool of leucine from 18 to 59 μmol and in depressions in the total free pools of valine from 30 to 10 μmol, of isoleucine from 14 to 4 μmol, of phenylalanine from 9.8 to 4.7 μmol and of tyrosine from 17.9 to 11.9 μmol. In subsequent studies the BCKA followed patterns similar to those of their corresponding BCAA after leucine loading (18, 206).

Snyderman et al (215) observed dramatic drops in the plasma concentrations of valine, phenylalanine, tyrosine, threonine, and proline in 2 adult males three hours after oral administration of 25 g of L-leucine, approximately 25 times the normal requirement. Many other studies of the effects of leucine loading since then have shown these characteristic plasma pool changes. Swendseid et al (220) reported that plasma pools of valine, isoleucine, phenylalanine, tyrosine, and methionine were depleted after oral administration of 10 g of leucine to adults fasted overnight. These effects were not associated with changes in blood glucose, which indicates that the changes could not be attributed to increased insulin release. In 14 healthy, fasted subjects, intravenous infusions of up to 5.9 g of leucine were accompanied by marked drops in arterial blood concentrations of valine, isoleucine, methionine, tryosine, and phenylalanine (83) and by significant increases in splanchnic uptake of isoleucine and valine. No significant changes in splanchnic blood flow, leg blood flow, or oxygen consumption were observed. Arterial concentrations of insulin were elevated by 25%, which according to Pozefsky et al (187), is not enough to influence tissue exchange of amino acids. Similar changes have been observed in other studies cited below. Thus the changes observed in amino acids appear to result from a direct action of leucine. Abumrad et al (2) infused 1.2–5.8 g of leucine intravenously into 13 adult males in the postabsorptive state and observed that

arterial blood concentrations of leucine rose three- to fourfold; concentrations of valine, isoleucine, methionine, and tyrosine dropped significantly; KIC concentration rose 122%; and KIV concentration fell 60%. This study contrasts with that of Hagenfeldt et al (83) in that infusion of leucine resulted in a 55% drop in α-amino nitrogen release from the forearm; in four hours, isoleucine output had dropped by 80% and valine output had dropped by 60%. The circulating insulin level in the leucine-treated subjects had increased 81% after five hours, while blood glucose level had dropped 11%. Abumrad et al (2) suggested that decreased release of amino acids from muscle could account for the changes observed in the plasma pools. Hutson et al (103) found that perfusate isoleucine and valine concentrations were markedly depressed after perfusing rat hindquarter with five times the normal plasma concentration of leucine.

These effects of leucine are unique (18, 56, 206, 220). In a study with the rat, Block & Harper (18) did not detect a significant effect of excess dietary isoleucine on the plasma concentrations of leucine, KIC, or valine, although plasma KIV concentration was depressed moderately. Shinnick & Harper (206) observed no depressing action of excess isoleucine on plasma valine in rats given amino acid solutions intragastrically. Swendseid et al (220) were the first to report that an oral load of leucine, but not of isoleucine or valine, depleted the plasma pools of other BCAA and large neutral amino acids (LNAA) in humans. More recently, Eriksson et al (56) reported that intravenous infusion of adult human subjects with leucine resulted in the characteristic drops in plasma concentrations of the other BCAA, methionine, and aromatic amino acids, whereas infusion of isoleucine or valine was without effect. Infusion of all three BCAA gave plasma amino acid patterns similar to those observed when only leucine was infused.

Another set of observations deserves consideration in relation to effects of leucine on free LNAA concentrations. When diets deficient in leucine are fed to rats and humans, plasma and tissue concentrations of isoleucine and KMV, valine and KIV, and other LNAA are elevated (38, 85, 89, 215, 242). Feeding diets devoid of isoleucine or valine does not significantly affect the plasma concentrations of other LNAA. This fine-tuning of plasma isoleucine and valine concentrations by both low and high dietary levels of leucine appears to be the result of a unique control mechanism.

Probable Basis for Plasma Amino Acid Changes

Efforts to explain the lowering of free amino acid pools by high dietary levels of leucine in terms of classical antimetabolite action have not been successful. Although competition among BCAA has been demonstrated in vitro, significant impairment of [^{14}C]isoleucine or valine absorption during high-leucine feeding has not been detected in vivo (18, 31, 86, 88, 212). Competition among

BCAA for absorption by intestinal tissue (84) and for reabsorption by the kidney tubules was demonstrated in 1951 (117), but in studies with rats (18, 86, 88) and chicks (31, 212), no significant effect of excess leucine on the excretion of radiolabeled BCAA has been observed. Recently, Hagenfeldt et al (83), reported that intravenous administration of excess leucine to men did not affect renal clearance or tubular reabsorption of amino acids.

Three mechanisms which could explain the leucine-induced changes in free amino acid pools are (*a*) increased net tissue protein accumulation (increased synthesis, decreased degradation, or both), (*b*) transport phenomena involving the L-carrier system that would alter body amino acid distribution, and (*c*) increased BCAA oxidation.

Evidence of a role for leucine in stimulating protein synthesis and suppressing protein degradation in vitro has come from studies with isolated rat diaphragm (28, 71, 225) and perfused rat hindquarter (130), heart (36, 37), and liver (186). In these studies, isoleucine and valine each had little effect on protein turnover. Despite convincing results in studies with isolated tissues and perfused organs, observations on effects of leucine on protein synthesis and degradation in vivo are conflicting. McNurlan et al (149) observed no effect of excess leucine on the fractional rate of protein synthesis in tissues from intact rats in a variety of nutritional states despite marked lowering of plasma isoleucine and valine pools in their study. Freund et al (68) demonstrated that valine was more effective then leucine in stimulating protein synthesis in laparotomized rats in vivo.

Studies of effects of leucine on protein degradation have also given conflicting results. Infusion of leucine into normal or obese adults had no effect on the urinary excretion of 3-methylhistidine (204). More recently, Marchesini et al (140) infused BCAA-enriched solutions into patients suffering from liver cirrhosis and observed a significant decrease in 3-methylhistidine excretion. Despite these conflicting results, infusions of KIC or BCAA have improved nitrogen balance in a number of studies on traumatized rats and post-operative patients (67). Leucine (57) and KIC (107) are potent insulin secretagogues, so in addition to any direct effect it might have, leucine could increase net protein synthesis in vivo by stimulating insulin release.

The assumption that increased net protein synthesis is the mechanism responsible for the leucine-induced lowering of free amino acid pools is problematic. Pools of the amino acids affected by leucine loading differ considerably in size, so absolute changes in pool sizes, not per cent changes, must be compared to analyze the effects accurately. Clark et al (39) found that the free muscle pools of isoleucine, valine, and phenylalanine dropped by 9, 18, and 5 μmol respectively after leucine loading. Animal muscle contains 0.395 μmol isoleucine, 0.448 μmol valine, and 0.244 μmol phenylalanine per mg protein (168). The theory that increased protein synthesis contributes to the depletion

of amino acid pools is supported by the observation that the ratio of isoleucine to phenylalanine in muscle protein, 1.6, is similar to the ratio of isoleucine to phenylalanine calculated for the free muscle pool changes after leucine loading, 1.8. Assuming that the depletion of free muscle isoleucine and phenylalanine pools is solely the result of increased protein synthesis, the anticipated drop in the muscle free pool of valine, based on the ratio of muscle protein valine to isoleucine would be 1.1; based on the ratio of valine to phenylalanine, it would be 1.8. However, the observed drop in the free muscle pools of valine expressed in this manner is approximately twice that predicted from increased protein synthesis alone. The studies discussed above that compared plasma amino acid pool changes in humans after leucine administration also revealed that isoleucine and valine concentrations declined to a much greater extent than would be predicted from stimulation of net protein synthesis. Another discrepancy is encountered in the results of experiments in which rats were fed a 9% casein plus 5% leucine diet ad libitum for 7–12 days (222; K. P. Block and A. E. Harper, unpublished results). In these studies, only the isoleucine and valine pools in plasma were depleted. These discrepancies can be explained only if BCAA concentrations are affected selectively by mechanisms other than increased incorporation into protein.

In addition to having small pools, most amino acids affected by leucine administration have in common the L-transport system (208). The possibility that leucine influences the distribution of LNAA between intracellular and extracellular pools has not been examined extensively. Exchange and countertransport systems exist by which an increased flux of leucine from the intracellular to extracellular compartments could facilitate the inward transport of other LNAA; however, although extracellular and intracellular tissue LNAA concentrations have not been measured in humans during leucine infusions, studies of the rat have shown that tissue and plasma pools changed similarly, so this explanation is unlikely.

Toward an Explanation of Leucine: Isoleucine and Valine Antagonism

Specific stimulation of BCAA oxidation by consumption of excess leucine could account for depletion of isoleucine and valine pools. Several studies of isoleucine or valine oxidation in vivo failed to demonstrate a significant stimulative effect of excess dietary leucine (20, 88, 206), but there were methodological problems in all of the studies (18). Austic and associates (31, 212) reported an apparent increase in L-[1–^{14}C]valine and L-[1–^{14}C]isoleucine oxidation in vivo in chicks fed high-leucine diets, but true oxidation rates could not be calculated because of a lack of specific radioactivity measurements. Also, because $^{14}CO_2$ was collected in 24-hour periods, the time interval was too long for the sequence of the changes in plasma LNAA and rates of oxidation

to be detected. More recently, Block & Harper (18) demonstrated that the acute depressions in plasma isoleucine and valine concentrations in rats fed high-leucine meals were accompanied within one to three hours by a 50% increase in whole-body L-[1–^{14}C]valine oxidation, from 26±3 to 39±2 μmol/hr Consumption of a high-isoleucine meal had no effect on whole body L-[1–^{14}C]valine catabolism. Meguid et al (150) have shown that excess leucine increases oxidation of L-[1–^{14}C]valine in vivo in humans. Excess valine had no significant effect on L-[1–^{14}]leucine oxidation.

A leucine-induced increase in BCAA catabolism was also demonstrated in studies of isolated tissues. Increasing perfusate leucine concentration in perfused rat hindquarter from 0.2 to 1.0 mM resulted in rapid stimulation of L-[1–^{14}C]valine oxidation (243). Incubating rat adipocytes with 0.2–0.5 mM leucine increased oxidation of L-[1–^{14}C]valine; increasing isoleucine concentration depressed valine oxidation (70). In experiments on isolated intact epitrochlearis muscles from rats, increasing the leucine concentration of the medium from 0 to 1 mM resulted in more than a fourfold increase in L-[1–^{14}C]valine oxidation (150). These studies all indicate that excess leucine stimulates the flux of valine through the catabolic pathway.

Based on classical competitive inhibition between substrates in the BCAAT or BCKAD reactions, depression of isoleucine and valine oxidation by excess leucine would be predicted, and enlarged rather than diminished isoleucine and valine pools would be anticipated. Also, although there are some reports that BCAAT activity can be influenced by leucine (212, 240) and KIC (34, 156), responses have not been consistent and no unique regulator of this reaction has been described. Recently, we have been unable to detect a change in total liver or muscle BCAAT activity after feeding high levels of leucine, despite an elevated rate of valine oxidation (18).

In contrast to BCAAT, BCKAD is highly regulated by a phosphorylation (inactivation)-dephosphorylation (activation) mechanism. Early work with liver (115), heart (171), skeletal muscle (162, 165), and kidney (125) indicated that the ATP-mediated inhibition of BCKAD could be retarded by including BCKA, particularly KIC, in the media. Subsequent studies with the purified enzyme from liver (102, 180, 181), kidney (102, 126), and heart (102) indicated that KIC protected the BCKAD against inactivation by inhibiting BCKAD kinase and hence phosphorylation of the complex. Recently, Paxton et al (181) reported that values for 50% inhibition of rabbit liver BCKAD kinase by BCKA are 65 μM for KIC, 650 μM for KMV, and 1.9 mM for KIV. Based on a study with ox kidney BCKAD, Lau et al (126) reported noncompetitive inhibition of the kinase by BCKA with K_i's as follows: KIC, 0.48 mM; KMV, 0.92 mM; and KIV 8.9 mM. Accordingly, KIC is a more potent inhibitor of the kinase than KMV or KIV, so it should retard phosphorylation and hence inactivation of the BCKAD complex. BCKAD in adipose tissue is activated

when the tissue is incubated in a medium containing extra leucine or KIC (70). BCKAD is also activated when hearts are perfused with KIC (236). In the perfusion studies, KIC is a more potent activator of BCKAD than KMV or KIV. The marked activation of heart BCKAD by KIC is associated with dephosphorylation of the α-chain of the E_1 subunit (30). Thus, KIC appears to shift the kinase-phosphatase balance in favor of dephosphorylation. Reports of hepatic BCKAD modulation by dietary BCAA (18, 206, 240) and BCKA (120) have appeared in the literature. It is not known at present whether consumption of excess leucine results in the activation of BCKAD from tissues other than liver. Activation of BCKAD and consequent stimulation of BCKA oxidation by excess dietary leucine could explain the depletion of plasma isoleucine and valine pools by dietary excesses of leucine.

Based on information currently available, we propose the following explanation for the depletion of isoleucine and valine pools by administration of excess leucine: Consumption of excess leucine results in stimulation of BCAA oxidation and probably in a small increase in net protein accumulation. Evidence for an effect of increased net protein synthesis on amino acid pools is readily detected only in subjects administered leucine in the fasting state, when pools are not being replenished. The elevated rate of BCAA oxidation would be expected to continue as long as high concentrations of tissue leucine are maintained.

Excess Leucine and Food Intake Depression

Some of the metabolic changes that result from a high leucine intake would also be expected to provide the basis for an explanation of the depressed food intake of animals consuming high-leucine diets. As early as 1957, a distorted plasma amino acid pattern was thought to play a role in food intake control, and relationships between plasma and brain amino acid patterns and feeding behavior have been studied extensively over the years (88, 93). The depressed food intakes of animals consuming diets in which the amino acid patterns are out of balance usually are associated with depletion of brain amino acid pools. Peng et al (183) demonstrated that consumption of a diet containing excess leucine resulted in depletion of brain pools of LNAA. This effect, which is not evident in other tissues, is most likely the result of strong competition between leucine and other LNAA for uptake into brain because of the low K_m's of brain transport systems for amino acids (169). Of further interest are observations that consumption of excess leucine results in a decrease in brain serotonin (190) and dopamine concentrations (K. P. Block and A. E. Harper, unpublished data).

The food intake depression caused by excess leucine can be dissociated from its effect on plasma isoleucine and valine pools. Consumption of excess phenylalanine, a molecule similar to leucine in molecular weight and transport

characteristics, results in depression of food intake and alterations in brain amino acid profiles similar to those observed after high-leucine feeding. However, consumption of excess phenylalanine does not result in the characteristic depressions of plasma isoleucine and valine concentrations observed after high-leucine feeding. These observations suggest that the antagonism between leucine-isoleucine and valine observed in animals does not fit the classical antimetabolite concept. The blood and tissue changes discussed above appear to result from a direct stimulative effect of leucine on BCAA metabolism. The food intake depression appears to result independently from depletion of brain pools of LNAA caused by excess leucine inhibiting transport of LNAA into the brain. However, stimulation of isoleucine and valine oxidation and subsequent depletion of plasma isoleucine and valine pools would be expected to increase the degree of competition between leucine and the other BCAA for entry into brain.

The observations on effects of excess leucine have some bearing on the use of BCAA-enriched solutions in the treatment of hepatic encephalopathy. This condition is characterized by elevated concentrations of methionine and aromatic amino acids and by depressed BCAA concentrations in plasma (16). Normalization of patients' blood amino acid patterns by infusing with high BCAA, low phe, and low met solutions is reported to improve their mental state (64). The studies reported thus far appear to indicate that leucine alone may be efficacious in lowering plasma and brain methionine concentrations and aromatic amino acid concentrations, which are postulated to rise in brain and contribute to the mental impairment of hepatic encephalopathy. It should be stressed, however, that infusion of leucine alone or even infusion of a mixture of BCAA alone has the potential to cause further derangement of blood and brain amino acid pools. Depletion of isoleucine and valine pools after leucine administration could result in accelerated rates of protein wasting. Infusion of all three BCAA in a complete amino acid solution appears to be the appropriate approach to normalizing deranged blood amino acid levels (64) and ensuring an adequate supply of all amino acids. Wahren et al (229) recently questioned the effectiveness of using BCAA-enriched solutions to treat hepatic encephalopathy; it should be noted that subjects in this study were infused with a mixture of BCAA and glucose but not with the other amino acids needed for protein synthesis.

CONCLUSIONS

The BCAA are unique among the nutritionally indispensable amino acids in that the enzymes for their catabolism are distributed throughout the body rather than being confined to liver. The initial catabolic reaction is a reversible transamination yielding glutamate and BCKA. The steady-state concentrations

of BCKA in tissues are low, presumably in part because high intracellular steady-state concentrations of glutamate favor reamination of the BCKA. Also, the second catabolic reaction, an irreversible oxidative decarboxylation for which BCKA are substrates, leads to their further degradation. Despite this, BCKA are released into the media by perfused hindlimb and kidney preparations and into the bloodstreams of mammals. In organs and tissues throughout the body, nitrogen from BCAA is incorporated into alanine and glutamine, which are also released into the blood. As a result of the ready exchange of BCAA, BCKA, alanine, and glutamine across cell membranes, metabolism of both the nitrogen and the carbon skeletons of BCAA involves extensive interorgan relationships. The extent to which BCKA are released by various organs and tissues remains to be established, as does the quantitative importance of the liver, which has a limited capacity for transamination of BCAA but a great capacity for BCKA oxidation in overall BCAA metabolism.

BCKAD is the second enzyme in the pathway for BCAA catabolism, and it is subject to regulation by phosphorylation (inactivation) and dephosphorylation (activation). Knowledge of the extent of activation of BCKAD in tissues in vivo is limited, but current evidence indicates that it is influenced by both nutritional and endocrine factors. There is also evidence, at least in liver, for a protein factor in mitochondria that can influence the activity of this enzyme independently of its phosphorylation state. Establishing the role of nutritional, endocrine, and pathological states in the regulation of BCKAD and in the control of BCAA catabolism in general is a fertile field for investigation.

Establishing the relative importance of different regulatory systems on BCAA catabolism presents a challenge. The rate of BCAA degradation in vivo is highly responsive to changes in dietary and blood BCAA and BCKA concentrations; intracellular BCKA concentrations depend in turn on transport of BCAA and BCKA across cell membranes and on the rate and direction of transamination. The rate of BCKA oxidation depends in turn upon BCKA concentrations within the mitochondria and hence on transport of BCAA and BCKA to site of oxidation; it also depends on the degree of activation of the BCKA dehydrogenase. It seems highly probable that the relative importance of these potential regulatory systems will vary with the physiologic, nutritional, and pathologic state of the organism.

Disproportions of BCAA in the diet or body fluids, especially high leucine concentrations, can influence insulin release, tissue protein synthesis and degradation, catabolism of the other BCAA, and transport of large neutral amino acids into tissues, especially brain. Thus, the BCAA or their respective BCKA appear to exert a number of regulatory actions. The bases for these effects and their physiological significance are being investigated actively. Stimulation of BCKA oxidation by leucine or its ketoacid has been demonstrated and appears to be mediated through control of BCKAD inactivation.

Effects of high BCAA concentrations on feeding behavior and possibly on other behaviors appear to depend on depletion of brain pools of one or more of the large neutral amino acids, some of which are precursors of neurotransmitters, as the result of competition for transport across the blood brain barrier.

Stimulation of protein synthesis and suppression of protein degradation by high tissue leucine concentrations have been demonstrated in vitro, but results in vivo have not been consistent. The bases for these effects remains to be established. Each of these sets of observations represents an independent research area for further investigation.

Finally, these advances in basic knowledge of BCAA metabolism and interactions bear on clinical applications of BCAA and BCKA. Safe and effective use of BCAA in restoring normal amino acid patterns in blood and brain for treatment of hepatic encephalopathy, in attempting to reduce nitrogen loss in the treatment of patients in hypercatabolic states, and in efforts to reduce nitrogen accumulation in patients with renal disease will be advanced by more thorough basic knowledge of the interrelationships of the BCAA in metabolism in the intact organism.

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Literature Cited

1. Abumrad, N. N., Jefferson, L. S., Rannels, S. R., Williams, P. E., Cherrington, A. D., Lacy, W. W. 1982. Role of insulin in the regulation of leucine kinetics in the conscious dog. *J. Clin. Invest.* 70:1031–41
2. Abumrad, N. N., Robinson, R. P., Gooch, B. R., Lacy, W. W. 1982. The effect of leucine infusion on the substrate flux across the human forearm. *J. Surg. Res.* 32:453–63
3. Abumrad, N. N., Wise, K. L., Williams, P. E., Abumrad, N. A., Lacy, W. W. 1982. Disposal of α-ketoisocaproate: Roles of liver, gut and kidney. *Am. J. Physiol.* 243:E123–31
4. Adibi, S. 1976. Metabolism of branched-chain amino acids in altered nutrition. *Metabolism* 25:1287–1302
5. Adibi, S. A. 1980. Roles of branched-chain amino acids in metabolic regulation. *J. Lab. Clin. Med.* 95:475–84
6. Adibi, S. A., Krzysik, B. A., Morse, E. L., Amin, P. M., Allen, E. R. 1974. Oxidative energy metabolism in the skeletal muscle: Biochemical and ultrastructural evidence for adaptive changes. *J. Lab. Clin. Med.* 83:548–62
7. Adibi, S. A., Peterson, J. A., Krzysik, B. A. 1975. Modulation of leucine transaminase activity by dietary means. *Am. J. Physiol.* 228:432–35
8. Aftring, R. P., May, M. E., Manos, P. N., Buse, M. G. 1982. Regulation of α-ketoisocaproate oxidation in liver mitochondria by adenine nucleotides and calcium. *J. Biol. Chem.* 257:6156–63
9. Aki, K., Ogawa, K., Ichihara, A. 1968. Transaminases of branched chain amino acids. IV. Purification and properties of two enzymes from rat liver. *Biochim. Biophys. Acta* 159:276–84
10. Amen, R. J., Yoshimura, N. N. 1981. The pharmacology of branched-chain amino acids. In *Nutritional Pharmacology*, ed. G. A. Spiller, pp. 73–116. New York: A. R. Liss
11. Anderson, H. L., Benevenga, N. J., Harper, A. E. 1968. Associations among food and protein intake, serine dehydratase and plasma amino acids. *Am. J. Physiol.* 214:1008–13
12. Aoki, T. T., Brennan, M. F., Fitzpatrick, G. F., Knight, D. C. 1981. Leucine meal increases glutamine and total nitro-

gen release from forearm muscle. *J. Clin. Invest.* 68:1522–28
13. Bavetta, L. A., Nimni, M. E. 1964. Tissue distribution of α-aminoisobutyric acid and nitrogen metabolism in the rat. II. Effects of environmental temperature and dietary imbalance. *J. Nutr.* 82:379–84
14. Benton, D. A., Harper, A. E., Spivey, H. E., Elvehjem, C. A. 1956. Leucine, isoleucine, and valine relationships in the rat. *Arch. Biochem. Biophys.* 60:147–55
15. Berger, M., Zimmerman-Telschow, H., Berchtold, P. 1978. Blood amino acid levels in patients with insulin excess (functioning insulinoma) and insulin deficiency (diabetic ketosis). *Metabolism* 27:793–99
16. Bernardini, P., Fischer, J. E. 1982. Amino acid imbalance and hepatic encephalopathy. *Ann. Rev. Nutr.* 2:419–54
17. Blackshear, P. J., Holloway, P. A. H., Alberti, G. M. M. 1975. Factors regulating amino acid release from extrasplanchnic tissues in the rat. *Biochem. J.* 150:379–87
18. Block, K. P., Harper, A. E. 1984. Valine metabolism in vivo: Effects of high dietary levels of leucine and isoleucine. *Metabolism* 33: In press
19. Bloomgarden, Z. T., Liljenquist, J., Lacy, W., Rabin, D. 1981. Amino acid disposition by liver and gastrointestinal tract after protein and glucose ingestion. *Am. J. Physiol.* 241:E90–99
20. Boldizsár, H. K., Boorman, K. N., Buttery, P. J. 1973. The effect of excess leucine on valine catabolism in the chick. *Br. J. Nutr.* 30:501–10
21. Bowden, J. A., Connelly, J. L. 1968. Branched chain α-keto acid metabolism II. Evidence for the common identity of α-ketoisocaproic acid and α-keto-β-methylvaleric acid dehydrogenases. *J. Biol. Chem.* 243:3526–31
22. Bower, R. H., Fischer, J. E. 1983. Nutritional management of hepatic encephalopathy. *Adv. Nutr. Res.* 5:1–11
23. Brand, K. 1981. Metabolism of 2-oxoacid analogues of leucine, valine and phenylalanine by heart muscle, brain and kidney of the rat. *Biochim. Biophys. Acta* 677:126–32
24. Buffington, C. K., DeBuysere, M. S., Olson, M. S. 1979. Studies on the regulation of the branched chain α-keto acid dehydrogenase in the perfused rat heart. *J. Biol. Chem.* 254:10453–58
25. Buse, M. G., Biggers, J. F., Drier, C., Buse, J. F. 1973. The effect of epinephrine, glucagon, and the nutritional state on the oxidation of branched chain amino acids and pyruvate by isolated hearts and diaphragms of the rat. *J. Biol. Chem.* 248:697–706
26. Buse, M. G., Biggers, J. F., Friderici, K. H., Buse, J. F. 1972. Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat. *J. Biol. Chem.* 247:8085–96
27. Buse, M. G., Herlong, H. F., Weigand, D. A. 1976. The effect of diabetes, insulin and the redox potential on leucine metabolism by isolated rat hemidiaphragm. *Endocrinology* 98:1166–75
28. Buse, M. G., Reid, S. S. 1975. Leucine. A possible regulator of protein turnover in muscle. *J. Clin. Invest.* 56:1250–61
29. Buxton, P., Barron, L. L., Olson, M. S. 1982. The effects of α-adrenergic agonists on the regulation of the branched-chain α-ketoacid oxidation in the perfused rat liver. *J. Biol. Chem.* 257: 14318–23
30. Buxton, D. B., Olson, M. S. 1982. Regulation of the branched chain α-ketoacid and pyruvate dehydrogenases in the perfused rat heart. *J. Biol. Chem.* 257: 15026–29
31. Calvert, C. C., Klasing, K. C., Austic, R. E. 1982. Involvement of food intake and amino acid catabolism in the branched-chain amino acid antagonism in chicks. *J. Nutr.* 112:627–35
32. Cappuccino, C. C., Kadowaki, H., Knox, W. E. 1978. Assay of leucine aminotransferase in rat tissues and tumors. *Enzyme* 23:328–38
33. Cerra, F. B. 1983. Review of branched-chain amino acid supplementation in trauma. In *Advances in Clinical Nutrition*, ed. I. D. A. Johnson, pp. 51–64. Boston: MTP
34. Chan, W., Walser, M. 1978. Effect of branched-chain ketoacids and dietary protein content on the activity of branched-chain amino acid transferase in rat tissues. *J. Nutr.* 108:40–45
35. Chaplin, E. R., Goldberg, A. L., Diamond, I. 1976. Leucine oxidation in brain slices and nerve endings. *J. Neurochem.* 26:701–7
36. Chua, B., Siehl, D. L., Morgan, H. E. 1979. Effect of leucine and metabolites of branched chain amino acids on protein turnover in heart. *J. Biol. Chem.* 254: 8358–62
37. Chua, B. H. L., Siehl, D. L., Morgan, H. E. 1980. A role for leucine in regulation of protein turnover in working rat hearts. *Am. J. Physiol.* 239:E510–14
38. Clark, A. J., Peng, Y., Swendseid, M. E. 1966. Effect of different essential amino acid deficiencies on amino acid pools in rats. *J. Nutr.* 90:228–34
39. Clark, A. J., Yamada, C., Swendseid,

M. E. 1968. Effect of L-leucine on amino acid levels in plasma and tissue of normal and diabetic rats. *Am. J. Physiol.* 215:1324–28
40. Coiro, V., Frick, G. P., Braverman, L. E., Goodman, H. M. 1981. Effects of hypophysectomy and thyroidectomy on leucine metabolism in adipose tissue. *Am. J. Physiol.* 240:E669–76
41. Connelly, J. L., Danner, D. J., Bowden, J. A. 1968. Branched chain α-keto-isocaproic: α-keto-β-methylvaleric acid dehydrogenase. *J. Biol. Chem.* 243: 1198–203
42. Cook, K. G., Lawson, R., Yeaman, S. J. 1983. Multisite phosphorylation of bovine kidney branched-chain 2-oxoacid dehydrogenase complex. *FEBS Lett.* 157:59–62
43. Corkey, B. E., Martin-Requero, A., Walajtys-Rode, E., Williams, R. J., Williamson, J. R. 1982. Regulation of the branched chain α-ketoacid pathway in liver. *J. Biol. Chem.* 257:9668–76
44. Corkey, B. E., Martin-Requero, A., Walajtys-Rode, E., Williamson, M. T., Williamson, J. R. 1983. Regulation of flux through branched-chain ketoacid dehydrogenase in isolated rat liver mitochondria. *Fed. Proc.* 42:1978 (Abstr.)
45. Crabb, D. W., Harris, R. A. 1978. Studies on the regulation of leucine catabolism in the liver. *J. Biol. Chem.* 253: 1481–87
46. Cree, T. C. 1980. *Studies on the degradation of the branched-chain amino acids in the perfused rat hindquarter and rat skeletal muscle extracts.* PhD thesis. Univ. Wisc., Madison, pp. 51–83
47. Dancis, J., Hutzler, J., Cox, R. P. 1969. Enzyme defect in skin fibroblasts in intermittent branched-chain ketonuria and in maple syrup urine disease. *Biochem. Med.* 2:407–11
48. Danner, D. J., Lemmon, S. K., Elsas, L. J. 1978. Substrate specificity and stabilization by thiamine pyrophosphate of rat liver branched chain α-ketoacid dehydrogenase. *Biochem. Med.* 19:27–38
49. Dawson, A. G., Hird, F. J. R., Morton, D. J. 1967. Oxidation of leucine by rat liver and kidney. *Arch. Biochem. Biophys.* 122:426–33
50. Dien, L. T. H., Ravel, J. M., Shive, W. 1954. Some inhibitory interrelationships among leucine, isoleucine and valine. *Arch. Biochem. Biophys.* 49:283–92
51. Dixon, J. L. 1982. *The effect of dietary protein and meal-feeding on the branched-chain α-keto acid dehydrogenase of rat liver and skeletal muscle.* PhD thesis. Univ. Wisc., Madison. pp. 97–166
52. Dixon, J. L., Harper, A. E. 1981. Effect of dietary protein and meal feeding on subcellular activity of α-ketoisocaproate (KIC) and α-keto-γ-methiolbutyrate (KMBA) decarboxylation in rat liver. *Fed. Proc.* 40:900 (Abstr.)
53. Dixon, J. L., Harper, A. E. 1984. Effects on plasma amino acid concentrations and branched-chain α-ketoacid dehydrogenase of feeding rats diets containing 9% or 50% casein. *J. Nutr.* 114: In press
54. Elia, M., Farrell, R., Ilic, V., Smith, R., Williamson, D. H. 1980. The removal of infused leucine after injury, starvation and other conditions in man. *Clin. Sci.* 59:275–83
55. Elia, M., Livesey, G. 1983. Effects of ingested steak and infused leucine on forelimb metabolism in man and the fate of the carbon skeletons and amino groups of branched-chain amino acids. *Clin. Sci.* 64:517–26
56. Eriksson, S., Hagenfeldt, L., Wahren, J. 1981. A comparison of the effects of intravenous infusion of individual branched-chain amino acids on blood amino acid levels in man. *Clin. Sci.* 60:95–100
57. Fajans, S. S., Floyd, J. C., Knopf, R. F., Conn, J. W. 1967. Effect of amino acids and proteins on insulin secretion in man. *Rec. Prog. Horm. Res.* 23:617–62
58. Fatania, H. R., Lau, K. S., Randle, P. J. 1982. Activation of phosphorylated branched chain 2-oxoacid dehydrogenase complex. *FEBS Lett.* 147:35–39
59. Featherstone, W. R., Hom, G. W. 1973. Dietary influences on the activities of enzymes involved in branched-chain amino acid catabolism in the chick. *J. Nutr.* 103:757–65
60. Felig, P. 1975. Amino acid metabolism in man. *Ann. Rev. Biochem.* 44:933–55
61. Felig, P., Pozefsky, T., Marliss, E., Cahill, G. F. Jr. 1970. Alanine: Key role in gluconeogenesis. *Science* 167:1003–4
62. Fernstrom, J. D. 1981. Dietary precursors and brain neurotransmitter formation. *Ann. Rev. Med.* 32:413–25
63. Fernstrom, J. D., Wurtman, R. J., Hammarstrom-Wickland, B., Rand, W. M., Munro, H. N., Davidson, C. S. 1979. Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino acids: Effect of dietary protein intake. *Am. J. Clin. Nutr.* 32:1912–22
64. Fischer, J. E., Rosen, H. M., Ebeid, A. M., James, J. H., Keane, J. M., Soeters, P. B. 1976. The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 80:77–91
65. Frame, E. G. 1958. The levels of individual free amino acids in the plasma of

normal man at various intervals after a high-protein meal. *J. Clin. Invest.* 37: 1710–23
66. Freedland, R. A., Szepesi, B. 1971. Control of enzyme activity: Nutritional factors. In *Enzyme Synthesis and Degradation in Mammalian Systems,* ed. M. Recheigl, pp. 103–40. Baltimore: Univ. Park
67. Freund, H. R.; Gimmon, Z., Fischer, J. E. 1983. Nitrogen sparing effects and mechanisms of branched chain amino acids: Experimental and clinical experience. In *New Aspects of Clinical Nutrition,* ed. G. Kleinberger, E. Deutsch, pp. 346–60. Basel: Karger
68. Freund, H. R., James, J. H., Fischer, J. E. 1981. Nitrogen-sparing mechanisms of singly administered branched-chain amino acids in the injured rat. *Surgery* 90:237–43
69. Frick, G. P., Goodman, H. M. 1980. Insulin regulation of branched chain α-keto acid dehydrogenase in adipose tissue. *J. Biol. Chem.* 255:6186–92
70. Frick, G. P., Tai, L-R., Blinder, L., Goodman, H. M. 1981. L-leucine activates branched-chain α-keto acid dehydrogenase in rat adipose tissue. *J. Biol. Chem.* 256:2618–20
71. Fulks, R. M., Li, J. B., Goldberg, A. L. 1975. Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. *J. Biol. Chem.* 250:290–98
72. Fürst, P., Alvestrand, A., Bergström, J. Askanazi, J., Elwyn, D., Kinney, J. 1983. Intracellular and plasma branched-chain amino acid interrelationships. See Ref. 33, pp. 25–34
73. Galim, E. B., Hruska, K., Bier, D. M., Matthews, D. E., Haymond, M. V. 1980. Branched-chain amino acid nitrogen transfer to alanine in vivo in dogs. *J. Clin. Invest.* 66:1295–1304
74. Ganong, W. F. 1981. *Review of Medical Physiology,* p. 476. Los Altos, Calif.: Lange Medical. 628 pp. 10th ed.
75. Garber, A. J., Karl, I. E., Kipnis, D. M. 1976. Alanine and glutamine synthesis and release from skeletal muscle. I. *J. Biol. Chem.* 251:826-35
76. Garber, A. J., Karl, I. E., Kipnis, D. M. 1976. Alanine and glutamine synthesis and release from skeletal muscle. II. *J. Biol. Chem.* 251:836–43
77. Gillim, S. E., Paxton, R., Cook, G. A., Harris, R. 1983. Activity state of the branched chain α-ketoacid dehydrogenase complex in heart, liver, and kidney of normal, fasted, diabetic, and protein-starved rats. *Biochem. Biophys. Res. Commun.* 111:74–81
78. Goldberg, A. L., Chang, T. W. 1978. Regulation and significance of amino acid metabolism in skeletal muscle. *Fed. Proc.* 37:2301–7
79. Golden, M. H. N. 1981. Metabolism of branched chain amino acids. In *Nitrogen Metabolism in Man,* ed. J. C. Waterlow, J. M. L. Stephen, pp. 109–10. London: Applied Science
80. Goodman, H. M. 1978. The effects of growth hormone on the utilization of L-leucine in adipose tissue. *Endocrinology* 102:210–17
81. Gopalan, C., Jaya Rao, K. S. 1975. Pellagra and amino acid imbalance. *Vitam. Horm.* 33:505–28
82. Goto, M., Shinno, H., Ichihara, A. 1977. Isozyme patterns of branched-chain amino acid transaminase in human tissues and tumors. *Gann* 68:663–67
83. Hagenfeldt, L., Eriksson, S., Wahren, J. 1980. Influence of leucine on arterial concentrations and regional exchange of amino acids in healthy subjects. *Clin. Sci.* 59:173–81
84. Hagihira, H., Ogata, M., Takedatsu, N., Suda, M. 1960. Intestinal absorption of amino acids. III. Interference between amino acids during intestinal absorption. *J. Biochem.* 47:139–43
85. Hambraeus, L., Bilmazes, C., Dippel, C., Scrimshaw, N., Young, V. R. 1976. Regulatory role of dietary leucine on plasma branched-chain amino acid levels in young men. *J. Nutr.* 106:230–40
86. Harper, A. E. 1974. Control mechanisms in amino acid metabolism. In *The Control of Metabolism,* ed. J. D. Sink. pp. 49–74. University Park: Penn. State Univ. Press
87. Harper, A. E. 1983. Some recent developments in the study of amino acid metabolism. *Proc. Nutr. Soc.* 42:361–73
88. Harper, A. E., Benevenga, N. J., Wohlhueter, R. M. 1970. Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.* 50:428–558
89. Harper, A. E., Benjamin, E. 1984. Relationship between intake and rate of oxidation of leucine and α-ketoisocaproate in vivo in the rat. *J. Nutr.* 114: In press
90. Harper, A. E., Benton, D. A., Elvehjem, C. A. 1955. L-leucine, an isoleucine antagonist in the rat. *Arch. Biochem. Biophys.* 57:1–12
91. Harper, A. E., Benton, D. A., Winje, M. E., Elvehjem, C. A. 1954. Leucine-isoleucine antagonism in the rat. *Arch. Biochem. Biophys.* 51:523–24
92. Harper, A. E., Block, K. P., Cree, T. C. 1983. Branched-chain amino acids: Nutritional and metabolic interrelationships. In *Protein Metabolism and Nutrition, 4th Int. Symp.,* ed. M. Arnal, R. Pion, D.

Bonin, 1:159–81. Paris: Inst. Natl. Rech. Agron.
93. Harper, A. E., Peters, J. C. 1983. Amino acid signals and food intake and preference: Relation to body protein metabolism. *Experientia* 44:107–34
94. Harper, A. E., Zapalowski, C. 1981. Interorgan relationships in the metabolism of the branched-chain amino and α-ketoacids. See Ref. 234, pp. 195–203.
95. Harper, A. E., Zapalowski, C. 1981. Metabolism of branched-chain amino acids. See Ref. 79, pp. 97–115
96. Harris, R. A., Crabb, D. W., Sans, R. M. 1978. Studies on the regulation of leucine catabolism. *Arch. Biochem. Biophys.* 190:8–16
97. Hauschildt, S., Brand, K. 1980. Effects of branched-chain α-keto acids on enzymes involved in branched-chain α-keto acid metabolism in rat tissues. *J. Nutr.* 110:1709–16
98. Hauschildt, S., Lüjthje, J., Brand, K. 1981. Influence of dietary nitrogen intake on mammalian branched-chain α-keto acid dehydrogenase activity. *J. Nutr.* 111:2188–94
99. Haymond, M. W., Miles, J. M. 1982. Branched chain amino acids as a major source of alanine nitrogen in man. *Diabetes.* 31:86–89
100. Hems, D. A. 1972. Metabolism of glutamine and glutamic acid by isolated perfused kidneys of normal and acidotic rats. *Biochem. J.* 130:671–80
101. Herlin, P. M., James, J. H., Gimmon, Z., Fischer, J. E. 1982. Combined or individual administration of branched-chain amino acids following total hepatectomy in the rat: Effects on amino acids and indoleamines in brain. *J. Par. Ent. Nutr.* 6:383–87
102. Hughes, W. A., Halestrap, A. P. 1981. The regulation of branched-chain 2-oxo acid dehydrogenase of liver, kidney and heart by phosphorylation. *Biochem. J.* 196:459–69
103. Hutson, S. M., Cree, T. C., Harper, A. E. 1978. Regulation of leucine and α-ketoisocaproate metabolism in skeletal muscle. *J. Biol. Chem.* 253:8126–33
104. Hutson, S. M., Harper, A. E. 1981. Blood and tissue branched-chain amino and α-keto acid concentrations. Effect of diet, starvation and disease. *Am. J. Clin. Nutr.* 34:173–83
105. Hutson, S. M., Zapalowski, C. 1981. Relationship of branched-chain amino acids (BCAA) to skeletal muscle gluconeogenic amino acids. See Ref. 234, pp. 245–50
106. Hutson, S. M., Zapalowski, C., Cree, T. C., Harper, A. E. 1980. Regulation of leucine and α-ketoisocaproic acid metabolism in skeletal muscle. Effects of starvation and insulin. *J. Biol. Chem.* 255:2418–26
107. Hutton, J. C., Sener, A., Malaisse, W. J. 1980. Interaction of branched chain amino acids and keto acids upon pancreatic islet metabolism and insulin secretion. *J. Biol. Chem.* 255:7340–46
108. Ichihara, A. 1985. Aminotransferases of branched-chain amino acids. In *Transaminases*, ed. P. Christen, D. E. Metzler. New York: John Wiley. In press
109. Ichihara, A., Koyama, E. 1966. Transaminase of branched chain amino acids. *J. Biochem.* 59:160–69
110. Ichihara, A., Noda, C., Ogawa, K. 1973. Control of leucine metabolism with special reference to branched-chain amino acid transaminase isozymes. *Adv. Enz. Regul.* 11:155–66
111. Ichihara, A., Noda, C., Tanaka, K. 1981. Oxidation of branched chain amino acids with special reference to their transaminase. See Ref. 234, pp. 227–31
112. Ichihara, A., Takahashi, H., Aki, K., Shirai, A. 1967. Transaminase of branched-chain amino acids. II. Physiological change in enzyme activity in rat liver and kidney. *Biochem. Biophys. Res. Commun.* 26:674–78
113. Ichihara, A., Yamasaki, Y., Masuji, H., Sato, J. 1975. Isozyme patterns of branched chain amino acid transaminase during cellular differentiation and carcinogenesis. In *Isozymes III, Developmental Biology*, ed. C. L. Markert, pp. 875–89. New York: Academic
114. Ikeda, T., Konishi, Y., Ichihara, A. 1976. Transaminase of branched-chain amino acids. XI. Leucine (methionine) transaminase of rat liver mitochondria. *Biochim. Biophys. Acta* 445:622–31
115. Johnson, W. A., Connelly, J. L. 1972. Cellular localization and characterization of bovine liver branched-chain α-keto acid dehydrogenases. *Biochemistry* 11:1967–73
116. Kadowaki, H., Knox, W. E. 1982. Cytosolic and mitochondrial isozymes of branched-chain aminotransferase during development of the rat. *Biochem. J.* 202:777–83
117. Kamin, H., Handler, P. 1951. Effect of infusion of single amino acids upon excretion of other amino acids. *Am. J. Physiol.* 164:654–61
118. Käser, H., Käser, R., Lestradet, H. 1960. Separation and quantitative estimation of new alpha-keto-acids in human blood for paper chromatography. *Metabolism* 9:926–31
119. Khatra, B. S., Chawla, R. K., Sewell, C.

W., Rudman, D. 1977. Distribution of branched-chain α-ketoacid dehydrogenase in primate tissues. *J. Clin. Invest.* 59:558–64

120. Khatra, B. S., Chawla, R. K., Wadsworth, A. D. 1977. Effect of dietary branched-chain α-ketoacids on hepatic branched-chain α-ketoacid dehydrogenase in the rat. *J. Nutr.* 107:1528–36
121. Kinney, J. M., Elwyn, D. H. 1983. Protein metabolism and injury. *Ann. Rev. Nutr.* 3:433–66
122. Krebs, H. A. 1972. Some aspects of the regulation of fuel supply in omnivorous animals. *Adv. Enz. Regul.* 10:397–420
123. Krebs, H. A. 1975. The role of chemical equilibrium in organ function. *Adv. Enz. Regul.* 13:449–72
124. Krebs, H. A., Lund, P. 1977. Aspects of the regulation of the metabolism of branched-chain amino acids. *Adv. Enz. Regul.* 15:375–94
125. Lau, K. S., Fantania, H. R., Randle, P. J. 1981. Inactivation of rat liver and kidney branched chain 2-oxoacid dehydrogenase complex by adenosine triphosphate. *FEBS Lett.* 126:66–70
126. Lau, K. S., Fatania, H. R., Randle, P. J. 1982. Regulation of the branched-chain 2-oxoacid dehydrogenase kinase reaction. *FEBS Lett.* 144:57–62
127. Lau, K. S., Phillips, C. E., Randle, P. J. 1983. Multisite phosphorylation in oxkidney branched-chain 2-oxoacid dehydrogenase complex. *FEBS Lett.* 160: 149–52
128. Leathwood, P. D., Ashley, D. V. M. 1983. Strategies of protein selection by weanling and adult rats. *Appetite* 4:97–112
129. Li, E. T. S., Anderson, G. H. 1983. Amino acids in the regulation of food intake. *Nutr. Abstr. Rev.* 53:169–81
130. Li, J. B., Jefferson, L. S. 1978. Influence of amino acid availability on protein turnover in perfused skeletal muscle. *Biochim. Biophys. Acta* 544:351–59
131. Lindsay, D. B. 1980. Amino acids as energy sources. *Proc. Nutr. Soc.* 39:53–59
132. Livesey, G. 1983. Bovine serum albumin decreases 4-methyl-2-oxovalerate utilization by isolated rat hepatocytes. *Biochem. J.* 212:655–58
133. Livesey, G., Lund, P. 1980. Enzymic determination of branched-chain amino acids and 2-oxoacids in rat tissues. *Biochem. J.* 188:705–13
134. Livesey, G., Lund, P. 1982. Binding of branched-chain 2-oxoacids to bovine serum albumin. *Biochem. J.* 204:265–72
135. Lowenstein, J. M. 1972. Ammonia production in muscle and other tissues: The purine nucleotide cycle. *Physiol. Rev.* 52:382–414
136. Lund, P. 1978. Ketoleucine (α-ketoisocaproic acid) as a precursor of ketone bodies. In *Biochemical and Clinical Aspects of Ketone Body Metabolism*, ed. H-D. Söling, C-D. Seufert, pp. 98–107. Stuttgart: Georg Thieme
137. Mallette, L. E., Exton, J. H., Park, C. R. 1969. Control of gluconeogenesis from amino acids in the perfused rat liver. *J. Biol. Chem.* 244:5713–23
138. Manson, J. A., Carpenter, K. J. 1978. The effect of a high level of dietary leucine on the niacin status of chicks and rats. *J. Nutr.* 108:1883–88
139. Manson, J. A., Carpenter, K. J. 1978. The effect of a high level of dietary leucine on the niacin status of dogs. *J. Nutr.* 108:1889–98
140. Marchesini, G., Zoli, M., Dondi, C., Bianchi, G., Cirulli, M., Pisi, E. 1982. Anticatabolic effect of branched-chain amino acid-enriched solutions in patients with liver cirrhosis. *Hepatology* 2:420–25
141. Marliss, E. B., Aoki, T. T., Pozefsky, T., Most, A. S., Cahill, G. F. Jr. 1971. Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. *J. Clin. Invest.* 50:814–17
142. Matthews, D. E., Bier, D. M., Rennie, M. J., Edwards, R. A. T., Halliday, D., Millward, D. J., Clugston, G. A. 1981. Regulation of leucine metabolism in man: A stable isotope study. *Science* 214:1129–31
143. Matthews, D. E., Schwarz, H. P., Yang, R. D., Motil, K. J., Young, V. R., Bier, D. M. 1982. Relationship of plasma leucine and α-ketoisocaproate during a L-[1-^{13}C]leucine infusion in man: A method for measuring human intracellular leucine tracer enrichment. *Metabolism* 31:1105–12
144. May, M. E., Aftring, R. P., Buse, M. G. 1980. Mechanism of the stimulation of branched chain oxoacid oxidation in liver by carnitine. *J. Biol. Chem.* 255:8394–97
145. May, M. E., Mancusi, J. J., Aftring, R. P., Buse, M. G. 1980. Effects of diabetes on oxidative decarboxylation of branched-chain keto acids. *Am. J. Physiol.* 239:E215–22
146. McFarlane, I. G., von Holt, C. 1969. Metabolism of amino acids in protein-calorie-deficient rats. *Biochem. J.* 111: 557–63
147. McFarlane, I. B., von Holt, C. 1969. Metabolism of leucine in protein-calorie-deficient rats. *Biochem. J.* 111:565–71

148. McMenamy, R. H., Vang, J., Drapanas, T. 1965. Amino acid and α-ketoacid concentrations in plasma and blood of the liverless dog. *Am. J. Physiol.* 209:1046–52
149. McNurlan, M. A., Fern, E. B., Garlick, P. J. 1982. Failure of leucine to stimulate protein synthesis in vivo. *Biochem. J.* 204:831–38
150. Meguid, M. M., Schwarz, H., Matthews, D. E., Karl, I. E., Young, V. R., Bier, D. M. 1983. In vivo and in vitro branched-chain amino acid interactions. In *Amino Acids. Metabolism and Medical Applications,* ed. G. L. Blackburn, J. P. Grant, V. R. Young, pp. 147–54. Boston: John Wright
151. Meikle, A. W., Klein, G. J. 1972. Effect of fasting and fasting-refeeding on conversion of leucine into CO_2 and lipids in rats. *Am. J. Physiol.* 222:1246–50
152. Miller, L. L. 1962. The role of the liver and the non-hepatic tissues in the regulation of free amino acid levels in the blood. In *Amino Acid Pools,* ed. J. T. Holden, pp. 708–21. Amsterdam: Elsevier
153. Miller, R. H., Harper, A. E. 1983. Branched-chain amino acid (BCAA) metabolism in the isolated perfused rat kidney. *Fed. Proc.* 42:543 (Abstr.)
154. Mimura, T., Yamada, C., Swendseid, M. E. 1968. Influence of dietary protein levels and hydrocortisone administration on the branched-chain amino acid transaminase activity in rat tissues. *J. Nutr.* 95:493–98
155. Mitch, W. E., Chan, W. 1978. Transamination of branched-chain keto acids by isolated perfused rat kidney. *Am. J. Physiol.* 235:E47–52
156. Mitch, W. E., Chan, W. 1979. α-Ketoisocaproate stimulates branched-chain amino acid transaminase in kidney and muscle. *Am. J. Physiol.* 236:E514–18
157. Neale, R. J., Waterlow, J. C. 1974. The metabolism of ^{14}C-labelled essential amino acids given by intragastric or intravenous infusion to rats on normal and protein-free diets. *Br. J. Nutr.* 32:11–25
158. Neale, R. J., Waterlow, J. C. 1974. Critical evaluation of a method for estimating amino acid requirements for maintenance in the rat by measurement of the rate of ^{14}C-labelled amino acid oxidation in vivo. *Br. J. Nutr.* 32:257–72
159. Nissen, S., Haymond, M. W. 1981. Effects of fasting on flux and interconversion of leucine and α-ketoisocaproate in vivo. *Am. J. Physiol.* 241:E72–75
160. Nissen, S. L., Miles, J. M., Gerich, J. E., Haymond, M. W. 1982. Regulation of α-ketoisocaproate binding to albumin in vivo by free fatty acids. *Am. J. Physiol.* 242:E67–71
161. Noda, C., Ichihara, A. 1976. Control of ketogenesis from amino acids. *J. Biochem.* 80:1159–64
162. Odessey, R. 1980. Reversible ATP-induced inactivation of branched-chain 2-oxo acid dehydrogenase. *Biochem. J.* 192:155–63
163. Odessey, R. 1982. Purification of rat kidney branched-chain oxo acid dehydrogenase complex with endogenous kinase activity. *Biochem. J.* 204:353–56
164. Odessey, R., Goldberg, A. L. 1972. Oxidation of leucine by rat skeletal muscle. *Am. J. Physiol.* 223:1376–83
165. Odessey, R., Goldberg, A. L. 1979. Leucine degradation in cell-free extracts of skeletal muscle. *Biochem. J.* 178:475–89
166. Odessey, R., Khairallah, E. A., Goldberg, A. L. 1974. Origin and possible significance alanine production by skeletal muscle. *J. Biol. Chem.* 249:7623–29
167. Olson, M. S., Scholz, R., Buffington, C., Dennis, S. C., Padma, A., Patel, T. B., Waymack, P., DeBuysere, M. S. 1981. Regulation of α-ketoacid dehydrogenase multienzyme complexes in isolated perfused organs. In *The Regulation of Carbohydrate Formation and Utilization in Mammals,* ed. C. M. Veneziale, pp. 153–88. Baltimore: Univ. Park
168. Orr, M. L., Watt, B. K. 1957. *Home Econ. Res. Rep. No. 4.* Washington D.C.: US Govt. Print. Off. 82 pp.
169. Pardridge, W. M. 1977. Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. *J. Neurochem.* 28:103–8
170. Parker, P. J., Randle, P. J. 1978. Branched-chain 2-oxoacid dehydrogenase complex of rat liver. *FEBS Lett.* 90: 183–86
171. Parker, P. J., Randle, P. J. 1978. Inactivation of rat heart branched-chain 2-oxoacid dehydrogenase complex by adenosine triphosphate. *FEBS Lett.* 95:153–56
172. Parker, P. J., Randle, P. J. 1980. Active and inactive forms of branched-chain 2-oxoacid dehydrogenase complex in rat heart and skeletal muscle. *FEBS Lett.* 112:186–90
173. Patel, T. B., DeBuysere, M. S., Barron, L. L., Olson, M. S. 1981. Studies on the regulation of the branched-chain α-ketoacid dehydrogenase in the perfused rat liver. *J. Biol. Chem.* 256:9009–15
174. Patel, T. B., Olson, M. S. 1982. Evidence for the regulation of the branched-

chain α-keto acid dehydrogenase multienzyme complex by a phosphorylation/dephosphorylation mechanism. *Biochemistry* 21:4259–65
175. Paul, H. S., Adibi, S. A. 1976. Assessment of effect of starvation, glucose, fatty acids and hormones on α-decarboxylation of leucine in skeletal muscle of rat. *J. Nutr.* 106:1079–88
176. Paul, H. S., Adibi, S. A. 1978. Leucine oxidation in diabetes and starvation: Effects of ketone bodies on branched-chain amino acid oxidation in vitro. *Metabolism* 27:185–200
177. Paul, H. S., Adibi, S. A. 1982. Role of ATP in the regulation of branched-chain α-keto acid dehydrogenase activity in liver and muscle mitochondria offed, fasted and diabetic rats. *J. Biol. Chem.* 257: 4875–81
178. Paul, H. S., Adibi, S. A. 1982. Activation of hepatic branched chain α-keto acid dehydrogenase by a skeletal muscle factor. *J. Biol. Chem.* 257: 12581–88
179. Paul, H. S., Adibi, S. A. 1983. Mechanism of activation of hepatic branched-chain α-ketoacid dehydrogenase by a muscle factor. *J. Biol. Chem.* 258: 11471–75
180. Paxton, R., Harris, R. A. 1982. Isolation of rabbit liver branched-chain α-keto dehydrogenase and regulation by phosphorylation. *J. Biol. Chem.* 257:14433–39
181. Paxton, R., Harris, R. A. 1983. Regulation of phosphorylation and inactivation of isolated rabbit liver branched chain α-ketoacid dehydrogenase. *Fed. Proc.* 42:543 (Abstr.)
182. Pearce, F. J., Baptista, M. 1980. Branched chain ketoacid metabolism in the perfused rat heart. *Fed. Proc.* 39:2140 (Abstr.)
183. Peng, Y., Gubin, J., Harper, A. E., Vavich, M. G., Kemmerer, A. R. 1973. Food intake regulation: Amino acid toxicity and changes in rat brain and plasma amino acids. *J. Nutr.* 103:608–17
184. Pettit, F. H., Yeaman, S. J., Reed, L. J. 1978. Purification and characterization of branched chain α-ketoacid dehydrogenase complex of bovine kidney. *Proc. Natl. Acad. Sci. USA* 75:4881–85
185. Pitot, H. C., Yatvin, M. B. 1973. Interrelationships of mammalian hormones and enzyme levels in vivo. *Physiol. Rev.* 53:228–325
186. Pösö, A. R., Wert, J. J., Mortimore, G. E. 1982. Multifunctional control by amino acids of deprivation-induced proteolysis in liver. *J. Biol. Chem.* 257:12114–20
187. Pozefsky, T., Felig, P., Tobin, J. D., Soeldner, J. S., Cahill, G. F. Jr. 1969. Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. *J. Clin. Invest.* 48:2273–82
188. Pozefsky, T., Tancredi, R. G., Moxley, R. T., Dupre, J., Tobin, J. D. 1976. Effects of brief starvation on muscle amino acid metabolism in nonobese man. *J. Clin. Invest.* 57:444–49
189. Pozefsky, T., Walser, M. 1977. Effect of intraarterial infusion of the ketoanalogue of leucine on amino acid release by forearm muscle. *Metabolism* 26:807–15
190. Ramanamurthy, P. S. V., Srikantia, S. G. 1970. Effects of leucine on brain serotonin. *J. Neurochem.* 17:27–32
191. Randle, P. J., Fatania, H. R., Lau, K. S. 1984. Regulation of the mitochondrial branched chain 2-oxoacid dehydrogenase complex of animal tissues by reversible phosphorylation. *Mol. Asp. Cell Regul.* 3: In press
192. Reed, L. J., Pettit, F. H., Yeaman, S. J., Teague, W. M., Bleile, D. M. 1980. Structure, function and regulation of the mammalian pyruvate dehydrogenase complex. In *Trends in Enzymology: Enzyme Regulation and Mechanism of Action,* ed. P. Mildner, B. Ries, 60:47–56. Oxford: Pergamon
193. Reeds, P. J. 1974. The catabolism of valine in the malnourished rat. Studies in vivo and in vitro with different labelled forms of valine. *Br. J. Nutr.* 31:259–70
194. Rogers, Q. R., Tannous, R. I., Harper, A. E. 1967. Effects of excess leucine on growth and food selection. *J. Nutr.* 91:561–72
195. Ruderman, N. B. 1975. Muscle amino acid metabolism and gluconeogenesis. *Ann. Rev. Med.* 26:245–58
196. Ruderman, N. B., Berger, M. 1974. The formation of glutamine and alanine in skeletal muscle. *J. Biol. Chem.* 249:5500–06
197. Sabourin, P. J., Bieber, L. L. 1981. Subcellular distribution and partial characterization of an α-ketoisocaproate oxidase of rat liver: Formation of β-hydroxyisovaleric acid. *Arch. Biochem. Biophys.* 206:132–44
198. Sabourin, P. J., Bieber, L. L. 1982. Purification and characterization of an α-ketoisocaproate oxygenase of rat liver. *J. Biol. Chem.* 257:7460–67
199. Sans, R. M., Jolly, W. W., Harris, R. A. 1980. Studies on the regulation of leucine catabolism. Mechanism responsible for oxidizable substrate inhibition and dichloroacetate stimulation of leucine oxidation by the heart. *Arch. Biochem. Biophys.* 200:336–45

200. Sans, R. M., Jolly, W. W., Harris, R. A. 1980. Studies on the regulation of leucine catabolism. III. Effects of dichloroacetate and 2-chloropropionate on leucine oxidation by the heart. *J. Mol. Cell Cardiol.* 12:1–16
201. Schauder, P., Schröder, K., Matthaei, D., Henning, H. V., Langenbeck, U. 1983. Influence of insulin on blood levels of branched chain keto and amino acids in man. *Metabolism* 32:323–27
202. Schauder, P., Schröder, K., Matthaei, D., Henning, H. V., Scheler, F., Herbertz, L., Langenbeck, U. 1981. Blood levels of branched-chain amino and keto acids in patients with diabetes mellitus, renal failure and liver cirrhosis. See Ref. 234, pp. 569–79
203. Shambaugh, G. E., Koehler, R. A. 1983. Fetal fuels VI. Metabolism of α-ketoisocaproic acid in fetal rat brain. *Metabolism* 32:421–27
204. Sherwin, R. S. 1978. Effect of starvation on the turnover and metabolic response to leucine. *J. Clin. Invest.* 61:1471–81
205. Shinnick, F. L., Harper, A. E. 1976. Branched-chain amino acid oxidation by isolated rat tissue preparations. *Biochim Biophys. Acta* 437:477–86
206. Shinnick, F. L., Harper, A. E. 1977. Effects of branched-chain amino acid antagonism in the rat on tissue amino acid and keto acid concentrations. *J. Nutr.* 107:887–95
207. Shirai, A., Ichihara, A. 1971. Transaminase of branched chain amino acids. *J. Biochem.* 70:741–48
208. Shotwell, M. A., Kilberg, M. S., Oxender, D. L. 1983. The regulation of neutral amino acid transport in mammalian cells. *Biochim. Biophys. Acta* 737:267–84
209. Sketcher, R. D., Fern, E. B., James, W. P. T. 1974. The adaptation in muscle oxidation of leucine to dietary protein and energy intake. *Br. J. Nutr.* 31:333–42
210. Sketcher, R. D., James, W. P. T. 1974. Branched-chain amino acid oxidation in relation to catabolic enzyme activities in rats given a protein-free diet at different stages of development. *Br. J. Nutr.* 32: 615–23
211. Sketcher, R. D., James, W. P. T. 1976. Estimation of muscle leucine oxidation in the perfused hind-limb of the rat: Effect of feeding with a protein-free diet. *Proc. Nutr. Soc.* 35:48A
212. Smith, T. K., Austic, R. E. 1978. The branched-chain amino acid antagonism in chicks. *J. Nutr.* 108:1180–91
213. Snell, K. 1980. Muscle alanine synthesis and hepatic gluconeogenesis. *Biochem. Soc. Trans.* 8:205–13
214. Snell, K., Duff, D. A. 1979. Muscle phosphoenolpyruvate carboxykinase activity and alanine release in progressively starved rats. *Int. J. Biochem.* 10:423–26
215. Snyderman, S. E., Cusworth, D. C., Roitman, E., Holt, L. E. 1959. Amino acid interrelationship: The effect of variations in leucine intake. *Fed. Proc.* 18:546 (Abstr.)
216. Spolter, P. D., Harper, A. E. 1961. Leucine-isoleucine antagonism in the rat. *Am J. Physiol.* 200:513–18
217. Spydevold, Ø. 1979. The effect of octanoate and palmitate on the metabolism of valine in perfused hindquarter of rat. *Eur. J. Biochem.* 97:389–94
218. Spydevold, Ø., Hokland, B. 1981. Oxidation of branched-chain amino acids in skeletal muscle and liver of rat. Effects of octanoate and energy state. *Biochim. Biophys. Acta* 676:279–88
219. Sullivan, S. G., Dancis, J., Cox, R. P. 1978. Transient and long-term differential modulations of branched-chain α-keto acid decarboxylase activity in hypophysectomized rats. *Biochim. Biophys. Acta* 539:135–41
220. Swendseid, M. E., Villalobos, J., Figueroa, W. S., Drenick, E. J. 1965. The effects of test doses of leucine, isoleucine or valine on plasma amino acid levels. The unique effect of leucine. *Am. J. Clin. Nutr.* 17:317–21
221. Tanaka, K., Rosenberg, L. E. 1983. Disorders of branched-chain amino acid and organic acid metabolism. In *The Metabolic Basis of Inherited Disease*, ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, M. S. Brown, pp. 440–73. New York: McGraw-Hill. 5th ed.
222. Tannous, R. I., Rogers, Q. R., Harper, A. E. 1966. Effect of leucine-isoleucine antagonism on the amino acid pattern of plasma and tissues of the rat. *Arch. Biochem. Biophys.* 113:356–61
223. Taylor, R. T., Jenkins, W. T. 1966. Leucine aminotransferase. I. Colorimetric assays. *J. Biol. Chem.* 241: 4391–95
224. Taylor, R. T., Jenkins, W. T. 1966. Leucine aminotransferase. II. Purification and characterization. *J. Biol. Chem.* 241:4396–4405
225. Tischler, M. E., Desautels, M., Goldberg, A. L. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? *J. Biol. Chem.* 257:1613–21
226. Tischler, M. E., Goldberg, A. L. 1980. Amino acid degradation and effect of

leucine on pyruvate oxidation in rat atrial muscle. *Am. J. Physiol.* 238:E480-86
227. van Hinsbergh, V. W. M., Veerkamp, J. H., Glatz, J. F. C. 1979. 4-methyl-2-oxopentanoate oxidation by rat skeletal-muscle mitochondria. *Biochem. J.* 182: 353–60
228. Wagenmakers, A. J. M., Veerkamp, J. H. 1982. Degradation of branched-chain amino acids and their derived 2-oxoacids and fatty acids in human and rat heart and skeletal muscle. *Biochem. Med.* 28:16–31
229. Wahren, J., Denis, J., Desurmont, P., Eriksson, L. S., Escoffier, J-M., Gauthier, A. P., Hagenfeldt, L., Michel, H., Opolon, P., Pans, J-C., Veyrac, M. 1983. Is intravenous administration of branched chain amino acids effective in the treatment of hepatic encephalopathy? A multi center study. *Hepatology* 3:475–80
230. Wahren, J., Felig, P., Hagenfeldt, L. 1976. Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patient with diabetes mellitus. *J. Clin. Invest.* 57:987–99
231. Walser, M. 1978. Keto acid therapy in chronic renal failure. *Nephron* 21:57–74
232. Walser, M. 1983. Nutrition in renal failure. *Ann. Rev. Nutr.* 3:125–54
233. Walser, M., Lund, P., Ruderman, N. B., Coulter, A. W. 1973. Synthesis of essential amino acids from their α-keto analogues by perfused rat liver and muscle. *J. Clin. Invest.* 52:2865–77
234. Walser, M., Williamson, J. R., eds. 1981. *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids*. New York: Elsevier/North Holland. 631 pp.
235. Waterlow, J. C., Harper, A. E. 1975. Assessment of protein nutrition. In *Total Parenteral Nutrition: Premises and Promises*, ed. H. Ghadimi, pp. 231–58. New York: John Wiley
236. Waymack, P. P., DeBuysere, M. S., Olson, M. S. 1980. Studies on the activation and inactivation of the branched chain α-keto acid dehydrogenase in the perfused rat heart. *J. Biol. Chem.* 255:9773–81
237. Wergedal, J. E., Harper, A. E. 1964. Metabolic adaptations in higher animals: X. Glutamic dehydrogenase activity of rats consuming high protein diets. *Proc. Soc. Exp. Biol. Med.* 116:600–4
238. Williamson, J. R., Walajtys-Rode, E., Coll, K. E. 1979. Effects of branched chain α-ketoacids on the metabolism of isolated rat liver cells. *J. Biol. Chem.* 254:11511–20
239. Windmueller, H. G., Spaeth, A. E. 1980. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. *J. Biol. Chem.* 255:107–12
240. Wohlhueter, R. M., Harper, A. E. 1970. Coinduction of rat liver branched chain α-keto acid dehydrogenase activities. *J. Biol. Chem.* 245:2391–2401
241. Wurtman, R. J., Hefti, F., Melamed, E. 1981. Precursor control of neurotransmitter synthesis. *Pharmacol. Rev.* 32: 315–35
242. Young, V. R. 1981. Metabolism of branched chain amino acids. In *Nitrogen Metabolism in Man*, See Ref. 79, pp. 108–9
243. Zapalowski, C. 1981. *Studies on the metabolism of the branched-chain amino acids in the isolated rat hindquarter*. PhD Thesis. Univ. Wisc., Madison. pp. 213–39
244. Zapalowski, C., Hutson, S. M., Harper, A. E. 1981. Effects of starvation and diabetes on leucine and valine metabolism in the perfused rat hindquarter. See Ref. 234, pp. 239–44