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Role of branched-chain ketoacids in protein metabolism

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Ketoanalogues of three branched-chain amino acids (leucine, isoleucine, and valine) are the principal constituents of ketoacid-amino acid mixtures currently under clinical trial in the United States [1], Canada, and France [2] as dietary supplements for patients with chronic renal failure. They have also been studied as agents of possible therapeutic value in portal-systemic encephalopathy [3], congenital hyperammonemia [4], post-operative N wasting [5], muscular dystrophy [6], McArdle's disease [7], and as feed additives for farm animals [8–11].

In addition to their possible uses, these branched-chain ketoacids (BCKA) play a role in normal amino acid metabolism, owing to their rapid interconvertibility (by transamination) with branched-chain amino acids (BCAA). Most of their potential therapeutic uses stem from effects of these compounds on protein turnover.

The purpose of this review is to summarize the current knowledge concerning the role of endogenous BCKA in protein metabolism and the effect of exogenous BCKA on N balance, with particular reference to renal failure. Other aspects of BCKA metabolism have been reviewed recently [12–15].

Effects of BCKA on growth and N balance

Measurement of nutritional efficiency of BCKA relative to BCAA

Numerous early studies established that BCKA (and also branched-chain hydroxyacids) can serve as dietary substitutes for BCAA in supporting the growth of rats on diets lacking one BCAA (reviewed by Close [16] and Baker [17]). Chawla, Stackhouse, and Wadsworth [18] were the first to analyze the efficiency of BCKA as substitutes for BCAA in quantitative terms. They proposed that nutritional efficiency of a specific BCKA be defined as the ratio of the dose of the corresponding BCAA (on a purified diet) to the substituted BCKA dose required to achieve the same rate of growth. They found that this ratio was 0.20 to 0.27 for ketoisocaproate (KIC) as a substitute for leucine, and was independent of dose. With ketoisovalerate (KIV) and valine, however, the ratio varied from 0.3 to 0.8, depending on the dose used [19]. This technique for determining nutritional efficiency is quite cumbersome.

Kang and Walser [20] showed that an isotopic technique yielded the same result. The principle of this technique is as follows. Keto analogues may be transaminated to amino acids or may be oxidatively decarboxylated. The portion of an administered ketoacid that undergoes transamination will have the same fate in the body as the corresponding amino acid. Since amino acids, but not ketoacids, can become incorporated into protein, determination of the extent of incorporation of label into whole body protein following administration of a labelled amino acid can be used as a measure of the fate of the amino acid. Simultaneous determination of the extent of incorporation of a different isotope into whole body protein, following administration of a labelled ketoacid, would then give the fraction transaminated. This fraction can be expressed as a ratio, R, defined as the ratio of the fraction of label derived from ketoacid that becomes incorporated into protein divided by the fraction of labelled amino acid incorporated into protein. Clearly this ratio will depend on relative rates of transport across cell membranes, relative rates of transamination, rates of loss of label by oxidative decarboxylation, and so forth. R expresses the global result of all of these processes. It is (precisely) the ratio of probability of a ketoacid molecule at the site of administration becoming incorporated into protein to the probability of an amino acid molecule at the same site becoming incorporated into the same protein. We showed that R for whole body protein averaged 0.39 in rats fed labelled KIC and leucine in moderate dosage, and did not differ from nutritional efficiency of KIC relative to leucine, assessed by the growth rate technique of Chawla and associates [18, 19] (Fig. 1).

R is constant in whole body protein for one hour onwards for at least a week after intragastric injection of labelled leucine and labelled KIC, despite many differences in the metabolism of these two compounds [21]. When the labelled compounds are given intravenously, R values are much higher for both KIC versus leucine and KIV versus valine, because oral administration leads to substantial first pass oxidation of BCKA but not BCAA in splanchnic organs [22]. Hence the nutritional efficiency of BCKA relative to BCAA is considerably greater when given parenterally.

In normal human subjects, R for KIC versus leucine in plasma albumin and fibrinogen is about 0.6 (similar to the value seen in free leucine), but higher values are seen in red cell globin and salivary mucin, suggesting that these proteins derive a portion of their leucine from circulating KIC, transaminated locally, rather than from circulating leucine [23]. Further evidence on this point is discussed below.

Imura et al [24] developed a technique for estimating R based on expired air. The principle of this technique is as follows. R, defined above, is the ratio of the fraction, f, of the dose of

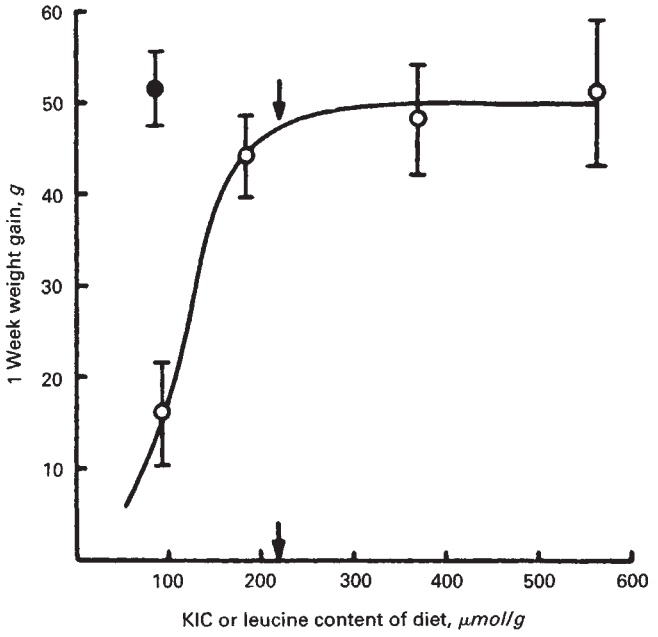

Fig. 1. *Growth of rats fed varying levels of KIC (○) as a substitute for dietary leucine (●).* Growth on a leucine-containing diet is indicated by the solid circle. Equimolar substitution of KIC leads to reduced growth. At a KIC excess of approximately 70% (arrow), growth is restored to the rate seen on leucine. This value, 1/1.7 = 0.6, is the same as the "R" value seen in whole body protein of these rats (see text). Drawn from data in reference 20.

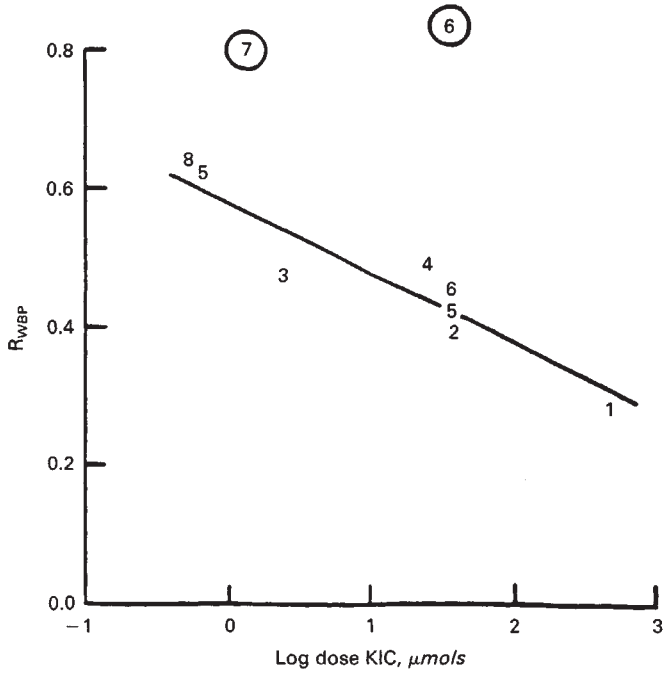

Fig. 2. *Nutritional efficiency of KIC relative to leucine, R, in whole body protein as a function of log dose of KIC in several reports from this laboratory.* Encircled numbers are from intravenous isotope injections; others are from oral isotope injections. R is nearly independent of dosage when given i.v., but decreases progressively with KIC dosage by the oral route, owing to increasing first pass oxidation. References are: *1*, 78; *2*, 20; *3*, 53; *4*, 76; *5*, 24; *6*, 22; *7*, 110; *8*, 21.

labelled ketoacid incorporated into whole body protein to the fraction, F, of the dose of labelled amino acid incorporated into whole body protein. Since only a trivial fraction of the administered compounds can remain as free amino acid or free ketoacid several hours after the injection, the fraction of labelled ketoacid oxidized is essentially 1-f and the fraction of labelled amino acid oxidized is 1-F. Therefore (1-f)/(1-F) can be estimated from expired air without analysis of the carcass, and R (= f/F) can be calculated. Using [1 − ^{14}C]leucine or [1 − ^{14}C]KIC (in different groups of rats) we found that the same average value for R as found by counting whole body protein could be derived from measurements of expired CO_2 [24].

Application of this technique to man, in whom recovery of labeled CO_2 in expired air is variable and incomplete [25], poses more problems than in the rat, in which recovery of labeled CO_2 is 95% or greater [24, 26, 27]. Even if both ^{14}C-labelled and ^{13}C-labelled compounds are used, two experiments in each subject will be necessary to obtain a value for CO_2 recovery from labelled bicarbonate, from a labelled BCAA, and from the corresponding labelled BCKA. Such studies have yet to be reported.

When non-tracer doses of BCKA are employed in studies of nutritional efficiency, as in the work of Chawla and associates [18, 19], the results may be affected by the N-sparing actions of these compounds. Thus nutritional efficiency of a given BCKA in a given species, whether assessed by growth and N balance or by relative rates of incorporation of labelled BCKA versus labelled BCAA into protein, might increase as the dose of BCKA is raised progressively from a tracer level into the pharmacologic range. However, when oral doses of labelled BCKA and labelled BCAA are used, a progressively greater fraction of the dose of BCKA is oxidized in the splanchnic bed with increasing dosage [22, 24]. This has the effect to reduce nutritional efficiency of BCKA relative to BCAA. This is illustrated in Figure 2, which summarizes R values observed in several of our studies. R decreases with oral KIC dose.

Non-isotopic studies should exhibit similar trends. Thus, N-sparing by BCKA, when given orally, should be easier to demonstrate at low dosage than at high dosage. On the other hand, when BCKA are given intravenously, N-sparing should not decrease with increasing dosage. As shown in Figure 2, limited data obtained by the isotopic technique show a slight increase in nutritional efficiency of KIC relative to leucine with an increment in KIC dosage, given intravenously. This point clearly deserves further investigation.

In growth experiments, ornithinine and histidine salts of KIC were equally effective but the lysine salt was less effective in replacing dietary leucine [28].

Utilization of diastereoisomers of KMV

S-KMV, the ketoanalogue of isoleucine, is more effective than R-KMV, the ketoanalogue of alloisoleucine, in supporting rat growth [28], but the latter compound can be utilized to some extent in rats [29] but not in chicks [30, 31]. In vivo racemization about the β-carbon atom occurs in dogs [32] and perhaps in rats [33] and man [34], but not in isolated rat muscle [35, 36]. The ornithine and lysine salts of racemic KMV are as effective as the sodium salt in supporting growth [28]. Large doses of

racemic KMV may reduce plasma isoleucine levels [34]. At commonly employed dosages of racemic KMV, plasma alloisoleucine gradually accumulates to levels higher than those of isoleucine, and disappears with a half-life of 7 to 12 hours [37].

N-sparing effects of BCKA in normal animals and man

N-sparing by KIC-containing mixtures given intravenously in man was first shown by Sapir et al [38]. In this study obese subjects in the seventh week of a total fast were given intravenously a complete mixture containing BCKA, the keto-analogues of phenylalanine and methionine, and the other four essential amino acids (lysine, threonine, tryptophan, and histidine) as such. Urine urea fell 39% during a week of daily infusions and remained below control values during the following week. Later, the same effects (including "carryover") were demonstrated early in total starvation by infusion of BCKA alone [39]. Subsequently Mitch, Walser, and Sapir [40] obtained the same result with KIC alone, and showed that leucine infusions had no such effect. These findings were confirmed by Cersosimo et al [41].

As noted earlier, a number of studies have documented growth rates of rats in which individual BCKA's have been substituted for the corresponding BCAA's [16, 17]. Effects of supplemental BCKA's, added to a complete diet, have been reported more recently, but not in final form. Flakoll and Nissen [42] fed rats KIC at three levels (0.0, 0.1, and 0.5% by weight of sodium salt) in conjunction with two energy levels and two protein levels (adequate and high). On the adequate protein diet, growth improved slightly but significantly (up to 8%) with increasing KIC dosage. Nissen and his associates [8, 9, 11, 43] have reported that KIC addition to a complete diet increases feed efficiency in lambs, milk production and milk fat content in cows, and egg production in hens. Abras and Walser [44] fed rats by continuous intragastric infusion with a mixture of BCKA, amino acids, and other nutrients. By carcass analysis we found that 65% of dietary N was retained for growth, a percentage far higher than previously reported for any nutrient regimen (on an ordinary diet, only 26% of dietary N is retained for growth by young rats). These results indicate a pronounced N-sparing effect of BCKA. KIC infusion reduced blood urea in normal subjects [45]. However, forearm intraarterial infusion of KIC failed to alter forearm release of lysine, tyrosine, or phenylalanine [46]. Yagi and Walser (unpublished observations) found that addition of KIC to a complete parenteral nutrient solution infused intravenously in rats reduced urinary urea excretion, and converted N balance from negative to positive. This was associated with an increase in steady-state plasma KIC concentration from 14.2 μM to 84.3 μM. Hauschildt and Brand [47] fed rats a diet in which all three BCKA's were substituted (at threefold higher levels) for the three BCAA's with a concomitant reduction in N intake. Growth was unaltered, compared to pair-fed controls receiving BCAA; urinary and plasma urea were lower. Laouari et al [48] fed rats diets in which KIC was substituted for leucine or KIV for valine at molar ratios of 1 to 3.5. Twofold increments in dosage restored weight gain, increase in length, and N retention as a fraction of N intake. Similar findings with respect to KIC versus leucine were reported by Kang and Walser (Fig. 1) [20].

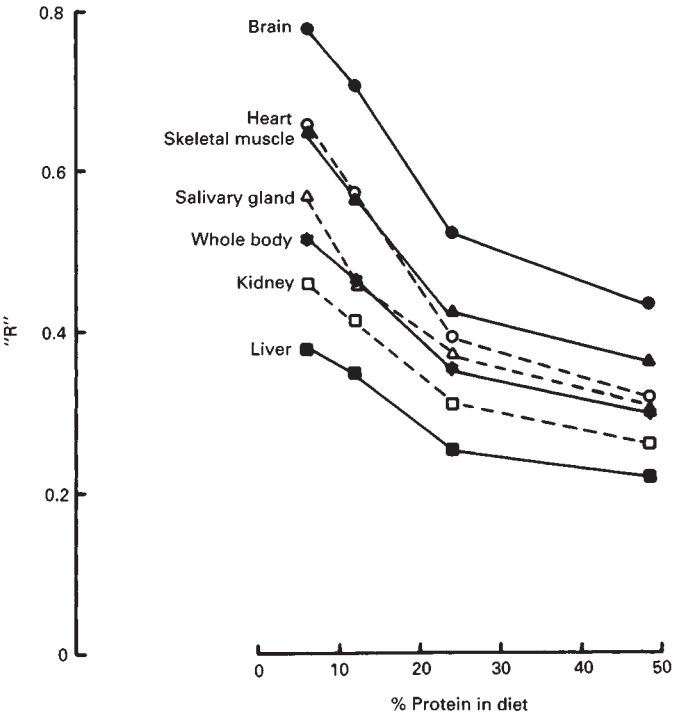

Fig. 3. *Relationship between the nutritional efficiency of ketoleucine relative to leucine, expressed as the ratio, R (see text), and protein intake.* Results in the protein of individual organs and in whole body protein are shown. Reprinted by permission from Kang, Tungsanga and Walser [53].

Effect of protein intake on nutritional efficiency of BCKA

It is well established that the enzyme responsible for degrading BCKA, branched-chain ketoacid hydrogenase (BCKAD), is activated by a high protein diet [47, 49]. Thus it is to be expected that protein restriction would reduce the fractional oxidation of BCKA and thereby improve their nutritional efficiency. This was demonstrated by Chow and Walser [50], who found no impairment of growth in rats consuming 6% or 10% amino acid diets when valine was replaced by KIV. In contrast, when rats were fed a 15% amino acid diet, equimolar substitution of KIV for valine led to reduced growth [51]. Epstein et al [52] observed only 13 to 32% decarboxylation of ingested KIV in subjects on a low protein intake, in contrast with 44 to 53% decarboxylation on a high protein diet. A more complete analysis of the effect of dietary protein on nutritional efficiency of KIC was reported by Kang, Tungsanga, and Walser [53]. Our results, summarized in Figure 3, show parallel variations in R values with protein intake in all organs studied, as well as in the body as a whole.

N-sparing by BCKA in stress

Sapir et al [5] randomized patients undergoing major abdominal surgery to receive daily intravenous infusions of glucose + $NaHCO_3$, leucine plus $NaHCO_3$, or sodium KIC. No other calories were given. N balance was less negative and 3-methylhistidine excretion (an index of protein breakdown) was lower in those receiving KIC. François, Rose and associates [54, 55] infused patients undergoing elective gynecological surgery to receive daily infusions of glucose (3 g/kg/day), glucose plus

leucine (90 mg/kg/day), or glucose plus sodium KIC (100 or 200 mg/kg/day). 3-methylhistidine excretion was lower in those receiving KIC, but N excretion did not differ. Whether the difference between these results and those of Sapir et al [5] is attributable to the concomitant infusion of significant quantities of glucose remains to be established. In septic [56] or injured [57] rats, two studies have reported no N-sparing by KIC. However, no increase in plasma leucine or KIC was observed in one of these studies [56]. This indicates that KIC decomposed before infusion, since at least ten studies have documented substantial increases in leucine and/or KIC in plasma when KIC is infused. In the second study, neither leucine or KIC was measured. Thus further study of this question is indicated.

Effects of BCKA on growth and N balance in uremia

Richards et al [58] fed normal and uremic subjects diets containing amino acids in place of protein. When valine was absent, N balance was negative, but when KIV (2 g/day) was added, it improved significantly. Similar results (in normal subjects) were obtained by Rudman [59] and by Gallina et al [60]. Walser et al [61] demonstrated maintenance of N balance in uremic patients fed various mixtures of BCKA, other analogues, and amino acids as supplements to a very low protein diet, for 15 to 18 days. Because N balance was more positive than with BCAA, the possibility of "altered metabolic pathways" was suggested. Rippich et al [62] also showed that N balance became positive when a BCKA-containing mixture of ketoacids and amino acids was added to an inadequately low protein diet in uremic patients: a "carryover" effect on N balance was again observed for two weeks after the analogues were withdrawn. Bauerdick, Spellerberg, and Lamberts [63] also showed that addition of either BCKA-containing supplements or BCAA-containing supplements to a 25 g protein diet caused improvement in N balance. Schmicker et al [64] followed 93 patients for an average of eight months on either ketoacid or essential amino acid supplements to a low protein diet. N balance, measured every three months, was usually less negative in those on ketoacids than in those on amino acids. Kampf, Fischer, and Kessel [65], in a crossover comparison of BCAA supplementation versus BCKA supplementation, observed better nutritional parameters on BCKA exclusively in patients with severe renal insufficiency. Mariani et al [66] administered ketoacids and a low protein diet to uremic subjects for 6 to 15 months. Albumin pools and levels of other serum proteins were maintained; fractional catabolic rate of albumin decreased. Lemke, Lindenau and Fröhling [67] treated 96 children with a low protein diet supplemented by either amino acids or ketoacids. N balance was more positive with the ketoacid supplement. Jureidini et al [68] found growth of children with chronic renal failure receiving ketoacid supplements to be faster than on conventional therapy. Heidland et al [69] switched uremic patients from an amino acid supplement to a ketoacid supplement after six months: blood urea fell, and serum levels of transferrin and other proteins rose, as did blood hemoglobin concentration. Ell et al [70] observed improvement in N balance in patients with chronic renal failure when a ketoacid supplement was added to a 31 g protein diet.

On the other hand, Burns et al [71] observed no difference in N balance between a BCKA-containing supplement and a BCAA-containing supplement in patients with chronic renal failure who were consuming an average of 44 g/day of protein. Hecking et al [72] administered 0.55 g protein per kg to uremic patients. After three months, either ketoacids or placebo was added. There was no evidence for protein deficiency before or during the treatment periods; the ketoacids essentially had no effect on protein metabolism. Lee and Jackson [73] found no difference in N balance between ketoacid supplements as compared with amino acid supplements to a 39 g protein diet. In all three of these studies, the BCAA content of the diet was probably sufficient to meet BCAA requirements. A greater fraction of administered BCKA would be oxidized on such diets, as noted earlier.

In uremic rats, Barsotti et al [74] found that addition to a standard 20% protein diet of a supplement containing predominantly BCKA improved growth and serum protein levels; urea N appearance fell. Friedrich et al [75] replaced part of the casein in the diet of rats with chronic renal failure with either BCKA-containing or branched-chain hydroxyacid-containing supplements; growth was improved by both supplements compared with controls. Laouari et al [48] varied KIC or KIV dosage in uremic rats. They found that nutritional efficiency of these analogues was the same as in non-uremic rats. However, Tungsanga, Kang and Walser [76], using the isotopic technique described earlier, found that the utilization of KIC for protein synthesis in various organs and in the body as a whole was greater in uremic rats than in control rats, despite equal protein intakes.

Abras and Walser [134] found that rats reinfused intragastrically with 90% of their urine output, receiving a nutrient mixture containing BCKA by constant intragastric infusion, grew as well as non-reinfused rats and utilized 67% of their dietary N for growth. Abras and Walser [77] also employed constant nasogastric infusion of a BCKA-containing nutrient mixture in patients with severe renal failure. Three-fourths of daily caloric intake was by this route. Total N intake averaged only 3.3 g/day, but N balance was nevertheless positive (+1.22 g/day). As in rats, N conservation on this regimen was higher than has been reported on any regimen (N requirement 2 g/day).

Utilization of BCKA for protein synthesis in liver disease

In rats with experimentally induced cirrhosis, portal-systemic shunts, or acute liver failure, incorporation of labeled KIC given orally into proteins is increased, in comparison with normal rats, except in the liver itself [78]. In patients with cirrhosis, similar results are seen [23].

Effects of BCKA on protein degradation in muscular dystrophy

Oral administration of the three BCKA's as ornithine salts for four days reduced urinary 3-methyhistidine excretion by a small but highly significant amount in boys with muscular dystrophy [6]. However, KIC supplementation failed to attenuate denervation atrophy in rats [79].

Interorgan metabolism of BCKA

Gastrointestinal absorption

Abumrad et al [80] reported that KIC instilled in the stomach of the dog appears in the circulation in a few minutes, and our

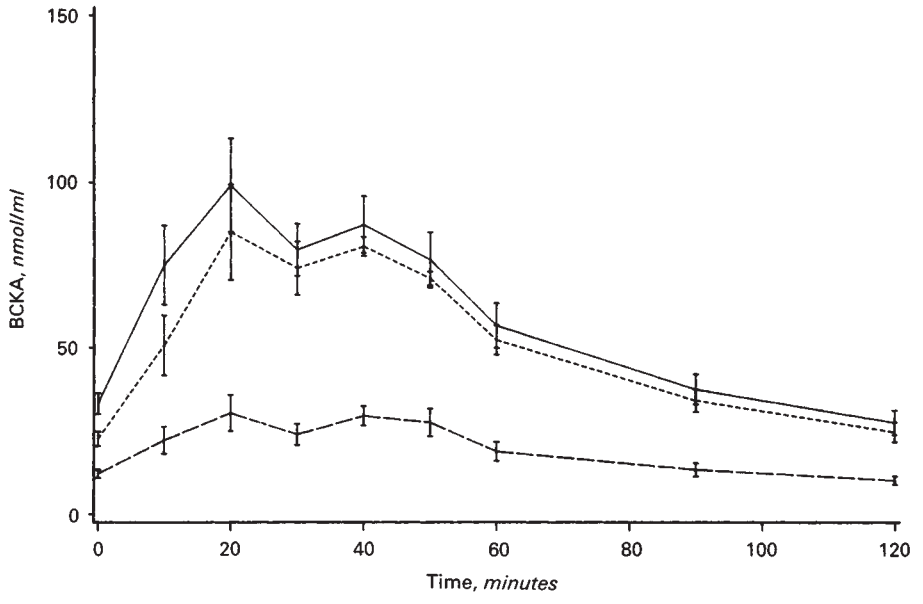

Fig. 4. *Response, in normal subjects, of plasma branched-chain ketoacid (BCKA) levels to oral ingestion of a mixture containing KIC (—), KMV (---), and KIV (– –).* The vertical lines represent only 1 SEM for clarity. Reprinted by permission from Walser, Jarskog and Hill [84].

study [24] of labeled CO_2 excretion following injection of ^{14}C-KIC or ^{14}C-leucine also provided evidence for rapid absorption of KIC from the GI tract. Ketoacids are relatively strong organic acids, with pK_a's in the range of 2 to 3 (unpublished observations), but would nevertheless exist largely in the undissociated form in the fasting stomach of normal individuals and normal rats, and might be absorbed by non-ionic diffusion.

We found that when ^{14}C-KIC and ^{3}H-leucine were administered together orally in rats, ^{14}C incorporation into stomach protein was many times greater than ^{3}H incorporation [21]. We concluded that KIC was rapidly absorbed by the stomach mucosa, transaminated to leucine, and utilized for synthesis of stomach tissue protein more rapidly than was labelled leucine administered as such.

BCKA's instilled into dog jejunum appear in mesenteric venous blood chiefly as such and also as the corresponding BCAA's [81]; about 1/3 of KIC is apparently oxidized by the jejunal wall. Rates of absorption of BCKA in rat jejunum and ileum are somewhat slower than those of the corresponding BCAA [82].

In man, BCKA given orally appear in the venous blood in a few minutes, and peak levels following a single dose occur at 30 to 60 minutes [83, 84]. When equal doses of KIV or valine are given, the peak increment in plasma concentration of KIV plus valine is about twice as great when valine is given as when KIV is given [85]. Similar comparative results are seen with the other two BCKA's and BCAA's [83]. However, when all three BCKA's are given together orally (as salts of basic amino acids in doses of 4.7 to 6.0 nmol each), the area under the plasma concentration curve for KIV is only about 1/4 as great as the corresponding areas for KIC and KMV (Fig. 4) [84]. The explanation of this observation may be that the muscle/plasma ratio and hence the volume of distribution of KIV exceeds that of KMV or KIC [86]. Another possible explanation of the lower plasma curve for KIV is competition between the three BCKA's for absorption. Weber, Deak and Laine [82] found that KIV absorption from rat small intestine was depressed 57% by the addition of KMV. If this is the explanation, larger doses of KIV might be required when using BCKA as supplements to an inadequately low protein diet. However, the possibility that BCKA's may be less than completely absorbed from the gut under any circumstances seems unlikely, except when small intestinal function is seriously compromised.

Plasma protein binding of BCKA

Albumin binds BCKA, at a site that also binds free fatty acids [81, 87]. Consequently, a major portion of circulating BCKA is bound to plasma proteins, although less so in the rat [88]. In normal human plasma, we have found fasting concentrations of KIC, KMV, and KIV are 29 ± 8 (SD) μM, 18 ± 4 (SD) μM, and 12 ± 3 (SD) μM, respectively [89], but considerable variability is apparent among different reports. The relative proportion that each BCKA comprises of total BCKA concentration is less variable: 49 ± 3 (SD)%, 30 ± 2 (SD)%, and 21 ± 2 (SD)%, respectively [89]. In the rat, concentrations and proportions are nearly the same [89; Matsuo, Yagi and Walser, unpublished observations]. Erythrocyte levels are low [90].

Tissue BCKA levels

Measurement of BCKA in tissues has proven to be exceptionally difficult. Livesey and Lund [91] measured the total of all three BCKA's in rat tissues enzymatically. They could not detect BCKA in liver, kidney, or mammary gland; levels in heart were very low; levels in muscle were similar to aortic blood or plasma. Hutson and Harper [86] developed a gas chromatographic procedure for analysis of individual BCKA's in tissues. They found muscle levels 1/3 to 1/2 of plasma. In heart and liver, far lower concentrations were found. However, a subsequent summary chapter from the same laboratory [92] states that "All three BCKA are present at about 5 nmol/g in skeletal muscle but are undetectable in brain." We have found levels in muscle half of those in plasma in rat and dog, lower concentrations in liver, heart, and kidney, and even lower levels in brain (Matsuo, Yagi and Walser, unpublished obser-

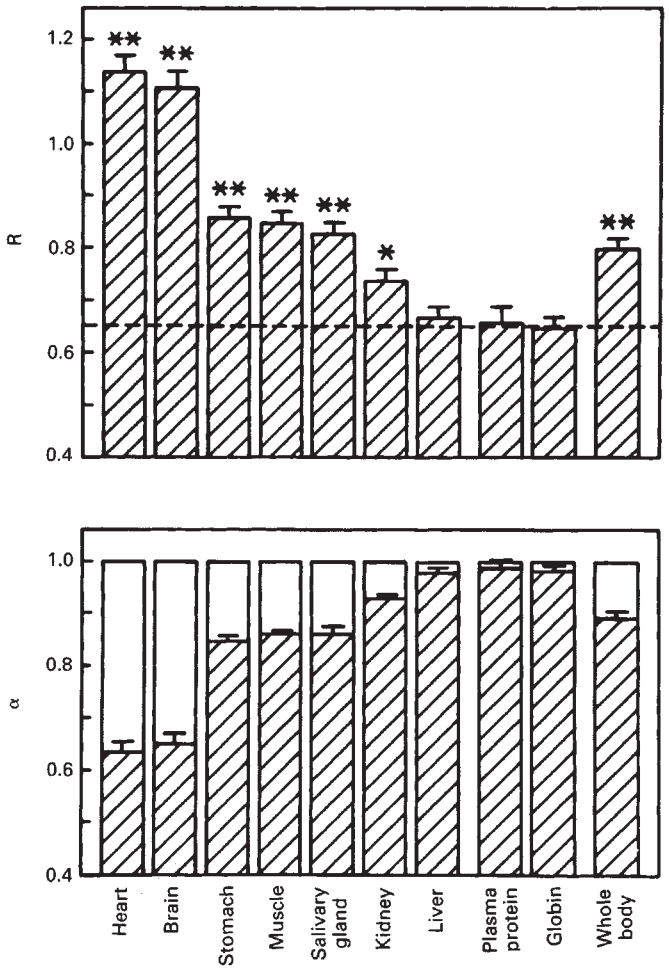

Fig. 5. *The $^{14}C/^{3}H$ ratio (R) of leucine incorporation into the protein of various tissues, blood, and whole body, and the fraction (α) of extracellular leucine + KIC incorporated into individual protein that is derived from extracellular leucine.* Dotted line shows the mean R_{leu} in plasma. Significantly different from R_{leu} in plasma: $^{*}P < 0.05$, $^{**}P < 0.01$. All data are presented as the mean ± SE of seven to nine rats. Reprinted by permission from Shiota, Yagi and Walser [110].

vations), using a new HPLC method with nearly complete recovery of labelled BCKA. Brain uptake may nevertheless be relatively high, as indicated below (Fig. 5); a high-affinity transport mechanism is present in the blood-brain barrier [93]. BCKA are more effective than BCAA in elevating brain BCAA levels, when given intravenously to rats with experimental liver disease [94], but less effective in this respect when given orally [95]. This is probably attributable to increasing first pass oxidation (Fig. 2), even though they are still more effective than oral BCAA in improving EEG, blood ammonia, and brain tyrosine [95].

Interorgan flux of BCKA

Harper and Zapalowski [96] suggested that in the rat, BCKA may be released by skeletal muscle and oxidized by liver. This pattern would be consistent with the distribution of BCAA transaminase, which is predominantly in muscle and is low in liver, in contrast with BCKAD, which is predominantly in liver and is low in muscle [97]. This hypothesis was confirmed by Livesey and Lund [98], who showed that hepatic venous blood concentration of total BCKA in the rat was half of aortic or portal venous concentration. Femoral vein concentration, on the other hand, was 63% higher. No release or uptake could be demonstrated across kidney or gut. However, we have demonstrated gut uptake as well as muscle release of each of the three BCKA; lung release is nil (Matsuo, Yagi, and Walser, unpublished results). Livesey and Lund [98] estimated that muscle release and hepatic uptake of BCKA were both about 1.4 mmol/day in a 400 g rat. They inferred that the low tissue concentration observed in liver reflected relative impermeability of the hepatic cell membrane.

However, this inference seems inconsistent with earlier experiments of the present author's performed in the same laboratory [99], in which rapid transamination of each of the three BCKA's, when added to the medium of isolated perfused liver, was demonstrated. Another possible explanation is that liver BCKA concentration is kept at a low level by BCKAD in this organ, despite high permeability. In normal fed rats, BCKA uptake by the liver from portal blood is about 60% of BCAA uptake; KMV uptake is the least efficient [100; Matsuo, Yagi and Walser, unpublished results]. Portal BCKA loads greatly increase hepatic uptake [100]. Abumrad et al [80] gave oral loads of KIC to dogs and observed that 35% was taken up by the liver, where 2/3 was oxidized and 1/4 transaminated. The gut and the kidneys also took up significant fractions of the dose, leaving only 15% accessible to non-splanchnic extrarenal organs. Nissen et al [101] showed in dogs that KIC is released by the hindlimb and taken up by the liver, but according to their results, KIC taken up by the liver is transaminated to leucine instead of being oxidized. In ovine fetus [102] and in mature ewes [103] hindlimb release of BCKA is substantially smaller than hindlimb uptake of BCAA. In fasting man, by contrast with rat or dog, significant release by peripheral tissues of KIC and KIV is only marginally demonstrable and remains small even after protein feeding or amino acid infusion [45, 46, 90, 104–106].

Precursor pools of BCAA and BCKA for protein synthesis

Most methods for measuring the rate of protein synthesis in vivo depend on plasma sampling to quantitate the specific activity (or atoms % excess) of a precursor amino acid during constant infusion of its tracer. However, it has long been recognized that the precursor pool of amino acid for protein synthesis may have a specific activity different from that in plasma. To avoid this problem, we have described a technique for measuring whole body protein synthesis in rats that does not require plasma sampling [27].

In the case of labelled leucine infusion, it has been suggested that plasma KIC specific activity would be a better index of intracellular leucine specific activity than would plasma leucine, because KIC is formed intracellularly [107]. During infusion of ^{3}H-labelled leucine and ^{14}C-labelled KIC in dogs, the $^{3}H/^{14}C$ ratio in protein-bound leucine was reported to be close to the "reciprocal pool" ratio in plasma, that is, [^{3}H-KIC]/[^{14}C-leucine] [108, 109]. However, we have found this to be true in some proteins in the dog, such as IgG and tissue proteins of liver, heart, muscle and kidney, but not in others, such as brain protein, red cell globin, albumin, and fibrin (Campollo, Matsuo,

and Walser, unpublished observations). Even larger discrepancies are seen in rats infused with the same isotopes [110]. We interpret these data to show that (1) precursor pool specific activity cannot be reliably estimated by plasma sampling and (2) extracellular KIC rather than extracellular leucine serves as the source of leucine for synthesis of many proteins (12% of whole body protein synthesis in the rat) (Fig. 5).

Mechanism of protein sparing by BCKA

As a N-free source of essential amino acids

It has long been evident that substitution of the minimum daily requirements of essential amino acids by their ketoanalogues could reduce minimal N requirements, provided that nonessential N were not limiting. However, the amount of N so spared cannot exceed the N required to convert these analogues to amino acids. Since two of the essential amino acids (lysine and threonine) cannot be replaced by ketoanalogues [16, 17], and two others (histidine and tryptophan) are not readily available as analogues, the amount of N so spared will not exceed the N content of the minimum daily requirement of BCAA plus phenylalanine plus methionine, namely, about 0.5 g. This minor degree of N-sparing is not, strictly speaking, a form of protein sparing, because it does not involve any change in protein synthesis or breakdown. From the earliest clinical studies with mixture of analogues [61], it was recognized that N-sparing was greater than could be accounted for in this way.

Suppression of glucocorticoid production

Nissen [9, 10] reported that addition of KIC to feed reduces plasma cortisol levels in lambs. However, they observed no such effect in cows [43]. In patients with chronic renal failure, we found that urinary 17-hydroxycorticosteroid excretion (a measure of 24-hour glucocorticoid production) was lower when ketoacid supplements were administered than when essential amino acid supplements were administered, in conjunction with a low protein diet [111]. This latter finding, in contrast to Nissen's data in lambs [9, 10], could simply mean that ketoacids stimulate glucocorticoid production less than amino acids, since it has been established that glucocorticoid production varies with dietary protein intake [112].

We have recently found even minor changes in mean 24-hour glucocorticoid levels (in corticosterone-replaced adrenalectomized rats) may induce profound changes in N balance, N excretion, whole body protein synthesis, and whole body protein breakdown (Quan and Walser, unpublished observations). Furthermore, spontaneous rates of growth of farm animals are often negatively correlated with their spontaneous plasma cortisol levels [113]. Hence, if BCKA cause only a small reduction in daily glucocorticoid production, significant N-sparing could result.

Stimulation of ketone body production

In post-operative patients exhibiting N-sparing induced by daily KIC infusions, ketone bodies concentrations in plasma were higher than in patients receiving leucine infusions [5]. Similar results are seen in fasting normal subjects given KIC [40, 45], but not in those given BCKA [39] or a BCKA-containing mixture [38]. Metabolism of KIC and KMV leads to ketone bodies, but KIC in particular stimulates ketone body production in isolated perfused liver by more than a stoichiometrically equivalent amount [114]. Since there is some evidence that ketone bodies may exert N-sparing effects [115], they could be involved in N-sparing induced by BCKA.

Direct action to suppress protein breakdown or stimulate protein synthesis

In isolated muscle [116, 117], heart [118], and liver [119], KIC at high levels suppresses protein breakdown, although not in muscle from septic rats [117]. According to one report [120], BCKA stimulate albumin synthesis by perfused liver. Whether these effects occur at KIC levels attainable in vivo is uncertain. This effect is not reproduced in isolated muscle by KIV or KMV or by isovalerate, the first metabolic breakdown product of KIC [121].

Stimulation of insulin production and glucose utilization

KIC stimulates insulin production and inhibits glucagon production by isolated perfused pancreas [122] and is responsible for the stimulation induced by leucine [123]. However, plasma insulin levels are little altered by BCKA administration [38–40, 45]. Forearm glucose utilization is reduced by infusion of KIC or KIC plus insulin, effects not reproduced by leucine [124]. In uremic patients, BCKA-containing diets improve glucose tolerance and insulin sensitivity [125–127]. Conceivably improved protein balance could result.

Stimulation of hepatic glutamate output

Häussinger and Gerok [128] have demonstrated a marked increase in glutamate output from isolated perfused liver on adding KIC or KMV, but not KIV, and have suggested that this could result in N-sparing by diverting ammonia from urea synthesis to glutamate synthesis. The fate of the diverted glutamate remains uncertain.

Induction and activation of BCAA transaminase and BCKA dehydrogenase

BCKA, administered orally, induce these enzymes [129, 130]. KIC, but not KIV or KMV, stimulates BCAA transaminase in kidney and muscle in vitro [131, 132]. KIC activates BCKA dehydrogenase [133]. Conceivably these effects could play a role in N-sparing by KIC, but no detailed mechanism has yet been elucidated.

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