Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man

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SUMMARY

1. Using the forearm balance method, together with systemic infusions of l-[ring-2,6-3H]phenylalanine and l-[1-14C]leucine, we examined the effects of infused branched-chain amino acids on whole-body and skeletal muscle amino acid kinetics in 10 postabsorptive normal subjects; 10 control subjects received only saline.

2. Infusion of branched-chain amino acids caused a four-fold rise in arterial branched-chain amino acid levels and a two-fold rise in branched-chain keto acids; significant declines were observed in circulating levels of most other amino acids, including phenylalanine, which fell by 34%. Plasma insulin levels were unchanged from basal levels (8 ± 1 μ-units/ml).

3. Whole-body phenylalanine flux, an index of proteolysis, was significantly suppressed by branched-chain amino acid infusion (P< 0.002), and forearm phenylalanine production was also inhibited (P< 0.03). With branched-chain amino acid infusion total leucine flux rose, with marked increments in both oxidative and non-oxidative leucine disposal (P<0.001). Proteolysis, as measured by endogenous leucine production, showed a modest 12% decrease, although this was not significant when compared with saline controls. The net forearm balance of leucine and other branched-chain amino acids changed from a basal net output to a marked net uptake (P<0.001) during branched-chain amino acid infusion, with significant stimulation of local leucine disposal. Despite the rise in whole-body non-oxidative leucine disposal, and in forearm leucine uptake and disposal, forearm phenylalanine disposal, an index of muscle protein synthesis, was not stimulated by infusion of branched-chain amino acids.

4. The results suggest that in normal man branched-chain amino acid infusion suppresses skeletal muscle proteolysis independently of any rise of plasma insulin. Muscle branched-chain amino acid uptake rose dramatically in the absence of any apparent increase in muscle protein synthesis, as measured by phenylalanine disposal, or in branched-chain keto acid release. Thus, an increase in muscle branched-chain amino acid concentrations and/or local branched-chain amino acid oxidation must account for the increased disposal of branched-chain amino acids.

Key words: amino acid kinetics, branched-chain amino acids, leucine, metabolism, protein, proteolysis, skeletal muscle.

Abbreviations: BCAA, branched-chain amino acids; BCKA, branched-chain keto acids; KIC, α-ketoisocaproate; KIV, α-ketoisovalerate; KMV, α-ketomethylvalerate.

INTRODUCTION

The branched-chain amino acids (BCAA), valine, leucine and isoleucine, have been shown to stimulate protein synthesis and inhibit protein breakdown in skeletal muscle studied in vitro [1-5], including isolated human muscle [6]. Similar anabolic effects have been demonstrated in the isolated perfused heart [7, 8] and liver [9]. These properties of BCAA can be reproduced by leucine alone, but not by valine or isoleucine [2, 3, 7-9].

Although this evidence has suggested a unique anabolic regulatory role for BCAA, particularly leucine, the actual physiological relevance of these observations is uncertain [10, 11]. Studies in vivo of the effects of administered BCAA have yielded conflicting results. In the rat, leucine supplementation has been reported to either increase [12] or have no effect on [13] skeletal muscle protein synthesis; one study found little effect of BCAA alone, but reported an enhancement of insulin action on muscle protein [14]. In fasted man, leucine has been reported to either reduce [15] or have no effect on [16] net urinary nitrogen loss. A wealth of contradictory results have also been reported in
healthy, postabsorptive man, neither leucine [25, 26] nor clinical settings of injury or severe illness [17–24]. In a healthy, postabsorptive man, neither leucine [25, 26] nor its transamination product, α-ketoisocaproate (KIC) [27], appear to promote net muscle protein anabolism, based on limb net amino acid balance measurements. Splanchnic balance of most essential amino acids is also unaltered by short-term leucine infusion [26]. On the other hand, studies using whole-body leucine tracer kinetics suggest that postabsorptive leucine infusion increases leucine incorporation into protein and hence, whole-body protein synthesis [28].

To investigate the actions of BCAA and other regulatory factors on muscle protein metabolism in man, we have developed a method in vivo for assessing muscle protein synthesis and breakdown rates, using systemic L-[ring-2,6-3H]phenylalanine infusion in combination with steady-state forearm muscle balance measurements [29, 30]. Phenylalanine is a particularly suitable tracer for studying muscle protein turnover, since, unlike leucine, it is not catabolized in muscle [31, 32]. In the present context of BCAA administration, this method offers the additional advantage of employing as tracer an essential amino acid that is distinct from those being infused. This enables a more direct and straightforward analysis of endogenous amino acid kinetics than is possible using labelled leucine as tracer in settings of leucine infusion. The present study was designed to determine the acute effects of BCAA infusion on whole-body and skeletal muscle protein turnover in man, assessed by phenylalanine kinetics. For the purpose of comparison, L-[1-14C]leucine was also infused during the study.

METHODS

Subjects

A total of 20 healthy adult volunteers, aged 18–34 years, were studied. In 10 subjects (seven males, three females) we studied the effects of BCAA infusion; the other 10 (eight males, two females) received saline (150 mmol/l NaCl) alone and served as controls. All subjects were within 15% of ideal body weight [32a]. None had any history of endocrine or other major organ system disease, and none was taking any medications. Informed, written consent was obtained from each volunteer before participation in the study, which was approved by the Human Investigations Committee of Yale University. The radioactive tracer dosage was approved by the Yale New Haven Hospital Radioactive Drug Research Committee.

Experimental design

All subjects were studied in the postabsorptive, overnight fasted state. Catheters were introduced into a brachial artery and retrogradely into an ipsilateral deep forearm vein. Patency of the catheters was maintained by a slow infusion of saline. Through a contralateral arm vein subjects received a primed, continuous, 5 h infusion of L-[ring-2,6-3H]phenylalanine (~ 35 μCi, ~0.45 μCi/min) and L-[1-14C]leucine (~12 μCi, ~0.16 μCi/min), along with a 2 μCi bolus of sodium [14C]bicarbonate to prime the body bicarbonate pool. The total radiation exposure of the infused tracers was 0.40 mSv. This radiation dose is 40% of the nominal yearly background radiation exposure of 1 mSv. After a 2 h tracer equilibration period subjects received a 3 h systemic infusion of either saline or BCAA. The BCAA solution (Branchamin; 4% in water; Travenol Laboratories, Deerfield, IL, U.S.A., kindly provided by Dr D. Madsen) was an equimolar mixture of valine, leucine and isoleucine, infused at a rate of 1.66 μmol min⁻¹ kg⁻¹. To hasten the attainment of stable elevations in circulating BCAA, for the first 30 min a three-fold faster infusion rate (5.0 μmol min⁻¹ kg⁻¹) was used. For determination of steady-state forearm and whole-body fluxes, arterial and deep venous blood samples and samples of expired air were obtained at ~30, ~20, ~10 and 0 min in the basal state, and during the last hour of the BCAA or saline infusion (at 135, 150, 165 and 180 min), by which time all measurements displayed steady-state conditions. Arterial samples were also obtained at intermediate time points in order to document changes in systemic hormone and substrate levels. For 1 min before and during withdrawal of each deep venous blood sample, a paediatric sphygmomanometer cuff was inflated about the wrist to 200 mmHg to exclude blood flow to the hand. Forearm plasma flow was measured immediately after each arteriovenous sampling interval by the dilution of Indocyanine Green dye (Hyson, Westcott and Dunning, Baltimore, MD, U.S.A.), infused intraarterially for 5 min with the wrist cuff inflated; blood flow was derived by dividing plasma flow by (1 − packed cell volume). Forearm volume was measured by water displacement.

For measurement of whole-body leucine oxidation, expired CO₂ was collected by bubbling expired air through hyamine hydroxide trapping solution that contained a phenolphthalein indicator titrated to change colour when a known amount of CO₂ was trapped. The total rate of CO₂ production was measured with the ventilated hood technique, using a Beckman Metabolic Measurement Cart and a modified version of the Beckman Nutritional Assessment program (Sensor Medics, Anaheim, CA, U.S.A.).

Calculations

The net forearm balances for glucose and amino acids were calculated from the Fick principle:

\[ \text{Net balance} = ([A] - [V]) \times F \quad (1) \]

where [A] and [V] are the arterial and venous concentrations, respectively, and \( F \) is the forearm blood flow. The net balance, in turn, represents the difference between the rate of tissue disposal of arterial substrate (\( R_d \)) and the rate of tissue release into vein of endogenously produced substrate (\( R_s \)). That is:

\[ \text{Net balance} = R_d - R_s \quad (2) \]
For phenylalanine and leucine, tissue disposal can be calculated from the measured fractional extraction \((E)\) of tracer as:

\[
R_s = E \times [A] \times F
\]

where \(E\) is the arteriovenous difference in tracer radioactivity divided by the arterial tracer radioactivity (all in d.p.m./ml). Muscle production of new, unlabelled amino acid can then be calculated from eqns. (1-3):

\[
R_s = \frac{(E \times [A] \times F) - ([A] - [V]) \times F)}{1 - \frac{SA_v}{SA_a}}
\]

which reduces to

\[
R_s = F \times [V] \times (1 - \frac{SA_v}{SA_a})
\]

where \(SA_v\) and \(SA_a\) denote the specific activity (d.p.m./nmol) of the amino acid in artery and vein, respectively. Thus, the measured dilution of specific activity across the tissue [given by \((1 - \frac{SA_v}{SA_a})\)] indicates the fraction of the total venous amino acid outflow [given by \((F \times [V])\)] that was contributed by the tissue release of new, unlabelled amino acid.

It should be noted from eqns. (2) and (4) that tissue kinetics \((R_s\) and \(R_d\)) can be fully defined by measuring \([A], [V], F\) and the arteriovenous specific activity ratio. That is, \(R_s\) can be directly calculated using eqn. (4), and \(R_d\) is then given by:

\[
R_d = R_s + \text{net balance}
\]

Hence, if the venous-to-arterial specific activity ratio can be determined, measurement of the absolute radioactivity concentrations in artery and vein is not essential. This approach was employed in calculating phenylalanine kinetics, using measurements of arterial and venous phenylalanine specific activity by an h.p.l.c. technique (see the Analytical methods section below).

The above calculations define the kinetics of amino acid exchange between forearm muscle and circulating blood. For phenylalanine, which is neither synthesized nor metabolized in muscle [31, 32, 33], the measured rate of disappearance of tracer across the forearm at steady state should reflect its rate of incorporation into protein, while tissue release of new, unlabelled phenylalanine should reflect its release from the breakdown of tissue protein. For leucine, \(R_s\) represents the total disposal of leucine entering tissue via the artery, but it does not distinguish between its possible fates in muscle, namely incorporation into protein, transamination or complete oxidation to \(CO_2\).

Whole-body circulating leucine and phenylalanine flux rates \((Q)\) were calculated from the rate of tracer infusion \((IR, \text{in d.p.m.}/\text{min})\) divided by the steady-state amino acid specific activities in arterial blood:

\[
Q = \frac{IR}{SA_0}
\]

Under steady-state conditions, \(Q\) defines the rate of amino acid entry into and exit from the circulating blood. Since leucine and phenylalanine are both essential amino acids, in the absence of exogenous input, tissue protein represents the sole source of new amino acid entering the circulation, and \(Q\) then provides an index of amino acid release from protein breakdown \((B)\). During BCAA infusion, measured leucine flux \((Q)\) will represent the sum of the rates of entry into the circulation of infused leucine and endogenous leucine; \(B\) is then given by \(Q\) minus the rate of leucine infusion. Leucine oxidation \((C)\) was calculated as:

\[
C = \frac{V(14CO_2)}{(SA_a \times 0.8)}
\]

where \(V(14CO_2)\) is the rate of production of \(14CO_2\) (in d.p.m./min) calculated from the product of the steady-state activity of expired \(CO_2\) (in d.p.m./mmol) and the total rate of \(CO_2\) production. The factor 0.8 corrects for the non-expired \(14CO_2\) generated from \(L-[1-14C]\)leucine oxidation, which is retained within body bicarbonate stores. Non-oxidative leucine disposal \((S)\) was calculated as:

\[
S = Q - C
\]

### Analytical methods

Blood glucose concentration was measured by the glucose oxidase method with a glucose analyser (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Plasma insulin was determined by double-antibody radioimmunoassay. Concentrations of acidic and neutral amino acids were measured in sulphasalicylic acid extracts of whole blood with an automated ion-exchange chromatographic technique (Dionex D-500; Dionex, Sunnyvale, CA, U.S.A.).

For the determination of leucine and phenylalanine specific radioactivity, 2 ml of acidified plasma was placed on a Dowex 50G cation-exchange resin column (BioRad, Richmond, CA, U.S.A.). The column was washed with 0.01 mol/l HCl, and the acid eluate was collected and frozen for subsequent analysis of the concentration of the branched-chain keto acids (BCKA), \(\alpha\)-ketomethylvalerate (KMV), KIC and \(\alpha\)-ketoisovalerate (KIV), using the method of Nissen et al. [34]. The amino acids retained on the Dowex column were then eluted with 4 mol/l \(NH_4OH\). The eluate was vacuum centrifuged to dryness and the residue was redissolved in 800 \(\mu\)l of 2% \((w/v)\) trichloroacetic acid. After centrifugation, a 200 \(\mu\)l aliquot was removed and counted for \(14C\) radioactivity with a dual-channel Packard Tricarb scintillation counter (Packard, Downers Grove, IL, U.S.A.). Leucine specific activity was calculated from the \(14C\) radioactivity measurement and the corresponding leucine concentration measurement.

Phenylalanine specific activity was measured in the remaining trichloroacetic acid supernatant by an ion-pair, reverse-phase h.p.l.c. technique. The mobile phase consisted of 16% methanol \((v/v)\) containing a phosphoric acid buffer \((pH\) approximately 5) to which heptane sulphonic acid was added as an ion-pairing agent (Low UV-PIC B7 Reagent, Waters Associates, Milford, MA, U.S.A.). The column flow rate was 1.2 ml/min. The column eluant was monitored for u.v. absorbance at a wavelength of 214 nm. With samples of 200 \(\mu\)l injected on to a 4.6 mm \(\times\) 25 mm Beckman Ultrasphere ODS \((C_{18}, 5 \mu m)\) column, a sharply
separated phenylalanine peak eluted at 14–16 min. This fraction was collected in scintillation vials and subsequently counted for \(^{3}H\) radioactivity. The mass of phenylalanine in each sample was calculated by comparing its peak area with that of prepared standards using a Nelson Analytical chromatography software package (Cupertino, CA, U.S.A.). Specific activity was calculated as the \(^{3}H\) radioactivity divided by phenylalanine mass. The coefficient of variation of phenylalanine specific activity, calculated in this fashion, was 2–3%.

**Data presentation and statistical analysis**

All data are presented as means ± SEM. The values presented for the basal and BCAA infusion periods (−30 to 0 min, and 135–180 min, respectively) were each determined from the mean of four steady-state determinations in each subject. Comparisons between the basal and BCAA infusion periods, and between the responses of saline- and BCAA-infused subjects, were performed using analysis of variance with repeated measures (CRISP Statistical Package, CRUNCH Software, San Francisco, CA, U.S.A.). Comparisons between basal and the last hour of BCAA or saline infusion were performed by using a paired t-test.

**RESULTS**

**Blood concentrations of amino acids, glucose and insulin**

Systemic infusion of BCAA caused significant three-to-seven-fold elevations in arterial concentrations of valine, leucine and isoleucine, with near-plateau levels attained within 60 min (Fig. 1, Table 1). The BCKA derivatives

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<th>Table 1. Effect of BCAA infusion on arterial blood concentrations and net forearm balance of amino acids and BCKA</th>
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<td><strong>Arterial concentration</strong> (µmol/l)</td>
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also rose significantly (approximately two-fold), but by less than did the BCAA themselves (Table 1). Simultaneous with the BCAA elevations, significant reductions in the levels of many other essential and non-essential amino acids were observed (Table 1), in keeping with observations by others [26, 28, 35]. This fall occurred within the first 60–90 min after BCAA infusion. Over the 135–180 min period, when kinetic measurements were obtained, the levels of other amino acids changed by less than 5%. As shown in Fig. 1, glucose and insulin levels showed little change during the BCAA infusion. Glucose fell slightly, from 4.5 ± 0.1 to 4.3 ± 0.1 mol/l, a small but significant change (P < 0.02) compared with that observed with saline infusion (4.4 ± 0.1 to 4.4 ± 0.1 mmol/l). Insulin levels were not altered by BCAA infusion (basal 8 ± 1 versus 8 ± 1 μ-units/ml at 2–3 h of infusion).

**Forearm blood flow and glucose uptake**

Forearm blood flow (basal, 4.1 ± 0.4 ml min⁻¹ 100 ml⁻¹ forearm volume) did not change during BCAA infusion (4.3 ± 0.4 ml min⁻¹ 100 ml⁻¹). Forearm glucose uptake (basal 1.0 ± 0.1 μmol min⁻¹ 100 ml⁻¹) fell significantly during BCAA infusion (to 0.7 ± 0.1 μmol min⁻¹ 100 ml⁻¹, P < 0.04). However, although the magnitude of this fall was slightly greater than that seen with saline alone (0.9 ± 0.1 to 0.7 ± 0.1 μmol min⁻¹ 100 ml⁻¹), the difference between the two groups was not statistically significant (P > 0.3).

**Whole-body amino acid kinetics**

As shown in Fig. 2, arterial leucine and phenylalanine concentrations and specific activity were constant during the sampling intervals used for the basal and BCAA infusion periods. Although not shown, venous leucine and phenylalanine concentrations and specific activities were also constant. This permitted the application of steady-state calculations of amino acid kinetics. Table 2 shows the whole-body leucine and phenylalanine kinetic responses to BCAA compared with saline. BCAA infusion significantly increased whole-body leucine flux, with significant increments in both oxidative and non-oxidative pathways of leucine disposal. Endogenous leucine appearance or flux (B) showed a variable 12% fall, which was not significantly different from the 10% fall over time seen in the saline group. On the other hand, phenylalanine appearance or flux was suppressed by 22% during BCAA infusion, a decline that was several-fold greater (P < 0.002) than the modest 6% fall in flux seen in the saline control group.

**Forearm muscle amino acid exchange**

In the postabsorptive state there was a net release from forearm muscle of BCAA as well as phenylalanine and other essential amino acids (Table 1). Infusion of BCAA resulted in a marked net forearm uptake of BCAA, whereas the net balance of most other amino acids,
including phenylalanine, remained negative (Table 1). During BCAA infusion the total net forearm balance of measured (acidic and neutral) amino acids became positive, an effect attributable exclusively to the shift in net BCAA balance. Despite the marked increase in net BCAA uptake, BCKA balance across the forearm (which basally was not significantly different from zero) was unchanged by BCAA infusion (Table 1).

Table 3 and Fig. 3 show the changes in forearm leucine and phenylalanine kinetics that occurred during BCAA infusion. For leucine, the marked increase in net forearm balance was associated with a pronounced, three-fold rise in forearm leucine $R_d$. Although forearm leucine $R_d$ also fell substantially (by 40%), a change which could have contributed to the more positive net balance, the $R_d$ response was variable and did not achieve statistical significance when compared with saline infusion. It should be noted that with marked elevations in amino acid level, the measurement of $R_d$ becomes less accurate, since at any given turnover rate the difference between arterial and venous specific activity will diminish (see eqn. 4).

The net forearm balance of phenylalanine remained negative during BCAA infusion. There was a tendency for the net release to diminish, but the change was not significant. Although the net phenylalanine balance showed no significant change, the tracer data disclosed a marked alteration in forearm phenylalanine kinetics. Phenylalanine $R_d$ exhibited a significant 43% decline during BCAA infusion ($P<0.03$ versus saline). However, accompanying this change was a simultaneous 43% fall in phenylalanine $R_d$, which prevented the net balance measurement from manifesting any significant change in response to BCAA. Thus, despite the very marked rise in forearm leucine $R_d$ (Fig. 3), and in whole-body non-oxidative leucine disposal (Table 2), forearm phenylalanine $R_d$ showed no rise and actually tended to fall. The fall in forearm phenylalanine $R_d$ and $R_a$ is consistent with the observed decrease in whole-body phenylalanine flux.

DISCUSSION

The present study demonstrates that, in normal post-absorptive man, infusion of BCAA acts to restrain the rate of production of circulating phenylalanine, measured both across forearm muscle as well as in the body as a whole. Since phenylalanine is neither synthesized nor degraded in muscle, the forearm results strongly suggest a diminution in rates of degradation of muscle protein. Similarly, in keeping with the forearm observations, the reduced whole-body flux of this essential amino acid implies a fall in the rate of whole-body protein turnover.

Inferences about tissue protein physiology based on circulating amino acid kinetics must always be drawn with caution [36–38]. A key limitation of all these techniques derives from our inability to directly measure the specific activity of the true tissue precursor pool for protein synthesis, namely aminocetyl-tRNA. Thus, the calculated forearm phenylalanine disposal ($R_d$), and the forearm release of unlabelled phenylalanine into vein ($R_a$), will accurately reflect free phenylalanine exchange with tissue protein only to the extent that arterial phenylalanine specific activity approximates that of the phenylalanine-tRNA pool. Since the tRNA pool could be at lower specific activity than the arterial pool, due to dilution to phenylalanine released locally from protein breakdown ('internal recycling'), our calculations must be regarded as minimum estimates. As discussed previously [29, 30], an alternative approach involves the use of venous, rather than arterial, phenylalanine specific activity as the precursor pool in the kinetic calculations, since the venous specific activity, having been diluted by tissue-derived unlabelled phenylalanine, should more closely reflect that of the mixed intracellular pool. With this approach, $R_a = F \times [A] \times (S_A/S_{SA}-1)$ and $R_d = R_a + \text{net balance}$ (see [30]). Applying this method to the present data yields higher absolute rates for phenylalanine $R_a (70 \pm 3 \text{ nmol min}^{-1} \text{ kg}^{-1} \text{ basal})$ and $R_d (51 \pm 9 \text{ nmol min}^{-1} \text{ kg}^{-1})$.
Although it remains unclear whether arterial or venous (by approximately 40%) during BCAA infusion.

At steady-state, namely that BCAA infusion inhibits forearm muscle proteolysis. BCAA has been previously shown to cause a decrease in systemic levels of phenylalanine and other essential amino acids (e.g. incorporation into protein). An alternative possibility, however, is that a transient increase in protein synthesis led to the fall in free amino acid turnover. Since insulin levels were unaffected by the infusion of BCAA, the observed alterations in circulating amino acids and in muscle and whole-body amino acid kinetics appear to be independent of insulin, and are probably mediated by the elevated BCAA concentrations themselves. Other studies in man have shown that infusing a full mixture of amino acids also suppresses endogenous amino acid appearance rates [39-41], and that this effect, in agreement with our observations, administration of BCAA has been previously shown to cause a decrease in systemic levels of phenylalanine and other essential amino acids [26, 28, 35]. The present phenylalanine kinetic results suggest that this effect may be mediated by a reduction in amino acid release into the circulation, due to diminished proteolysis, rather than an increase in tissue disposal of essential amino acids (e.g. incorporation into protein). An alternative possibility, however, is that a transient increase in protein synthesis led to the fall in free amino acids, which in turn suppressed rates of both synthesis and breakdown to less than basal levels. Although we cannot exclude this possibility, our data indicate that once a new steady state is established, the major influence of BCAA infusion is to suppress, rather than stimulate, amino acid turnover.

Since insulin levels were unaffected by the infusion of BCAA, the observed alterations in circulating amino acids and in muscle and whole-body amino acid kinetics appear to be independent of insulin, and are probably mediated by the elevated BCAA concentrations themselves. Other studies in man have shown that infusing a full mixture of amino acids also suppresses endogenous amino acid appearance rates [39-41], and that this effect, in agreement with the present findings, does not require a rise in circulating insulin levels [40]. Our observations suggest that elevations in BCAA may be an important factor mediating the suppression of body protein turnover seen with more generalized hyperaminoacidemia. In the forearm, BCAA infusion caused a marked increase in net muscle leucine uptake and in local leucine $R_e$. This could reflect either leucine accumulation in the free tissue pools, leucine incorporation into protein, leucine catabolism, or some combination. Any stimulation of protein synthesis should be accompanied by an increase in the disposal of other essential amino acids, such as phenylalanine. Since phenylalanine $R_d$ was not stimulated (Fig. 3), our data imply that the marked rise in forearm leucine $R_d$ was mediated by a combination of tissue accumulation and local leucine catabolism. The fact that forearm KIC release did not increase (Table 1) suggests that terminal oxidation was the likely fate of the leucine catabolized by the forearm. This conclusion is consistent with the findings of previous limb balance studies performed during either leucine [42] or mixed amino acid [39, 43-45] infusion where selective muscle removal of BCAA is accompanied by neither increased uptake of all other essential amino acids (as would be required for local protein synthesis) nor increased release of BCKA.

We observed a significant (22%) decline in whole-body phenylalanine flux ($R_d$) during BCAA infusion (Table 2). Endogenous leucine flux also declined, but the change was not statistically significant. This is not altogether surprising since during exogenous BCAA infusion, the endogenous leucine $R_d$ is estimated from the difference between total $R_d$ (2.67 μmol min⁻¹ kg⁻¹) and the leucine infusion rate (1.66 μmol min⁻¹ kg⁻¹). If endogenous leucine $R_d$ declined by 22% this would be only a 9% fall in the total $R_d$ which could be difficult to quantify precisely.

The whole-body leucine kinetic data suggest that the increased leucine disposal that occurs during BCAA infusion results from an increase in both oxidative and non-oxidative pathways of leucine disposal (Table 2). Similar results in man have been found during infusion of either a mixture of amino acids [39-41, 46] or leucine alone [28]. The increase in non-oxidative leucine disposal appears to be a relatively consistent finding during infusion of leucine-containing solutions, despite the use of a diversity of leucine kinetic models, including the KIC reciprocal pool model [28, 40, 41], the present primary leucine pool model [39, 40] or other models [46]. Since at steady-state non-oxidative leucine disposal is presumed to reflect body protein synthesis, this finding appears to be at odds

| Table 3. Effects of BCAA infusion on forearm muscle amino acid kinetics |
|-----------------------------|-----------------------------|-----------------------------|
|                             | BCAA infusion               | Saline infusion             | $P$†               |
|                             | Basal 135-180 min           | Basal 135-180 min           |                   |
| Phenylalanine               |                             |                             |                   |
| Net balance (nmol min⁻¹ 100 ml⁻¹) | -20 ± 4  | -12 ± 3 | -18 ± 3 | -19 ± 3 | 0.16 |
| $R_e$ (nmol min⁻¹ 100 ml⁻¹) | 56 ± 6 | 32 ± 6* | 56 ± 5 | 50 ± 4 | <0.03 |
| $R_d$ (nmol min⁻¹ 100 ml⁻¹) | 37 ± 5 | 21 ± 3* | 38 ± 4 | 31 ± 8 | NS   |
| Leucine                     |                             |                             |                   |
| Net balance (nmol min⁻¹ 100 ml⁻¹) | -28 ± 9  | 188 ± 24* | -30 ± 8 | -23 ± 8 | <0.001 |
| $R_e$ (nmol min⁻¹ 100 ml⁻¹) | 119 ± 16 | 67 ± 33 | 143 ± 24 | 127 ± 18 | NS   |
| $R_d$ (nmol min⁻¹ 100 ml⁻¹) | 94 ± 12 | 286 ± 31* | 113 ± 18 | 104 ± 13 | <0.001 |

†Comparing the change from basal to 3 h during BCAA infusion with that seen during saline infusion, by repeated-measures analysis of variance.
Fig. 3. Comparison of the response of forearm phenylalanine (■) and leucine (□) kinetics to BCAA infusion. BCAA infusion caused a marked increase in net forearm leucine uptake, mediated predominantly by a rise in leucine $R_u$. In contrast, phenylalanine showed no rise in $R_u$; phenylalanine $R_o$ was significantly suppressed by BCAA infusion. Statistical significance of the observed change from basal during BCAA compared with that seen during saline infusion (repeated-measures analysis of variance): *$P<0.03$, **$P<0.001$. Values are means, with error bars representing SEM.

with the phenylalanine forearm kinetic results showing no stimulation, and if anything, a suppression, of forearm phenylalanine $R_o$, a marker of muscle protein synthesis [30]. One possible explanation for this discrepancy is that body protein synthesis is stimulated at non-muscle sites, since these may contribute substantially to the whole-body measurement [39]. Were this the case, however, one would anticipate an increase, rather than the observed decrease, in whole-body phenylalanine disposal, since phenylalanine would have to accompany the incorporation of large amounts of leucine into protein. In a recent study in man leucine infusion was reported to decrease the $R_u$ and $R_d$ of phenylalanine as well as of valine [47], consistent with our own findings.

It is possible that plasma leucine kinetic models of body protein turnover, which are best validated for basal, steady-state measurements, are less satisfactory in settings where substantial quantities of exogenous leucine are entering the plasma compartment for a relatively brief period of time. Under these circumstances, non-oxidative leucine disposal may less accurately reflect leucine incorporation into protein. For example, leucine accumulation in tissue pools may be contributing to non-oxidative disposal, or leucine oxidation may be underestimated, causing non-oxidative disposal to be overestimated. Although we cannot definitively distinguish these and other possibilities, a sustained acceleration of protein synthesis in response to administered BCAA or leucine alone seems physiologically unlikely in the absence of provision of a full complement of exogenous amino acids.

In summary, the present study demonstrates that in normal man BCAA infusion suppresses the rate of new phenylalanine appearance in the systemic circulation, and specifically its release from forearm muscle. Since phenylalanine is an essential amino acid that is not metabolized in muscle, these results imply a suppression of whole-body and forearm muscle proteolysis in response to BCAA. Although BCAA infusion markedly increases whole body and forearm muscle leucine disposal, the absence of a concomitant rise in phenylalanine disposal suggests that pathways other than incorporation into protein account for the fate of removed BCAA.

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REFERENCES

Branched-chain amino acids and muscle protein turnover


