4th Amino Acid Assessment Workshop

Observations of Branched-Chain Amino Acid Administration in Humans[1,2]

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ABSTRACT   Since the in vitro study of Buse and Reid in 1975 showing a stimulatory effect of leucine upon rat muscle protein synthesis and reduction in proteolysis, a similar effect has been sought in humans. In 1978, Sherwin demonstrated in humans an improvement in N balance with infusion of leucine in obese subjects fasting to lose weight. A variety of subsequent studies have been performed in humans where leucine alone or the BCAAs have been administered in varying amounts and durations, and the effect upon protein metabolism has been measured. Measurements of changes in muscle amino acid metabolism were made by arteriovenous difference measurements and by biopsies. An anabolic effect of leucine and the branched-chain amino acids (BCAAs) on reduction of muscle protein breakdown was found in these studies, with no measured effect upon muscle protein synthesis. Later studies using stable isotope tracers to define both whole-body protein turnover and leg or arm protein metabolism have similarly concluded that leucine administration specifically induces a reduction in protein breakdown without increasing protein synthesis. This anabolic effect, produced through a reduction of protein breakdown in vivo in humans by leucine is contrary to in vitro studies of rat muscle where stimulation of protein synthesis, has been demonstrated by leucine. Likewise an increase in protein synthesis has also been demonstrated by insulin in rat muscle that is not seen in humans. Of the various studies administering BCAAs or leucine to humans for varying periods of time and amount, the results have been consistent. In addition, no untoward effects have been reported in any of these studies from infusion of the BCAAs at upward 3 times basal flux or 6 times normal dietary intake during the fed portion of the day.   J. Nutr. 135: 1580S–1584S, 2005.

KEY WORDS: • *protein synthesis* • *protein breakdown* • *leucine*

The branched-chain amino acids (BCAAs) share common enzymes for the first 2 degradative steps, transamination and subsequent decarboxylation of the branched-chain keto acids (BCKAs),[4] and the BCAAs are the only amino acids to share common metabolic steps. The BCAAs are also the only indispensable amino acids to have degradative metabolic pathways active in muscle (1–3). After a meal containing protein, half of the BCAAs in the meal will pass through the splanchnic bed during absorption and will appear directly in the systemic circulation (4,5). These observations have been confirmed by us and by others (3) measuring the direct splanchnic extraction of tracer leucine in humans (6,7). Because muscle and other nonsplanchnic tissues are the major sites of postprandial uptake of these BCAAs, it is easy to hypothesize that the BCAAs should be good signals to indicate to peripheral tissues postprandial amino acid availability. As such, we could speculate that one or more of the BCAAs could have cell-signaling capabilities to tell tissues to increase protein synthesis or to reduce protein breakdown to store amino acids after a meal.

One of the earliest clues that one or more of the BCAAs may have signaling properties for promotion of anabolism came from an in vitro study of rat muscle protein metabolism in response to addition of the individual BCAAs (8). Rat hemi-diaphragm muscles were incubated with the individual amino acids and the uptake of ^{14}C-lysine into protein was measured, as an index of protein synthesis. Leucine increased ^{14}C-lysine incorporation into muscle protein, but neither isoleucine, valine, nor any other amino acid tested produced this effect. Studies blocking transcription with addition of actinomycin-D or translation with cycloheximide suggested that leucine both decreased protein breakdown and stimulated protein synthesis but not through stimulation of transcription or translation and that the effects observed were limited among amino acids to leucine (8).

A number of subsequent in vitro studies were completed in rat muscle and liver after the report of Buse and Reid (8). These studies were reviewed in 1989 (9). All of the papers listed for studies of muscle showed a positive effect of leucine upon stimulation of protein synthesis and reduction of protein breakdown. Similar studies in liver showed stimulation of protein synthesis by leucine and a reduction in protein break-

down. The initial report of Buse and Reid (8) and follow-up studies by others (9) set the stage for testing the effect of leucine in vivo in humans.

In 1976, Wahren et al. (4), picking up on the earlier observations that muscle extensively oxidizes BCAAs and releases significant amounts of glutamine and alanine, performed a study to measure splanchnic bed balance through use of an hepatic vein catheter and leg balance through arteriovenous (AV) measurements across the leg after feeding of a protein meal in normal postabsorptive humans. They determined that the protein meal produced a large absorption and release of amino acids from the splanchnic bed into systemic circulation, largely from the BCAAs (**Fig. 1**). The BCAAs made up half of the amino acids released after the protein meal. At the same time, there was a large uptake by muscle of the BCAAs. Alanine and glutamine were continuously released by muscle and were taken up by the splanchnic bed (presumably glutamine by the gut and alanine by the liver), but there was no significant stimulation of release of these amino acids by leg after the meal. The results of this paper established that dietary BCAAs are largely extracted by muscle after a meal and that other amino acids are not extracted postprandially by muscle to the same extent as the BCAAs (4).

Sherwin (10) infused normal subjects by i.v. for 3 h with leucine at 75 μmol $\cdot$ m^{-2} $\cdot$ min^{-1}. This leucine infusion rate corresponds to ~120 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1} in these subjects and is approximately equal to the endogenous leucine flux (3,7). Plasma leucine concentration approximately doubled during the leucine infusion, blood glucose concentration decreased slightly, and plasma insulin and glucagon remained unchanged. These results are typical to what others have found in subsequent studies: leucine infusion does not significantly alter hormone levels, including insulin. Sherwin (10) also noted that insulin infusion caused a significant fall in the plasma concentrations of the other 2 BCAAs, isoleucine and valine, and decreases in several other amino acids, of which declines in phenylalanine, tyrosine, methionine, threonine, and serine were particularly noted.

Sherwin (10) also infused obese subjects who were fasting for weight loss with leucine for 4 h at 75 μmol $\cdot$ m^{-2} $\cdot$ min^{-1} on d 4, 29, and 30 of fasting. He noted that urinary N loss was reduced by ~25% on the day that leucine was infused (d 4 of fasting) and again when leucine was infused on d 2 after a month of fasting. Because urinary 3-methylhistidine excretion was not altered on the days of leucine infusion, Sherwin (10) concluded that the reduction of urinary N loss was not because of reduced muscle protein breakdown and could be caused by a stimulation of protein synthesis, supporting the in vitro observations of Buse (9). However, there were no direct measures of protein breakdown in this study, and we cannot rule out the possibility that a reduction in nonmuscle protein breakdown (e.g., liver protein) could be partially responsible.

AV exchange measurements

In 1980, Hagenfeldt et al. (11) began a series of studies of leucine or BCAA infusions into normal subjects, measuring uptake by various organ beds using AV difference measurements across each bed. They first infused leucine, i.v., for 2.5 h at 300 μmol/min, i.e., ~260 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}, which is double the anticipated endogenous leucine turnover (causing leucine flux to triple). They noted large falls in the blood concentrations of valine and isoleucine, as well as significant declines in phenylalanine, tyrosine, and methionine. From their AV exchange measurements, they noted (**Fig. 2**) that half of the infused leucine was extracted by muscle (when leg uptake is extrapolated to whole-body muscle mass), one-fourth of the infused leucine was extracted by the splanchnic bed (gut and liver), and 10% was extracted by the brain. Presumably the remaining 10% was extracted by other tissues (e.g., kidney). They could account for much of the fall of blood concentration of valine and isoleucine through splanchnic bed uptake of these amino acids in response to leucine infusion, but the fall in blood phenylalanine and tyrosine, for example, could not be directly attributed to organ uptake. They concluded that elevated leucine through leucine infusion may be altering L-system amino acid transport, essentially restricting free movement of these amino acids, such as phenylalanine and tyrosine, out of cells and causing a reduction of blood concentrations.

A year later, Eriksson et al. (12) infused the individual BCAAs into postabsorptive subjects and measured the effect upon blood amino acid levels. The BCAAs were infused at rates relative to their plasma concentrations to each other (300 μmol/min leucine, 600 μmol/min valine, and 150 μmol/min isoleucine) for 2.5 h. They measured the change in concentration of other amino acids as a function of the change in concentration of the BCAAs and determined that leucine alone produced the most significant changes (decreases) on the concentrations of other amino acids such as phenylalanine, tyrosine, and methionine. Valine and isoleucine had little effect. They concluded that the depression of amino acid concentrations was a leucine-specific, not a BCAA-specific effect. They also concluded, as per the prior study, that the significant fall in the levels of specific amino acid could be from a leucine-specific alteration in L-system amino acid transport. Because there was no general fall in all amino acid concentrations, the investigators could not assign any stimulation of protein synthesis effect to the leucine infusion (11).

Because protein breakdown must exceed protein synthesis in the postabsorptive state to accommodate obligatory amino acid oxidation (3), there will be a net release of amino acids from protein into the intracellular compartments. If plasma
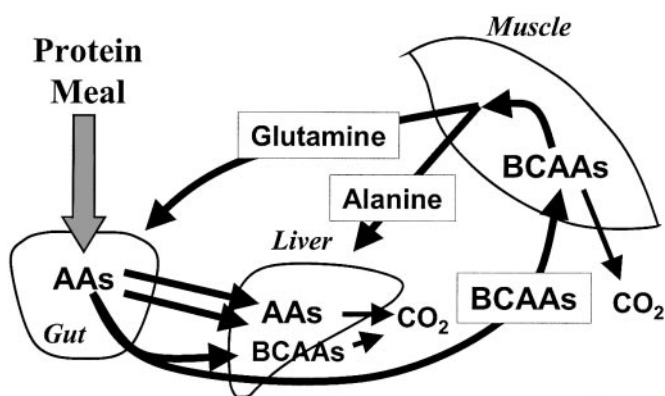

FIGURE 1 Diagram of the ingestion of protein on splanchnic and leg metabolism in humans. The figure is based upon data contained within the paper by Wahren et al. (4). Muscle released glutamine and alanine before the meal and during the meal, while the splanchnic bed extracted glutamine and alanine continuously. Many of the amino acids (AAs) and especially the indispensable AAs were retained in the splanchnic bed during meal absorption. The BCAAs accounted for about half of the AAs released from the splanchnic bed post meal, and muscle extracted most of the BCAAs that entered systemic circulation during meal absorption. Excess dietary amino acids that are not incorporated into protein are oxidized to CO_2, and muscle is a primary site for BCAA oxidation.
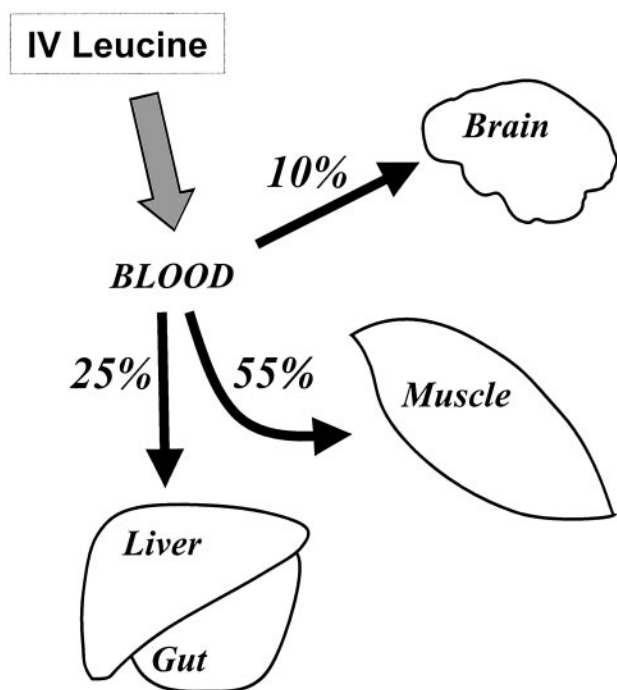
FIGURE 2 Diagram of the removal of i.v. infused leucine by different organ beds in humans. The figure is based upon data contained within the paper by Hagenfeldt et al. (11). Leucine was infused for 2.5 h into postabsorptive humans at 300 μmol/min. From AV balance measurements, Hagenfeldt et al. (11) determined that muscle extracted over half of the infused leucine, splanchnic bed extracted about one-fourth with the remainder removed by brain and other tissues.

concentrations of some amino acid fall as a result of leucine infusion restricting amino acid transport, then the intracellular concentrations of the affected amino acids must subsequently rise because of the net release of amino acids from protein. This hypothesis can be directly tested by measuring amino acid intracellular concentrations during a leucine infusion.

The Swedish group (13) then infused leucine, i.v., for 2.5 h at 300 μmol/min ($\sim$260 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1} and tripling the endogenous leucine flux). Besides measuring AV exchange of amino acids across the leg, they also obtained muscle biopsies before and at the end of the leucine infusion in half of the subjects to determine intracellular free amino acid concentrations. Based upon their leg AV exchange measurements, they concluded that $\sim$40% of the infused leucine was extracted by muscle and about half was accumulated as intracellular free leucine. Presumably, the remainder of the muscle-extracted leucine was oxidized or incorporated into protein. Plasma amino acid concentrations in general fell during leucine infusion with the greatest declines being in those previously shown to be affected. There was a reduced release of alanine and threonine, but no significant changes in the release of other amino acids from the leg were found with leucine infusion. The intracellular muscle concentration of leucine increased 3-fold with leucine infusion, but the concentration of most other intracellular muscle amino acids either had no change or decreased. These observations indicate that the depression in the plasma concentration of amino acids (e.g., phenylalanine, tyrosine, methionine, and threonine) cannot be caused by an altered intracellular–extracellular gradient from altered amino acid transport. The measurement of amino acid exchange

across the leg suggested an increase in uptake of amino acids because of a net increase in protein balance (either increased protein synthesis or decreased protein breakdown), but the uncertainty in the measurements precluded making any conclusions.

AV exchange measurement using tracers

Louard et al. (14) tested in humans the observation of Buse and Reid (8) in rats that elevating leucine increases muscle protein synthesis and decreases protein breakdown by performing an AV-balance study across the forearm but adding an infusion of [*phenyl*-2,6-^{3}H]phenylalanine and [1-^{14}C]leucine tracers. The tracers were used to determine both whole-body protein turnover (reflective of protein breakdown in the postabsorptive state) and to measure forearm muscle protein synthesis and breakdown using the phenylalanine tracer exchange measurements across the forearm model (15). They infused by i.v. either saline (control subjects) or BCAAs (100 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1} of each BCAA) into postabsorptive subjects for 3 h. This rate of BCAA infusion approximately matches endogenous leucine or valine flux and doubles isoleucine flux (3). BCAA infusion greatly increased the individual plasma concentrations of the BCAAs. Significant declines were observed in plasma concentrations of several amino acids, e.g., phenylalanine. The AV exchange across the forearm of the indispensable amino acids tended to move toward less release and more uptake. Whole-body protein breakdown measured with leucine declined during BCAA infusion, but a nearly similar decline was also noted in the saline control subjects, making this determination not significant. Subtraction of the large leucine infusion rate from the BCAA-infused subjects' leucine turnover made a clean measurement difficult for leucine kinetics. However, phenylalanine flux clearly declined $\sim$20% with BCAA infusion, defining a reduction in whole-body proteolysis. The forearm phenylalanine tracer exchange data supported this result: muscle phenylalanine appearance from protein breakdown declined about one-third with BCAA infusion. There was a nonsignificant decline in the rate of phenylalanine uptake for muscle protein synthesis with BCAA infusion. No stimulation of protein synthesis was measured with BCAA infusion (14). Note also that BCAA infusion did not alter insulin concentrations, thus assigning the reduction of protein breakdown to the leucine component of the BCAA infusion and independent of insulin, which is known to decrease protein breakdown (16,17).

Louard et al. (18) repeated their study, but this time addressed the question of whether the acute effects seen with a BCAA infusion would produce a longer-term effect or whether the acute effect would wane. Changes in intracellular pool sizes in response to BCAA infusion that could have affected some of the results seen during a short infusion period should stabilize during a longer infusion period. Thus, Louard et al. (18) infused BCAAs at a rate of 100 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1} per each BCAA, but this time as a 16-h overnight infusion. The tracers [^{3}H]phenylalanine and [1-^{14}C]leucine were infused at the end of the BCAA infusion period to determine both whole-body protein turnover and to measure forearm muscle protein synthesis and breakdown using the phenylalanine tracer exchange measurements across the forearm. Subjects who had been infused with saline for a shorter period were used as control subjects. The BCAA infusion increased the individual plasma concentrations of the BCAAs by 5–8 times. Significant declines were observed in plasma concentrations of most of the indispensable amino acids, e.g., phenylalanine. The AV exchange across the forearm of the indispensable

amino acids tended to move toward less release and more uptake but reached significance only for phenylalanine and methionine. Whole-body protein breakdown measured with leucine was not different between the subjects infused with BCAAs or saline, but a 37% decline was noted in phenylalanine appearance (a measure of protein breakdown) in the BCAA-infused subjects compared with the saline control subjects. The forearm phenylalanine tracer exchange data also showed a reduced rate of phenylalanine appearance from protein breakdown in the BCAA infusion group compared with the saline controls, and there was a nonsignificant decline in the rate of phenylalanine uptake for muscle protein synthesis with BCAA infusion. These results complement the earlier results and demonstrate that a BCAA infusion will produce a reduction in protein breakdown without a change in protein synthesis in humans infused with BCAAs for an extended 16-h period (18).

A similar study was performed by Nair et al. (19) using only a leucine infusion (150 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) or infusion of saline for 7 h into postabsorptive subjects. They measured AV balance across the arm during an infusion of [phenyl-^{2}H$_5$]phenylalanine and [1-^{13}C]valine tracers. Plasma leucine increased 4 times with the leucine infusion, and there was no change in any measured hormone. Significant declines were observed in plasma concentrations of the other BCAAs, phenylalanine, tyrosine, and methionine. The AV balance across the leg shifted toward less release and more uptake for indispensable amino acids, suggesting a change in muscle protein balance. The whole-body release of valine and phenylalanine from protein breakdown measured with tracers was significantly lower in the subjects infused with leucine compared with those receiving saline. The leg phenylalanine tracer exchange data showed a similar result: muscle phenylalanine appearance from protein breakdown was significantly lower in the leucine-infused subjects. There was also a nonsignificant decline in the rate of phenylalanine uptake for muscle protein synthesis with leucine infusion. These results, from Nair et al. (19), are concordant with both studies of Louard et al. (14,18) and also demonstrate that leucine administration produces an anabolic effect by reducing protein breakdown without causing an increase in protein synthesis.

Administration of BCAAs with other amino acids

One point that has been demonstrated from several studies is that insulin does not stimulate protein synthesis directly in humans. The fall in amino acid pools with insulin administration is attributed primarily to a reduction in protein breakdown (20). One thought is that protein synthesis cannot be stimulated without provision of amino acid substrate and that a protein stimulatory effect of insulin will not be seen when amino acids levels fall. To see a stimulatory effect, amino acids must also be infused with administration of insulin (21). Nonetheless, it appears that administration of amino acids promotes a stimulatory effect on protein synthesis, not on insulin (22).

In this context, McNurlan et al. (23) tested the effect of i.v. administration of BCAAs in addition to the provision of i.v. amino acids. The subjects in this study were patients who were to have colorectal tumors removed. As such, it gave these researchers a chance to also measure tumor rates of synthesis in response to BCAA administration. The patients received for 20 h before surgery either i) saline, ii) conventional amino acid formula infusion (0.2 g N/kg), or iii) amino acids with the addition of extra BCAAs (30% of the 0.2 g N/kg came from BCAAs). Amino acid infusion stimulated fractional protein synthetic rates equally in muscle and tumor. However, increasing the fraction of administered amino acids from BCAAs did not produce any additional stimulation. If anything, the synthetic rates were slightly reduced compared with the conventional amino acid infusion group. The results from this study showed that amino acid administration will stimulate muscle protein synthesis but that increasing the proportion of administered amino acids as BCAAs has no effect.

In vitro results of leucine stimulation of protein synthesis

Jefferson's group (24–28) have performed collectively a series of elegant studies investigating the signaling of protein synthesis stimulation in muscle. They have identified a leucine-mediated signaling pathway that stimulates protein synthesis in rat muscle through a stimulation of initiation of mRNA translation via activation of the mammalian target of rapamycin (mTOR) protein kinase. This pathway has also been shown to be stimulated through insulin's cell signaling pathway via the insulin receptor. The activation of mTOR results in the translational repression of eukaryotic initiation factor (eIF) 4E binding protein 1 that increases active 4F (eIF4F) complex for initiation of translation and simultaneously enhances phosphorylation of ribosomal protein S6 kinase. Anthony et al. (27) provides a good figure diagraming these signaling processes. The strong conclusion from this body of work is that leucine very clearly stimulates the processes increasing protein synthesis in at least 2 ways.

However, these observations from in vitro studies in rat muscle are not supported by results in humans. There are several reasons for these interspecies differences. One possibility is that rat physiology is regulated by different pathways than in humans and the pathway through mTOR does not show the same level of activity in human muscle. Another possibility is that in vivo regulation is more complex than that defined in in vitro preparations, and other factors may limit the ability of leucine to stimulate protein synthesis through mTOR. Regardless of the explanation, the original in vitro work of Buse and Reid (8) in 1975 through the current work in this decade from Jefferson's group (24–28) show a stimulatory effect of leucine on rat muscle protein synthesis that is not shown in studies in humans. The prominent effect in humans is a reduction of protein breakdown with administration of the BCAAs or leucine alone. This situation is analogous to the results found for insulin in humans (causing a reduction in proteolysis) vs. animal experiments (observation of stimulation of protein synthesis). It will take additional work to reconcile these differences.

Conclusions

BCAAs or leucine alone have been administered to humans in a variety of studies. Most studies have provided the BCAAs intravenously instead of enterally. Administration periods ranged from 1 or 2 h to almost 1 d. The amounts of the BCAAs administered were typically double to triple the normal turnover of the BCAAs and several-fold greater than that with respect to normal daily intake. None of these studies reported any untoward effects of either BCAA administration or administration of leucine alone. Neither BCAA nor leucine administration significantly altered concentrations of circulating hormones. However, both BCAA and leucine administration significantly reduced the plasma concentration of several indispensable amino acids. This reduction is not caused by a direct alteration of amino acid transport across cells but by a reduction of protein breakdown and reduced release of amino

acids from cells. This effect has been identified predominantly in muscle. No alteration or stimulation of protein synthesis with either BCAA or leucine administration has been defined in humans. The observed effects for BCAA administration can largely be reproduced by infusion of leucine alone.

The findings of the work to date suggest that leucine and the other BCAAs can be safely consumed in large amounts relative to the other amino acids in protein with no effect upon hormone or protein metabolism. Although large doses of leucine do not appear to stimulate protein synthesis in humans, the observations reported in animals and in vitro discussed above cannot be dismissed. Thus, any study designed to define the upper limits of tolerance in humans should include measurement of circulating hormones as well as measurement of protein metabolism using isotopically labeled tracers to confirm that high doses of BCAAs do not cause untoward effects with respect to hormonal regulation and protein metabolism in humans.

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