4th Amino Acid Assessment Workshop

Branched-Chain Amino Acids and Brain Function

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ABSTRACT   Branched-chain amino acids (BCAAs) influence brain function by modifying large, neutral amino acid (LNAA) transport at the blood–brain barrier. Transport is shared by several LNAAs, notably the BCAAs and the aromatic amino acids (ArAAs), and is competitive. Consequently, when plasma BCAA concentrations rise, which can occur in response to food ingestion or BCAA administration, or with the onset of certain metabolic diseases (e.g., uncontrolled diabetes), brain BCAA concentrations rise, and ArAA concentrations decline. Such effects occur acutely and chronically. Such reductions in brain ArAA concentrations have functional consequences: biochemically, they reduce the synthesis and the release of neurotransmitters derived from ArAAs, notably serotonin (from tryptophan) and catecholamines (from tyrosine and phenylalanine). The functional effects of such neurochemical changes include altered hormonal function, blood pressure, and affective state. Although the BCAAs thus have biochemical and functional effects in the brain, few attempts have been made to characterize time-course or dose-response relations for such effects. And, no studies have attempted to identify levels of BCAA intake that might produce adverse effects on the brain. The only "model" of very high BCAA exposure is a very rare genetic disorder, maple syrup urine disease, a feature of which is substantial brain dysfunction but that probably cannot serve as a useful model for excessive BCAA intake by normal individuals. Given the known biochemical and functional effects of the BCAAs, it should be a straightforward exercise to design studies to assess dose–response relations for biochemical and functional effects and, in this context, to explore for adverse effect thresholds.   J. Nutr. 135: 1539S–1546S, 2005.

KEY WORDS: • brain • blood–brain barrier • branched-chain amino acids • tyrosine • tryptophan • serotonin • catecholamines • rat • human • neurological diseases • metabolic diseases • exercise • toxicity

The branched-chain amino acids (BCAAs) leucine (LEU),[3] isoleucine (ILE), and valine (VAL) participate directly and indirectly in a variety of important biochemical functions in the brain. These include protein synthesis, the production of energy, the compartmentalization of glutamate (GLU; an excitatory amino acid neurotransmitter in the brain), and the synthesis of the amine neurotransmitters serotonin (5HT) and the catecholamines dopamine (DA) and norepinephrine (NE), which are derived from the aromatic amino acids

(ArAAs) tryptophan (TRP), phenylalanine (PHE), and tyrosine (TYR) (1–3). In relation to a connection between dietary BCAA intake and brain function, however, to date, only the production of the amine neurotransmitters appears clearly to have been linked to diet. This link occurs for several metabolic reasons: first, dietary protein contains considerable amounts of the BCAAs (e.g., 15–20% of the amino acid content of animal-based proteins) (4); second, a major fraction of ingested BCAAs is not metabolized by the liver and passes into the systemic circulation after a meal, causing plasma concentrations to rise appreciably and in proportion to the protein content of the meal (5,6); third, plasma BCAAs are transported into the brain [and other portions of the central nervous system (CNS)] by a transporter, located at the blood–brain barrier (BBB) on CNS capillary endothelial cells, that is almost fully saturated at normal plasma amino acid concentrations, competitive and shared by a number of large neutral amino acids (LNAAs), including the ArAAs TRP, TYR, and PHE (7–9). As a consequence of these relations, the ingestion of BCAAs causes rapid elevation of their plasma concentrations, increases their uptake into brain, and decreases the brain uptakes and levels of the ArAAs [e.g., see (10)]. And fourth, the synthesis and the release in the CNS of 5HT and the catecholamines varies directly and rapidly with changes in concentrations of their precursor amino acids (TRP, TYR, and

PHE, respectively) (3). Consequently, reductions in the brain levels of the ArAAs that follow the ingestion of amino acid mixtures containing BCAAs diminish the synthesis of these neurotransmitters (11–13). It is these neurotransmitter changes that largely drive the ongoing exploration for CNS functional changes after BCAA administration. It is also these relations that should form a part of any evaluation of the oral dose range in which humans can safely consume BCAAs.

Diet, BBB LNAA transport, and neurotransmitter synthesis

The relations between plasma LNAA concentrations, brain TRP and TYR uptake, and the formation of the amine neurotransmitters are summarized in **Figures 1** and **2**. The focus of each figure is the "revolving door," representing the BBB LNAA transporter, located at the capillary wall. Because this transporter is almost fully saturated at normal plasma LNAA concentrations and is competitive, the uptake of each LNAA into the brain will be affected not only by its own concentration in plasma but also by that of each of its competitors. For example, if plasma TRP (or TYR) declines or if plasma concentrations of the BCAAs (or other LNAAs) rise, brain TRP (or TYR) uptake and concentrations will fall. Conversely, if plasma TRP (or TYR) increases or if the plasma levels of the BCAAs (or other LNAAs) decrease, brain TRP (or TYR) uptake and concentrations will rise. Such changes in TRP or TYR lead rapidly to parallel alterations in the rates at which they are converted to their respective neurotransmitters, because the initial enzyme in the biosynthetic pathway of each (an ArAA hydroxylase, see Figs. 1 and 2) catalyzes the rate-limiting step in the pathway and is not fully saturated with substrate at normal brain concentrations. Consequently, raising or lowering brain TRP concentrations rapidly changes the rate of 5HT synthesis; the same relation holds for TYR and catecholamine synthesis, with some caveats (3). Moreover, precursor-related changes in 5HT and catecholamine synthesis rates directly modify the release of these transmitters from CNS neurons (14,15), which form the basis for thinking that brain functions might be modified as a result.
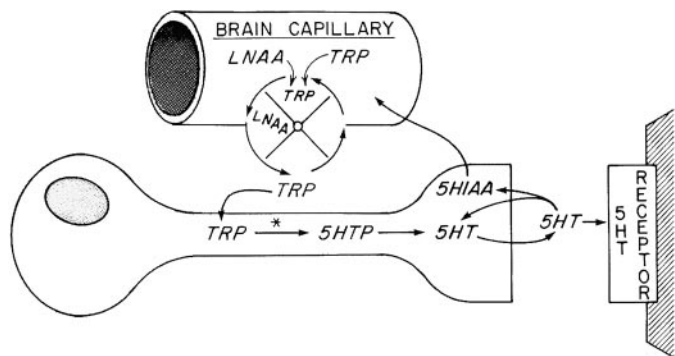

FIGURE 1   Brain tryptophan uptake and 5HT synthesis in neurons. TRP is converted to 5HT in neurons containing TRP hydroxylase (*), the rate-limiting enzyme in 5HT synthesis. TRP concentration controls synthesis, because the enzyme is normally unsaturated with TRP. Brain TRP uptake influences brain TRP levels and thus 5HT synthesis (and release by neurons). Brain TRP uptake depends on the serum levels of TRP and several other LNAA, which compete for transport at the BBB (revolving door in model). Meals and diet affect 5HT synthesis and release by directly influencing serum TRP and the other LNAA and thus brain TRP uptake and levels. 5HTP, 5-hydroxytryptophan, the product of TRP hydroxylation; 5HIAA, 5-hydroxyindoleacetic acid, the principal 5HT metabolite.
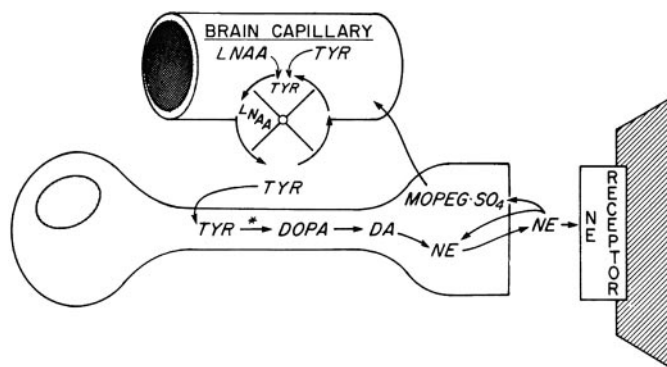

FIGURE 2   Brain TYR uptake and DA and NE synthesis in neurons. TYR is converted to DA and NE in neurons containing TYR hydroxylase (*), the rate-limiting enzyme in catecholamine synthesis. TYR concentration controls synthesis (in active neurons), because the enzyme is normally unsaturated with TYR. Brain TYR uptake influences brain TYR levels and thus catecholamine synthesis. Brain TYR uptake depends on the serum levels of TYR and its LNAA competitors for transport at the BBB (revolving door). Meals and diet affect DA and NE synthesis by directly influencing serum TYR and the other LNAA and thus brain TYR uptake and levels. DOPA, dihydroxyphenylalanine, the product of TYR hydroxylation; MOPEG-SO$_4$, a major NE metabolite in rat brain.

These relations suggest one further feature of interest. Because the uptake of an LNAA can be influenced by changes in the plasma concentrations of itself or any of the other LNAAs, any physiologic, pathophysiologic, or pharmacologic phenomenon that modifies the plasma LNAA pattern can modify LNAA uptake into the brain and thus, potentially, the synthesis and the release of the amine transmitters. Hence, oral or injected amino acid loads, normal foods, and certain metabolic diseases that modify amino acid metabolism all produce changes in the brain levels of TRP or TYR, and produce like modifications in their rates of conversion to their respective transmitters. Amino acid solutions containing LNAAs but lacking TRP or TYR, for example, when ingested by animals or humans, raise the plasma levels of the included LNAAs but not of TRP or TYR (indeed, their levels decline), and thus depress their brain uptake and the formation of 5HT or catecholamines (11–13). Or, a protein meal, fed to rats, raises the plasma concentrations of competing LNAAs relative to that of TRP and lowers brain TRP concentration and 5HT synthesis (16) but, at the same time, raises the plasma concentration of TYR relative to those of the other LNAAs, causing increases in CNS TYR concentrations and catecholamine synthesis (17). Chronic effects can be demonstrated as well, such as the observation that brain TYR concentrations rise as dietary protein content increases from very low (2% energy) to moderate (10% energy) levels, with catecholamine synthesis following the change in TYR (18). These effects parallel changes produced by the diet in the plasma level of TYR relative to that of its LNAA competitors. Finally, uncontrolled diabetes serves as a good example of another chronic metabolic setting, in which increases in the plasma concentrations of certain LNAAs produce a marked reduction in the brain uptake of other LNAAs and predictable effects on transmitter synthesis. The plasma concentrations of the BCAAs are high in uncontrolled diabetic rats, whereas those of the ArAAs are almost normal (**Fig. 3**). The predictable result is that brain concentrations of TRP, TYR, and PHE are abnormally low (19), causing reductions in both CNS 5HT and catecholamine synthesis (20,21). These effects cannot be attributed to direct changes in the LNAA transporter itself (22,23).
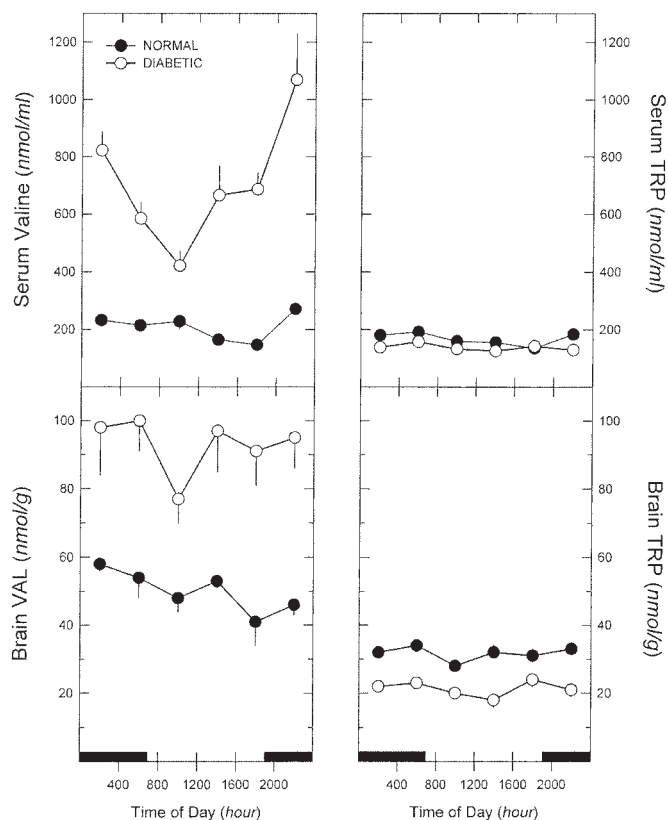
FIGURE 3 TRP and VAL concentrations in serum and brain of chronically diabetic rats. Rats (male, initial weight 150 g) were made diabetic with streptozotocin (65 mg/kg intracardiac) 3 wk before euthanasia. Food (standard rat diet, about 25% protein) and water were available ad libitum. They were killed at the indicated times. Horizontal black bars indicate daily dark period. Data are means ± SEM (n = 6 group). A significant group effect (P < 0.05; ANOVA) was present for each variable. Adapted from Crandall and Fernstrom (19).

Finally, functional effects of raising the plasma concentration of a BCAA can readily be demonstrated on 5HT- or catecholamine-linked brain functions that are modified by raising peripheral concentrations of TRP or TYR, respectively. One example is blood pressure, which is known to be influenced by catecholamine receptors in brain that, when stimulated, lower blood pressure (24). An injection of TYR [100 mg/kg (i.p.)] into spontaneously hypertensive rats produces a marked drop in blood pressure, an effect that can be blocked by coincident injection of an equimolar dose of VAL (**Fig. 4**). Another example is growth hormone secretion in the rat, which is stimulated by drugs that promote 5HT synaptic transmission in brain (25). Hence, an injection of TRP (100 mg/kg i.p.) into rats enhances the episodic secretion of growth hormone, and this effect can be blocked by an injection of VAL just before TRP administration (26). Together, such biochemical and functional effects in rats indicate that administration of BCAAs to elevate plasma BCAA concentrations should produce functional effects tied to reductions in brain TRP and TYR uptake, and the production and the release of their respective neurotransmitters.

Studies of BCAAs in humans

The BCAAs have been administered under a number of circumstances to healthy humans and to individuals with certain metabolic and neurological diseases. They have been given either alone or together with other amino acids, either as a single bolus or repeatedly for extended periods of time. In most (though not all) cases, when BCAAs have been given, they have been used to modify, indirectly, based on the competitive functioning of the BBB LNAA transport system, the concentration in the brain of one or more of the other LNAAs (e.g., TRP, TYR, PHE). In normal humans, the BCAAs have been used to improve mental and physical performance in athletes. In individuals with disease states such as phenylketonuria, hepatic encephalopathy, bipolar disorder, and other neurological diseases, they have been given to diminish or to retard the progression of CNS functional symptoms. Finally, individuals with a rare genetic disorder, maple syrup urine disease (MSUD), have also been studied, not as a target for BCAA administration but because they represent an unfortunate accident of nature in which plasma concentrations of the BCAAs are extremely high naturally (because of a defect in BCAA metabolism). The functional consequences of MSUD, therefore, could potentially provide insight into CNS aberrations that might be expected when excessive amounts of the BCAAs are ingested.

Athletes. Trained athletes use a variety of nutrients, including BCAAs, in an attempt to improve physical performance and mental focus during training and competition. The use of BCAAs is based on the notions that a) BCAAs can become depleted in muscle and plasma during exercise, producing a negative impact on muscle energy economy and promoting muscle fatigue, and b) plasma BCAA depletion can
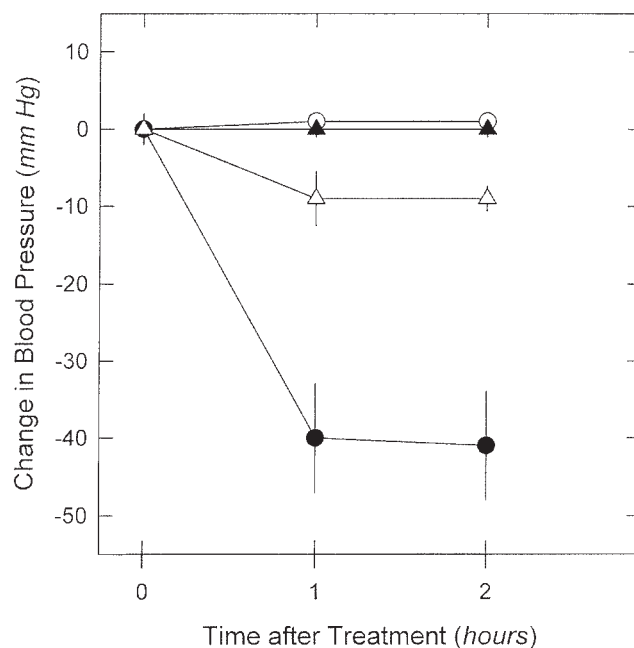
FIGURE 4 Effect of VAL on the antihypertensive action of L-tyrosine. TYR (100 mg/kg i.p., 0.55 mmol/kg; black circle), valine (0.55 mmol/kg, black triangle), TYR plus VAL (0.55 mmol/kg each; white triangle), or vehicle (white circle) was injected i.p. into spontaneously hypertensive rats (n = 4/group). Blood pressure was measured just before injection and then at the indicated times after injection. Data are expressed as the change in blood pressure from baseline values (mean ± SEM). TYR injection caused a significant decline in blood pressure (P < 0.01); treatment with TYR plus VAL produced a slight reduction in blood pressure (P < 0.05), but this effect was not as great as that produced by TYR alone. VAL and vehicle injections had no effect. Adapted from Sved et al. (62).

indirectly increase TRP uptake into brain, stimulate neuronal serotonin synthesis and release, and, as a consequence, cause central "fatigue" (27). Hence, supplying BCAAs during training and competition to counteract the BCAA-depleting action of exercise has been hypothesized to be beneficial to both muscle function and mental focus by preventing BCAA depletion. A number of studies have examined this idea in athletes, providing evidence in a population of normal (if highly fit) humans regarding the general tolerability of BCAA supplementation. For example, Strüder et al. (28) administered a total of 21 g of BCAAs (10.82 g LEU, 5.82 g VAL, 4.35 g ILE) to male cyclists in 2 doses (the first, just before a cycling test, and the second, 1 h later), and examined the effect (vs. a placebo) on their cycling performance over a 2.5-h period. Plasma BCAA concentrations (ΣBCAA = LEU + ILE + VAL) increased 4-fold, peaking at 1250 nmol/mL 90 min after the first dose. The authors noted that "supplementation did not induce any gastrointestinal discomfort nor were adverse side effects reported" (28). A similar cycle ergometry study was performed by van Hall et al. (29), in which the highest dose of the BCAAs was 23.4 g (equal amounts of VAL, ILE, and LEU in grams), spread at intervals over about a 2.5-h period. Plasma LEU, ILE, and VAL at the end of the exercise period (about 2.5 h after initiation) were 636, 561, and 1200 nmol/mL, respectively (ΣBCAA = 2397 nmol/m). No mention is made of any adverse effects in these athletes. And, Blomstrand et al. (27) conducted a study in experienced runners taking part in a lengthy race (30 or 42 km). The athletes running 42 km were given a total of 16 g (50% VAL, 30% LEU, 20% ILE), divided into 4 portions provided at equal intervals during the race (duration about 3.5 h; the 30-km athletes ingested 7.5 g in 5 divided doses). Blood samples were taken before and at the conclusion of the race. Plasma levels of each BCAA had doubled by the end of the race, and the total plasma BCAA concentration was 1250 nmol/mL. No mention was made in this report of any adverse events experienced by the runners. Other exercise studies involve chronic BCAA administration to athletes at significant doses (e.g., 200 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} for 30 d, or about 14 g $\cdot$ d^{-1} in a 70-kg subject) and make no mention of adverse effects (30). Because no performance decrements (and sometimes improvements) were noted, perhaps the subjects reported no treatment-related complaints (**Table 1**).

Phenylketonuria. Phenylketonuria (PKU) is an inherited metabolic disease involving a deficiency in the enzyme PHE hydroxylase. Individuals with this disease thus cannot hydroxylate PHE to TYR. As a result, in PKU patients eating a normal protein diet (at least 0.8 g protein $\cdot$ kg bw^{-1} $\cdot$ d^{-1} (31), in which the protein would contain about 5% PHE or at least 2.8 g PHE $\cdot$ d^{-1} $\cdot$ 70 kg^{-1}), plasma PHE levels become enormously elevated (20 times normal or more). PKU is successfully treated from birth by restricting PHE intake in the diet (low-PHE diet), indicating that either PHE or a metabolite is responsible for the devastating derangement of mental functions that results when the disease is untreated. Because of this suspected relation, the use of BCAA dietary "supplements" has been examined as a means to promote reductions (or further reductions) in brain PHE concentrations. The notion is that by elevating plasma concentrations of the BCAAs, brain PHE uptake can be diminished (or further diminished), thereby producing reductions in brain PHE concentrations and a beneficial effect to brain function. This concept is supported by recent work in human PKU subjects using magnetic resonance spectroscopy to follow brain PHE levels after an oral dose of PHE alone or together with other LNAAs (including the BCAAs): the increase in brain PHE that accompanied the

ingestion of PHE alone was prevented by coingesting the other LNAAs (32).

The chronic use of BCAA supplements has been evaluated in PKU subjects as either an adjunct to or substitute for a low-PHE diet (e.g., in patients unable to maintain the rather restrictive low-PHE diet). They were initially given to PKU subjects for up to 6 wk in 4 divided daily doses totaling 500 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}. This treatment significantly elevated plasma and cerebrospinal fluid (CSF) concentrations of the BCAAs and reduced CSF concentrations of both PHE and TYR in adolescents and adults (33). This treatment paradigm was associated with no adverse effects and, according to the investigators, has been used for up to 2 y in some patients (33). In later studies, in which this same dose regimen was examined over a 1-y treatment period, improvements in some cognitive functions were noted (34). In general, these and other studies have found that BCAA supplements in the 500 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} dose range are well tolerated and are associated with no adverse effects (33–36). In relation to plasma levels of LEU in patients with MSUD that are associated with neurologic effects (see below), plasma concentrations of LEU produced by BCAA ingestion by PKU subjects are comparatively low (<500 nmol/mL) (32,33).

Hepatic cirrhosis. Oral BCAA treatment has also been applied to patients with stable hepatic cirrhosis. This approach is based on the observation that liver failure produces elevated circulating levels of the ArAAs and depressed concentrations of the BCAAs. Such changes increase brain concentrations of the ArAAs, possibly stimulating the production of neurotransmitters and other biogenic amines that facilitate encephalopathy (37). Supplying BCAAs to elevate plasma BCAA concentrations is thus seen as a means to antagonize ArAA uptake into the brain and thus reduce the production of the biogenic amines derived from them. For example, in one study, patients ingested 250 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} of BCAA in divided doses at mealtime for 8 wk, or about 18 g $\cdot$ d^{-1} of BCAA (average patient weight was 70–75 kg). Indices of mental and motor function were significantly improved, and no adverse reactions were observed (38) (Table 1). In another study, subjects with advanced cirrhosis ingested for 12 mo 14.4 g BCAAs daily in divided doses at breakfast, lunch, and dinner. BCAA treatment reduced hospitalization, improved biochemical and pathophysiologic signs, and reduced anorexia (39). The principal side effects reported were gastrointestinal, but the incidence did not differ between treatment (BCAA) and control groups (there were two control groups, one receiving maltodextrin, and the other lactalbumin) (39). The absence of adverse effects (relative to a placebo) has recently been affirmed in a meta-analysis of a number of trials of BCAA use in hepatic encephalopathy (40).

Psychiatric, neurological, and other diseases. Oral BCAA supplements have been examined as a treatment for several neurologic diseases. For example, BCAAs have been given to bipolar subjects during periods of mania, on the presumption that this treatment will reduce brain TYR uptake and will slow catecholamine synthesis (increased catecholamine neuron activity is thought to be etiologic in mania) (41). The BCAAs (60 g) were administered daily for 7 d and produced a significant reduction in manic symptomology, consistent with an effect on brain catecholamines (42). In a dose-ranging study in normal volunteers, in which plasma amino acids were measured 300 min after dosing, plasma BCAA concentrations rose markedly (LEU to about 2000 nmol/mL), after ingestion of the 60-g BCAA dose (43). The BCAA drinks used "were well tolerated, and no adverse effects were reported by any of the volunteers" (43).

TABLE 1

BCAA studies in humans[1]

Subjects	Treatment	Effect on plasma BCAA	AE	Reference
Athletes adult, ♂	Acute treatment: 21 g BCAA (ratio V:I:L, 5.8:4.4:10.8); $n = 10$ (crossover design)	ΣBCAA = −1250 nmol/mL at 90 min	Reported No AEs	Struder et al. (28)
	Chronic: 200 mg · kg^{-1} · d^{-1} BCAA (ratio V:I:L, 1:1:2), 3 divided doses · d^{-1} for 30 d vs. placebo (71 kg bw ⇒ 14.2 g · d^{-1} BCAA); $n = 6$ BCAA; $n = 5$ placebo	Not measured	No comments regarding AEs	De Palo et al. (30)
	Acute: 7.5 g or 16 g BCAA during 30- to 42-km race (V:I:L, 50:15:35 or 50:20:30) in water with CHO; $n = 107$ BCAA; $n = 111$ control	Baseline ΣBCAA = −550 nmol/mL, end of race (3–3.5 h), low dose, ΣBCAA = −750 nmol/mL and high dose ΣBCAA = −1250 nmol/mL	No comments regarding AEs	Blomstrand et al. (27)
	Acute: 5.3 g total BCAA (V:I:L, 40:25:35) divided during marathon in water with CHO; $n = 24$ BCAA; $n = 28$ placebo	Baseline ΣBCAA = −520 nmol/mL, end of race, ΣBCAA = 650 nmol/mL	No comments regarding AEs	Hassmen et al. (63)
	Acute: 7.8 or 23.4 g BCAA (V:I:L, 1:1:1) in water with carbohydrates during cycling for 2 h; $n = 10$ (crossover design)	Baseline ΣBCAA = −500 nmol/mL, end of cycling, low dose, ΣBCAA = −950 nmol/mL and high dose ΣBCAA = −2400 nmol/mL	No comments regarding AEs	van Hall et al. (29)
Normal adult, ♀ + ♂	Acute: 10, 30, 60 g BCAA (ratio V:I:L, 3:3:4) (crossover design)	60-g dose: VAL −2350 nmol/mL, ILE −1300 nmol/mL, LEU −2000 nmol/mL at 300 min	Reported no AEs; "The drinks were well tolerated"	Gijsman et al. (43)
adult, ♀	Acute: 8-h infusion of Branchamin (BCAA; Travenol) at 4–6 g/h; $n = 6$ (crossover design)	5- to 14-fold rise in each BCAA in plasma	All infusions well tolerated; no AEs	Kirvela et al. (64)
adult, ♂	Acute: oral BCAA (55 g) as part 90 g total amino acid load with or without TYR or PHE; $n = 7$ (crossover design)	6 h after dosing, TYR + PHE ratio very low ⇒ BCAA very high (no data)	No comments regarding AEs	Montgomery et al. (42)
Bipolar adults	Chronic: 60 g oral BCAA/d (V:I:L:, 3:3:4) for 7 d during manic phase; $n = 8$ BCAA; $n = 10$ placebo	Not measured	Mania improved; BCAA generally well tolerated	Scarna et al. (41)
Tardive dyskinesia, adult, ♂	Chronic: 0, 222 mg/kg BCAA (V:I:L, 3:3:4) t.i.d. for 21 d 1 h before meals (80 kg bw ⇒ daily BCAA dose about 50 g); $n = 18$ BCAA; $n = 18$ placebo	No data presented; authors stated ΣBCAA about doubled 2 h after treatment administration (1 of 3 doses)	BCAA and placebo side effects same; no AEs on blood chemistries	Richardson et al. (45)
	Chronic: 150, 180, or 209 mg · kg BCAA^{-1} · d^{-1} (L:I:V, 4:3:3) in 3 divided doses for 14 d around mealtime; $n = 9$, not placebo controlled	Fasting ΣBCAA = −470 nmol/mL, ΣBCAA = −1240 nmol/mL 2 h post-AM high dose (−70 mg/kg)	"None of the subjects experienced side effects . . ."	Richardson et al. (44)
Spinocerebellar degeneration, adult, ♀ + ♂	Chronic: 0, 1.5, 3, or 6 g oral BCAA t.i.d. before meals for 4 wk; $n = 16$, all subjects received all treatments in random order	Not measured	"There were no remarkable side-effects"	Mori et al. (51)
Stable hepatic encephalopathy, adult, ♀ + ♂	Chronic: 250 mg BCAA · kg^{-1} · d^{-1} (L:I:V, 4.4:2.8:2.8) with meals for 8 wk; $n = 17$ (crossover design)	Placebo ΣBCAA = −240 nmol/mL, post-dosing/meal ΣBCAA = −450 nmol/mL, time post-meal not specified	No AEs observed. BCAA well-tolerated	Plauth et al. (38); Egberts et al. (65)
Hepatic cirrhosis, advanced, adult, ♀ + ♂	Chronic: 14.4 g BCAA · d^{-1} (L:I:V, 2:1:1) taken divided dose before meals for 12 mo ($n = 59$), vs. 2 placebos (lactalbumin, $n = 56$; maltodextrin, $n = 59$)	Not measured	"AEs usually mild and rapidly resolving . . ."; same in BCAA and placebo groups. (nausea, GI discomfort, diarrhea)	Marchesini et al. (39)
Cancer adult, ♀ + ♂	Chronic: 14.4 g BCAA · d^{-1} for 7 d as 4.8 g t.i.d. with meals (V:I:L, 1:1:2), vs. placebo; $n = 15$ BCAA; $n = 13$ placebo	Baseline ΣBCAA = −550 nmol/mL, 1 h after 4.8 g, ΣBCAA = −1250 nmol/mL	Appetite improved on BCAA; no comments about AEs	Cangiano et al. (52)
PKU adult & kids, ♀ + ♂	Chronic: 500 mg BCAA · kg^{-1} · d^{-1} (V:I:L, 3:3:4), 4 divided doses · d^{-1} for 2 d or 4–6 wk; $n = 11$, not placebo controlled	Adult ♀: Max V = −550 nmol/mL, I = −400 nmol/mL; L = −460 nmol/mL during daytime	Reported no AEs, even in Px on Rx for up to 2 y	Berry et al. (33)
adult	Acute: 0 or 90 mg BCAA · kg^{-1} (V:I:L, 1:1:1) with other LNAA (30 mg/kg each) every 3 h for 5 doses (total BCAA dose = 450 mg/kg); $n = 6$ (crossover design)	Baseline ΣBCAA = −430 nmol/mL, 2 h after dose 5, ΣBCAA = −1500 nmol/mL (L = 338 nmol/mL, I = 332 nmol/mL, V = 816 nmol/mL)	No AE, side effects reported	Pietz et al. (32)
young adult	Chronic: LNAA (800 mg · kg^{-1} · d^{-1}; 200 L + 146 I + 150 V, mg · kg^{-1} · d^{-1}) added to a low protein diet (0.6 g · kg^{-1} · d^{-1}) for 30 d; $n = 4$, not placebo controlled	Not measured	"The diet was well tolerated"	Dotremont et al. (35)
Adolescent & adult, ♀ + ♂	Chronic: oral BCAA (V = 150 + I = 150 + L = 200, mg · kg^{-1} · d^{-1}) in divided doses each day for 3 mo with meals and at bedtime; $n = 16$ (crossover design)	Nonfasting values, baseline ΣBCAA = −380; during chronic BCAA dosing; ΣBCAA = −820 nmol/mL (L = 228 nmol/mL, I = 144 nmol/mL, V = 448 nmol/mL)	No abnormal liver function; other metabolic safety measures normal	Berry et al. (34)
ALS, adult, ♀ + ♂	Chronic: 26.4 g/d BCAA (12 g L, 8 g I, 6.4 g V), 4 divided doses · d^{-1} for 12 mo vs. placebo; $n = 11$ BCAA; $n = 11$ placebo	Baseline ΣBCAA = 300 nmol/mL; ΣBCAA = 950 nmol/mL 60 min after ¼ daily dose	No comments regarding AEs; Rx was beneficial	Plaitakis et al. (49)
adult, ♀ + ♂	Chronic: 26.4 g/d BCAA (12 g L, 8 g I, 6.4 g V), 4 divided doses · d^{-1} with meals for 6 mo ($n = 31$) vs. THR (4 g/d + 160 mg/d pyridoxal P; $n = 32$) vs. placebo ($n = 32$)	Fasting ΣBCAA = −394 nmol/mL (V = 215 nmol/mL, I = 57 nmol/mL, L = 122 nmol/mL); 1 h after ¼ daily dose EBCAA 1957 nmol/mL (L = 554 nmol/mL, I = 795 nmol/mL, V = 608 nmol/mL)	Amino acids well tolerated; "no untoward effects"; but, faster loss of pulmonary function in BCAA and THR groups vs. placebo	Tandan et al. (50)
adult, ♀ + ♂	Chronic: 24 g/d BCAA (12 g L, 6 g I, 6 g V), 5 divided doses · d^{-1} before meals/food for 12 mo vs. placebo; $n = 13$ BCAA, $n = 11$ placebo	Fasting ΣBCAA = −400 nmol/mL (V = 220 nmol/mL, I = 60 nmol/mL, L = 120 nmol/mL); 1 h after ⅕ daily dose ΣBCAA = −1250 nmol/mL (L = 480 nmol/mL, I = 220 nmol/mL, V = 550 nmol/mL)	Study stopped because of increased mortality in patients on BCAA	Bastone et al. (47)
MSUD, children	MSUD patients during metabolic crisis and recovered vs. matched normal controls; no treatments; $n = 11$ MSUD, 10 controls	Control ΣBCAA = −475 nmol/mL; MSUD during crisis ΣBCAA = −5300 nmol/mL, MSUD recovered ΣBCAA = −1020 nmol/mL	Not applicable	Wajner et al. (55)

[1] Normal baseline plasma BCAA concentrations (nmol/mL): L, 100–120; I, 60–80; V, 200–300; varies with daily dietary protein intake (66, 67).

AE, adverse event; CHO, carbohydrate; GI, gastrointestinal; I, isoleucine, L, leucine; P$_x$, patients; R$_x$, treatment; ΣBCAA, sum of branched-chain amino acid concentrations; THR, threonine; t.i.d, three times a day; V, valine.

BCAAs have also been administered to patients with tardive dyskinesia, a notable aberration of voluntary motor control that develops in schizophrenic patients taking antipsychotic drugs. The application of oral BCAA therapy to this patient population followed from the observation that plasma PHE concentrations were high in these patients, possibly causing abnormally high PHE levels in the brain and adverse neurochemical effects. In one study, the doses examined ranged up to 209 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}, given in divided doses daily for 2 wk. The summed plasma BCAA concentration 2 h after the morning dose rose about 3-fold over baseline values to about 1250 nmol/mL (data for individual BCAAs not given); involuntary motor movements were diminished notably (44). Moreover, the investigators noted that "none of the subjects experienced adverse effects during the course of the trial" (44). In a later study, using a slightly higher dose (222 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}), administered for 3 wk, a similar outcome was obtained. The only adverse effect reported by subjects was occasional, mild gastrointestinal side effects; no clinically significant changes were found in routine physical examinations or in blood or urine chemistries (45).

The BCAAs have been studied as a treatment for amyotrophic lateral sclerosis (ALS; Lou Gherig disease), a progressive, debilitating and fatal neurological disease of the neurons that control the musculature (upper and lower motor neurons) (46). The logic for this application is that the ALS brain contains below-normal levels of GLU dehydrogenase, an enzyme that catabolizes GLU, suggesting that extracellular brain GLU levels may be abnormally high. In the brain, GLU is an excitatory neurotransmitter; excessive extracellular levels can overstimulate neurons, causing them to die (excitotoxicity). GLU dehydrogenase is activated by the BCAAs; hence, BCAA administration has been hypothesized to restore enzyme activity, increase brain GLU disposal rate, and thereby diminish the neurotoxic effects of excessive extracellular GLU (47) [this mechanism is now in dispute; see (48)]. The result would be to slow the progression of ALS. An initial study in ALS patients, providing 26.4 g $\cdot$ d^{-1} BCAA for 1 y, showed a slower rate of neurological decline in ALS patients (compared with control) and no side effects (49). However, later studies observed adverse effects in the active treatment group; for example, one study was stopped because of an increase in mortality [see (47)]. In another study, using 26.4 g BCAA administered daily in divided doses for 6 mo, BCAA treatment was noted to accelerate the decline in respiratory function (50), ultimately, the most common cause of death in ALS patients (46). The basis for such adverse effects is unknown, but a recent meta-analysis concluded that the use of BCAAs to treat ALS actually did not significantly increase the incidence of adverse effects or the death rate above that because of placebo (or provide significant benefit to the ALS patient) (48).

Patients with spinocerebellar degeneration (51) and cancer (52) have also been given BCAAs. In spinocerebellar patients, daily doses of up to 6 g for 4 wk significantly improved symptoms (compared with control) and were associated with no side effects. In an attempt to moderate anorexia, cancer patients were given 14.4 g BCAA (or a placebo) each day in 3 divided doses for 7 d. Food intake increased significantly in the BCAA group (the expectation was that the treatment would diminish TRP uptake into brain and thus 5HT release, an action that should increase hunger (53). Plasma total BCAA concentrations were measured 1 h after ingestion of one-third of a daily dose and before a meal; plasma BCAA levels increased about 2-fold, to a value about 1200 nmol/mL. No adverse effects were reported.

MSUD. MSUD, an inherited metabolic disease that occurs with a frequency of 1:200,000 births, presents an unfortunate example in nature of possible neurologic effects that may result from extremely high circulating levels of the BCAAs. MSUD is an enzyme deficiency disease in which BCAA metabolism is severely diminished, because of defects in the enzyme branched-chain α-keto acid dehydrogenase (54). As a consequence, plasma and tissue levels of the BCAAs (particularly LEU) and associated branched-chain keto acids are greatly elevated (55,56). This disease has catastrophic and life-threatening neurologic effects for newborns who survive, mostly attributed to LEU and its keto acid (plasma LEU concentrations can range well above 2000 nmol/mL during metabolic crises; a normal value is about 100 nmol/mL) (55). The underlying biochemical mechanisms that have been proposed to produce the neurotoxicity associated with this disorder include inhibition of creatine kinase, derailment of GLU handling by neurons and glia, including inhibition of synaptic GLU uptake (56–59), and possibly reduced synthesis of the monoamine neurotransmitters, secondary to reductions in the brain concentrations of their precursor amino acids (55). Many of these effects may result from alterations in the competitive uptake of the LNAA into brain and neurons, produced by the high circulating BCAA levels. Dietary treatments that lower circulating BCAA concentrations by restricting BCAA intake have been successful in controlling the symptoms of the disease (56). However, the onset of negative symptoms in MSUD patients does not correlate well with the absolute plasma LEU (or BCAA) concentrations achieved after BCAA loading such patients (56), suggesting that MSUD may not serve as a useful model for identifying threshold plasma BCAA concentrations at which adverse CNS effects might be expected in normal humans.

Relevance to upper limits of BCAA ingestion

Together, these studies generally indicate that the BCAAs can be consumed in considerable amounts by humans without adverse effect and, in some cases, with significant benefit to the study populations. To gain an impression of the size of the dose used in human studies, it is useful to reflect on the normal daily intake of BCAAs from the diet. The typical BCAA content of dietary proteins is 15–20 g $\cdot$ 100 g^{-1} protein (4). The current recommended dietary allowance (RDA) for protein is 0.8 g $\cdot$ kg^{-1} $\cdot$ d^{-1} for adults (31), or about 56 g protein $\cdot$ d^{-1} for a 70 kg person. The daily intake of the BCAAs in a 70-kg person consuming the RDA for protein would thus be 8.4–11.2 g. In athletes, for whom a common recommendation is 1.2 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} or more (60), the daily BCAA intake of a 70-kg individual would be 12.6–16.8 g. From this perspective, the highest doses of BCAAs that have been administered chronically to humans have been to PKU [up to 35 g/d (33,34)] and mania [up to 60 g/d (41,43)] patients. These doses thus represent 2- to 4-fold multiples of the daily BCAA intake for athletes, and 3- to 7-fold multiples for nonathletes (with reference to 70 kg body weight). In these studies, the authors reported minimal or no adverse effect or side effects (see Table 1). It would therefore appear that daily doses of up to 60 g of the BCAAs, in addition to the amounts consumed as a component of dietary protein, are safely consumed as by humans. Moreover, because no systematic evaluation of the safety of oral BCAA has been conducted in humans, the no observable adverse effect level (NOAEL) of BCAA intake may be considerably higher.

The possibility that humans can safely ingest supplemental amounts of the BCAA well in excess of 60 g/d (850 mg $\cdot$

kg$^{-1} \cdot$ d^{-1} for a 70-kg individual) provides an interesting contrast to a NOAEL dose that can be derived from recent subchronic studies in rats. Tsubuku et al. (61) recently conducted a 13-wk study, in which rats consumed individual BCAAs added to a standard diet at 1.25, 2.5, or 5 g $\cdot$ 100 g^{-1} diet. The outcome measures did not include brain effects but did include standard measures of potential toxicity. They concluded from their data that ingestion of the 2.5 g $\cdot$ 100 g^{-1} diet for each BCAA added to the diet in addition to the BCAAs already in the diet was associated with no observable adverse effects (combinations of the BCAAs were not evaluated). From this outcome, they calculated that the daily dose of each BCAA at the 2.5 g $\cdot$ 100 g^{-1} diet supplemental intake level was above 1500 mg $\cdot$ kg$^{-1} \cdot$ d^{-1} and, hence, was suggested as a NOAEL value. If the typical 100-fold safety factor is applied to this dose, to estimate a safe upper limit of intake in humans, the human NOAEL dose would be 15 mg $\cdot$ kg$^{-1} \cdot$ d^{-1}, or about 1 g/d for a 70-kg human. By this measure, even if one could assume that an acceptable daily dose for all 3 BCAAs might be 45 mg $\cdot$ kg$^{-1} \cdot$ d^{-1}, the total allowable dose would still be only about 3 g/d. This analysis thus vastly overestimates potential BCAA toxicity in the human population, because humans (healthy and diseased) have consumed up to 20-fold higher daily doses in addition to their daily protein loads (see above) without ill effect. Given that the bulk of the literature on the BCAAs already suggests that daily intakes of the BCAAs substantially in excess of those contained in dietary protein appear to be safe in humans, it might now be useful to conduct studies in normal humans to assess if accepted toxicologic indicators are normal in this dose range. This may ultimately prove to be the most effective strategy for establishing a safe range of intake in humans.

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