ORIGINAL ARTICLE

Branched-Chain Amino Acids Enhance the Cognitive Recovery of Patients With Severe Traumatic Brain Injury

Roberto Aquilani, MD, Paolo Iadarola, PhD, Antonella Contardi, MD, Mirella Boselli, MD, Manuela Verri, PhD, Ornella Pastoris, PhD, Federica Boschi, PhD, Patrizia Arcidiaco, PhD, Simona Viglio, PhD

ABSTRACT. Aquilani R, Iadarola P, Contardi A, Boselli M, Verri M, Pastoris O, Boschi F, Arcidiaco P, Viglio S. Branched-chain amino acids enhance the cognitive recovery of patients with severe traumatic brain injury. Arch Phys Med Rehabil 2005;86:1729-35.

Objective: To investigate whether supplementation with branched-chain amino acids (BCAAs) in patients with severe traumatic brain injury (TBI) improves recovery of cognition and influences plasma concentrations of tyrosine and tryptophan, which are precursors of, respectively, catecholamine and serotonin neurotransmitters in the brain.

Design: Forty patients with TBI were randomly assigned to 15 days of intravenous BCAA supplementation (19.6g/d) (n=20) or an isonitrogenous placebo (n=20).

Setting: Tertiary care rehabilitation setting in Italy.

Participants: Forty men (mean age, 32±15y) with TBI and 20 healthy subjects (controls) matched for age, sex, and sedentary lifestyle.

Intervention: Supplementation with BCAAs.

Main Outcome Measures: Disability Rating Scale (DRS) and plasma concentrations of BCAAs, tyrosine, and tryptophan.

Results: Fifteen days after admission to the rehabilitation department, the DRS score had improved significantly in both the placebo group ($P<.05$ vs baseline) and in the BCAA-supplemented group ($P<.01$ vs baseline). The difference between the 2 groups was significant ($P<.004$). Plasma tyrosine concentration improved in the group given BCAA supplementation, and tryptophan concentration increased in patients receiving placebo.

Conclusions: Supplemental BCAAs enhance the retrieval of DRS without causing negative effects on tyrosine and tryptophan concentration.

Key Words: Amino acids, branched-chain; Brain injuries; Rehabilitation.

From the Servizio di Fisiopatologia Metabolico-Nutrizionale e Nutrizione Clinica (Aquilani), Divisione I di Neuroriabilitazione (Contardi), and Divisione II di Neuroriabilitazione (Boselli), Istituto Scientifico di Montescano, IRCCS Fondazione S. Maugeri, Pavia; Dipartimento di Biochimica A. Castellani, Università di Pavia, Pavia (Iadarola, Viglio); Dipartimento di Scienze Fisiologiche e Farmacologiche, Sezione di Farmacologia, Università di Pavia, Pavia (Verri, Pastoris, Boschi); and Centro Grandi Strumenti, Università di Pavia, Pavia (Arcidiaco), Italy.

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Reprint requests to Simona Viglio, PhD, Dipartimento di Biochimica A. Castellani, Università di Pavia, Via Taramelli 3/B, I-27100 Pavia, Italy, e-mail: *simona.viglio@unipv.it.*

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BRANCHED-CHAIN AMINO ACIDS (BCAAs) (leucine, valine, isoleucine) are essential amino acids for humans, so they must be assimilated from the diet. BCAAs account for approximately 35% of the essential amino acids and 14% of the total amount of amino acids in skeletal muscle.[1] After a meal, BCAAs constitute at least 50% of the amino acid uptake by skeletal muscle.[2]

It is well documented that BCAAs may favorably influence protein metabolism by inhibiting muscle protein breakdown[3,4] and promoting muscle[4,5] and hepatic[6,7] protein synthesis. It has been reported that supplying BCAAs to injured and septic animals[8] and to stressed patients[9-12] has beneficial effects. Parenterally administered BCAAs are used clinically in nutritional support for postoperative,[13] traumatized,[14] and septic patients,[15] and the oral use of BCAAs suppresses whole-body proteolysis in tissues other than skeletal muscle in healthy men.[16] Beside these strictly nutritional aspects of BCAAs, numerous studies suggest that these amino acids may also have a notable effect on cognitive functions. In healthy, exercising people, BCCA supplementation improved cognitive performance.[17] Confirming this, subjects given BCAAs during prolonged exercise perceived lower exertional and mental fatigue than they did when given a placebo, as measured on 2 different Borg scales.[18] Moreover, BCCA supplementation improved physical performance during exercise in the heat, a situation in which the central component of fatigue is assumed to be increased.[19] In clinical settings, orally administered or parenterally infused BCAAs improved mental status, flapping, orientation, speech, and writing in patients with cirrhosis and chronic hepatic encephalopathy[20-23] and psychomotor functions (line tracing, tapping, steadiness, auditory reaction time), attention (digit table), and practical intelligence (digit symbol, number connection test) in patients with latent portosystemic encephalopathy,[24] although another study[25] failed to document these positive effects in patients with cirrhosis. Patients with Alzheimer's dementia had a significantly lower ratio of cerebrospinal fluid to plasma levels of valine (and other amino acids tested) than did control subjects, and significant correlations were found between memory and cognitive functions and cerebrospinal fluid–valine concentration.[26]

Further powerful evidence for the role of BCAAs in influencing cognition can be derived from animal and human studies on phenylketonuria (PKU). Learning deficits found in adult rats exposed prenatally to a PKU-inducing diet were significantly reduced by providing the animals with a supplement of valine, isoleucine, and leucine.[27] In another study,[28] neuropsychologic functioning in a group of patients with PKU improved significantly after 3 to 6 weeks of oral BCAA supplementation: abstract reasoning and tactile motor problem solving improved more than pure motor performance. Moreover, it was documented that the time adolescents and young adults with PKU took to complete a test requiring substantial attention with mental processing was shorter during leucine, valine, and isoleucine supplementation than during the periods when these

subjects received control mixtures.[29] Finally, a more recent investigation[30] found that when healthy volunteers drank BCAAs, their spatial recognition memory was impaired in a dose-dependent manner; this effect was consistent with lowered dopamine function, which could potentially be useful for treating disorders characterized by overactivity of dopamine pathways, such as maniac disorders and schizophrenia. Based on this previous research, we hypothesized that rehabilitation patients with severe traumatic brain injury (TBI), lacking plasma BCAAs,[31,32] might benefit from BCAA supplementation in their recovery of cognitive processes. We therefore undertook our investigation to determine whether supplementation of BCAAs improves cognitive recovery in rehabilitation patients with TBI (first priority), reverses plasma BCAA abnormalities (second priority), and affects concentrations of tyrosine (a brain catecholamine precursor) and tryptophan (a brain serotonin precursor).[31,32]

METHODS

Forty male patients with severe TBI, consecutively admitted to our rehabilitation department 64±32 days (range, 23–140d) after injury, were investigated in a random, double-blind, placebo-controlled trial.

Patients were admitted to our department from intensive care units. Their average age was 32±15 years (range, 14–64y). All had diffuse brain damage caused by road traffic collisions (n=39) or a gunshot wound (n=1). Their neurologic scores within the first 24 hours averaged 5.9±1.8 on the Glasgow Coma Scale (from referral information). On admission to our department, all patients were on antibiotic therapy for urinary and/or respiratory infections. Twenty patients were being enterally fed with polymeric formulas by nasogastric tube (12/20) or percutaneous endoscopic gastrostomy (8/20). The other 20 patients were on spontaneous alimentation (oral diet). Table 1 summarizes patients' clinical characteristics. After completion of routine laboratory and biohumoral investigations, all patients were weighted[31] and were then randomly assigned to the treatment group (n=20) or placebo group (n=20). The randomization process was performed using computer generation with Statistical Analysis System procedure plan for a completely randomized design for 2 treatments (by specifying 1 random factor of 40 levels). Both patients and physicians were blinded to treatment group. Patients were supplemented blindly with BCAA solution or fruit juice. The physician who evaluated the Disability Rating Scale (DRS) was blinded to the above-mentioned supplementation and was different from the physician who prescribed the supplementation itself.

The treatment group was supplied with BCAAs (4% Isoram[a]) given intravenously (V-BCAA group) by infusing 500mL of BCAA solution once a day over a 5-hour period through the antecubital vein, providing 19.6g of BCAAs (nitrogen, 3.13g; leucine, 7.50g; isoleucine, 3.01g; valine, 9.1g) and 1.6g of arginine (nitrogen, .26g). The amount of extra calories provided by this formula was 85kcal.

The placebo group was given 90kcal of energy in the form of fruit juice. Moreover, both groups of patients were adjusted in their protein calorie supplies to receive at least 0.9g/kg of protein per body weight and 28kcal/kg of body weight.

Before and after 15 days of BCAA supplementation or placebo, patients were submitted to the investigations reported below.

Determination of Plasma Amino Acid Concentrations

Within the first week of patients' admission to the rehabilitation center, venous blood samples were taken from the antecubital vein between 8:00 and 10:00 AM to determine plasma levels of total amino acids, although for the purposes of this study we focused on BCAAs and tyrosine (Tyr) and tryptophan (Trp), 2 precursors of brain neurotransmitters. We then calculated the tryptophan ratio:

$$\text{tryptophan ratio} = \text{Trp}/(\text{Tyr} + \text{Phe} + \text{BCAAs})$$

where Phe is phenylalanine. The tryptophan ratio reflects the brain tryptophan concentration, which is related to brain serotonin synthesis and concentration.[33] The concentration of free amino acids in plasma was measured by an AminoQuant II amino acid analyzer,[b] based on the HP 1090 HPLC system, with fully automated precolumn derivatization, by using both orthophthalaldehyde and 9-fluorenyl-methyl-chloroformate reaction chemistries according to the manufacturer's protocol. Determinations were performed essentially as reported earlier,[31] injecting 1μL of the derivatized mixture and measuring absorbance simultaneously at 338 and 262nm.

Table 1: Clinical Characteristics of the Study Patients

Parameters	Patients (n)
Neurologic injuries	
Brain injury	40
Hemorrhage on CT scan	
Left frontotemporoparietal	6
Bilateral frontoparietal + diffuse hemorrhagic lesions	9
Left frontoparietal	5
Left frontotemporoparietal + corpus callosum + mesencephalus	4
Left temporal + diffuse hemorrhagic lesions	5
Bilateral frontal	1
Associated injuries	
Multiple fractures	12
Hemothorax	5
Long bone fracture	9

Abbreviation: CT, computed tomography.

Calculation of Nitrogen Balance

A 24-hour urine sample was collected by catheter to determine daily urinary nitrogen excretion, expressed in grams (micro Kjeldahl technique). To estimate total nitrogen loss, 2g were added to the 24-hour urinary nitrogen excretion to compensate for fecal and transcutaneous nitrogen loss.[34] Nitrogen balance was defined as dietary nitrogen intake minus total nitrogen loss.

Evaluation of Patients' Disabilities

Patients' disabilities were evaluated using the DRS,[35] which is a sensitive, reliable, and valid measure of a patient's disability after a head injury.[36]

Control Subjects

To form a control group, we chose 20 healthy men in a similar age range to that of the patients. During the 3 months before their recruitment, these subjects had a stable weight (±1kg) and a sedentary lifestyle—that is, they did not engage in aerobic activity during the week.[37] Control subjects underwent all the procedures applied to patients with TBI. (The 24-h urine samples were collected from the healthy volunteer subjects without catheterization.)

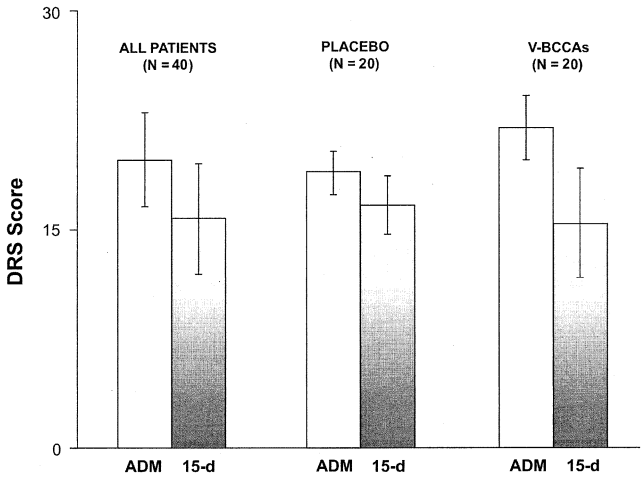

Fig 1. Patients' DRS scores at admission (ADM) to rehabilitation and 15 days after admission.

The study was approved by the Ethical, Technical, and Scientific Committee of our institute and written informed consent was obtained from patients or their caregivers.

Statistical Analysis

Repeated-measures analysis of variance (ANOVA) were applied to test differences over time in DRS score, plasma amino acid concentrations, tryptophan ratio, and nutritional parameters between the treatment group, placebo group, and healthy controls. Data are given as mean ± standard deviation (SD) or standard error (SE) for plasma amino acid levels only. Statistical significance was set at P less than .05.

RESULTS

Levels of Disability

Figure 1 shows the cognitive function as expressed by patients' DRS scores at their admission to rehabilitation and 15 days after admission. At admission, the patients as an entire group (n=40) had a DRS score of 20.1±2.0 (placebo group, 19.2±1.6; V-BCAA group, 21±2.1; not significant [NS]). Fifteen days after admission, DRS scores significantly improved in the patients as an entire group, averaging 15.5±3.3 ($P<.02$ vs baseline). However, improvement in the DRS scores was not as great in the placebo group (from baseline 19.2±1.6 to 16.4±3.3; $P<.05$) as in the V-BCAA group (from 21±2.1 to 14.7±3.1; $P<.01$), and the differences between the 2 groups reached statistical significance ($P<.004$).

Plasma Amino Acid Concentration

Tables 2 and 3 report plasma amino acid concentrations, tryptophan ratio, and statistical analysis, both in healthy controls and in patients with TBI—the latter at admission to rehabilitation and after 15 days. The results show that at admission, circulating total BCAAs, and within these leucine, isoleucine, valine, were lower in the patients than in controls ($P<.002$). Tyrosine concentration also was reduced when compared with the control value ($P<.001$). However, all these amino acids results were similar in the treatment and placebo groups. The plasma tryptophan concentration, precursor of brain serotonin, did not differ between patients and controls or between the 2 patient groups. Fifteen days after admission to rehabilitation, only patients given BCAA supplementation significantly improved their baseline total BCAAs ($P<.01$) and, within these, leucine ($P<.01$), isoleucine ($P<.02$), and valine ($P<.001$) (see tables 2, 3). After 15 days from admission to rehabilitation, the level of plasma tyrosine, the precursor of brain catecholamines, significantly improved in the V-BCAA group ($P<.01$) but remained lower than in healthy controls. After 15 days from admission, plasma tryptophan concentration was higher in patients on placebo than in the treatment group ($P<.01$).

Baseline tryptophan ratio significantly decreased after 15 days of intravenous BCAAs ($P<.01$). Patients on placebo had higher tryptophan ratios than the treatment patients at 15 days from admission ($P<.03$).

Body Weight, Nutritional Intake, and Nitrogen Balance

Table 4 reports patients' body weight, nutritional intake, and nitrogen balance at baseline and after 15 days.

Patients had an average body weight decrease of 15% after the acute event, their actual body weight being 85%±12% of their usual weight.

Nutritional intake and nitrogen balance tended to be higher in patients on placebo than in the treatment group, but the difference was not important. After nutritional correction, when necessary (see Methods), the energy and macronutrient intakes and nitrogen balance at day 15 were very similar in placebo and treatment groups of TBI patients. Body weight had not changed by day 15.

Table 2: Plasma Amino Acid Concentrations and Their Ratios in Healthy Subjects (Controls) and in Patients With TBI at Admission to Rehabilitation and 15 Days After Admission

		Patients (n=40)			
		Placebo (n=20)		V-BCAA (n=20)	
Amino Acids	Controls (n=20)	Admission	15 Days	Admission	15 Days
Total BCAAs	224±17	144±13	163±19	117±21	357±24
Single BCAAs					
Leucine	46.2±3.2	27.9±2.7	30.6±3.9	21.9±1.5	53.8±7.1
Valine	87±7.4	59±4.7	63±7	40.8±0.2	242±5.5
Isoleucine	21±1.7	17±1.7	20.7±2.7	13.5±1.3	25.4±6.5
Tyrosine	31±4.7	14.4±2.2	16.1±3.0	10.4±0.31	14±1.5
Tryptophan	10.6±0.5	11.4±2.3	16.5±2.0	7.0±3.8	8.0±3.3
Trp/LNAA	0.05±0.00	0.07±0.01	0.10±0.00	0.06±0.02	0.02±0.01

NOTE. Values are expressed as mean ± SE.
Abbreviation: Trp/LNAA, tryptophan/large neutral amino acid.

Table 3: Statistical Analysis Applied to the Groups of Patients and to Controls

	V-BCAA		Tyrosine		Tryptophan		Tryptophan Ratio	
Groups	Admission	15 Days	Admission	15 Days	Admission	15 Days	Admission	15 Days
Placebo vs controls	<.003	<.002	<.002	NS	NS	NS	NS	<.010
V-BCAA vs controls	<.002	<.010	<.001	<.001	NS	NS	NS	NS
Placebo vs V-BCAA	NS	<.002	NS	NS	NS	<.001	NS	<.010

DISCUSSION

This study shows that short-term intravenous supplementation of BCAAs in rehabilitation patients with TBI enhances recovery of cognitive function, induces a supraphysiologic plasma content of BCAAs, and increases tyrosine plasma concentration.

BCAA Supplementation and Cognitive Function

At present, we can only speculate about the mechanism underlying the improved cognition associated with BCAAs. However, some plausible mechanisms include a direct action of the BCAAs on brain function by providing substrates and an indirect action by increasing brain insulin availability.

It is reasonable to believe that normalization of plasma concentrations of BCAAs may lead to increased BCAA provision to the brain.[38] These amino acids may be used to produce energy and synthesize proteins in the central nervous system (CNS). Given that they are amino acids, BCAAs can enter the energy-producing oxidative pathway of the Krebs cycle[39,40] so that higher amounts of adenosine 5′-triphosphate (ATP) can be formed. The finding that processed amino acids in the Krebs cycle make a very large contribution to $^{14}CO_2$ production of brain cells supports this BCAA supplementation mechanism of effect.[41,42] An increase in brain ATP availability in TBI may represent an important factor, contrasting the cascade of biochemical alterations caused by the injury.[43] For instance, in severe brain injury, ATP depletion is responsible for alterations in ion pumps, which bring about a failure of cellular sodium, potassium, and calcium homeostasis. The loss of ion homeostasis contributes to the death of neurons in TBI.[44-48] Therefore, BCAA supplementation might protect and restore the function of those neurons that are still viable although metabolically altered.

The BCAAs, particularly leucine,[49-52] play an important role in mediating amino acid–regulated steps of protein synthesis. To get an idea of the importance of active protein synthesis for the brain structures of TBI patients, it is sufficient to mention that de novo protein synthesis is essential for brain tissue repair, sprouting, and circuitry remodeling.[53]

BCAAs may also favor the recovery of cognition indirectly by an insulin-mediated action. This hypothesis is highly plausible, both because BCAAs induce insulin secretion and release and because this hormone crosses the blood-brain barrier, exerting profound effects on the CNS.

It is well documented that BCAAs, particularly leucine, are essential for the regulation of insulin production by pancreatic beta cells.[54] Early studies found that leucine not only stimulates insulin release[55] but also is the sole indispensable amino acid capable of inducing insulin secretion, even in the absence of glucose.[56] In animals, leucine sensors in the portal-hepatic area appear to exert a reflex regulation on pancreatic hormone secretions through hepatic branch vagal afferents.[57,58] In contrast to what was previously believed, circulating insulin can cross the blood-brain barrier,[59] reaching many brain structures, and insulin can be found at high levels in the brain.[60,61] In the CNS, this hormone may modulate cognitive activity.[62] First, insulin can promote glucose utilization in some areas of the brain such as the hippocampus,[63] an important system for many types of learning and memory,[64] and it has been found that the hippocampus contains insulin receptors.[65,66] Second, the firing rates of neurons of the hypothalamus,[67] suprachiasmatic nucleus,[68] and hippocampus[69,70] are sensitive to insulin. Third, many enzymes involved in the energy-producing Krebs cycle,[71] as well as expression of the genes for many enzymes involved in metabolism, appear to be regulated by insulin.[72] Fourth, disruption of insulin activity is involved in the disturbance of cognitive function found in Alzheimer's dementia,[73,74] Huntington's disease,[75,76] and probably also in Parkinson's disease.[77,78] Moreover, patients with non–insulin-dependent diabetes mellitus show impairments in cognitive

Table 4: Body Weight, Energy and Macronutrient Intakes, and Nitrogen Balance of the Patients With TBI, at Admission to Rehabilitation and 15 Days After Admission

	Placebo (n=20)		V-BCAA (n=20)	
Parameters	Admission	15 Days	Admission	15 Days
Actual BW				
Kilograms	54.1±9.0	54.9±9.2	62.3±15.0	62.7±16.0
% habitual BW	85±10	86±9	85±13	87±17
Nutrient intakes				
Energy (kcal/kg BW)	34.2±6.9	34.0±6.9	25.6±7.8	32.3±6.2
CHO (g/kg BW)	4.7±1.4	4.8±1.4	3.3±1.0	3.9±1.0
Proteins (g/kg BW)	1.40±0.26	1.39±0.29	1.04±0.30	1.20±0.3
N_2 (g/kg BW)	0.22±0.00	0.22±0.05	0.16±0.04	0.19±0.04
Lipids (g/kg BW)	1.16±0.30	1.12±0.24	0.91±0.22	1.10±0.20
Nitrogen balance				
g/24h	3.80±3.60	3.50±2.00	1.12±7.10	3.84±1.40

NOTE. Values are mean ± SD. No differences were observed between treatment and placebo groups (repeated-measure ANOVA).
Abbreviations: BW, body weight; CHO, carbohydrates; N_2, as protein: 6.25.

tests.[79,80] Fifth, insulin can influence processes potentially affecting the response of the postsynaptic neurons. Indeed, the hormone modulates membrane potential by effects on sodium/potassium adenosine triphosphatase activity,[81] the potassium channel,[82] and calcium homeostasis.[83]

Therefore, together with a presumed increase in ATP formation induced directly by the BCAAs, insulin activity in the brain may contribute to mitigating the biochemical alterations caused by the injury.[43] Thus, the insulin-mediated mechanism might help explain why patients receiving BCAAs and those on placebo have different cognitive recoveries, despite similar carbohydrate, protein, and lipid intakes and nitrogen balance.

BCAA Supplementation and Plasma Tyrosine and Tryptophan Concentrations

This study shows that intravenous BCAA supplementation increases plasma concentration of tyrosine, precursor of brain catecholamines, but not of serotonin, precursor of brain tryptophan. Thus, BCAA supplementation reduces tryptophan ratio. These findings could not be anticipated because, in the case of reduced availability of one essential amino acid, the influx of other essential amino acids leads to increased protein synthesis, which results in rapid depletion of the limiting essential amino acids in the plasma.[84-86] Likely, the absence of plasma tryptophan depletion after BCAA supplementation is due to a normal tryptophan provision/intake with proteins in patients with normal protein calorie intake. The fact that patients with TBI were in anabolic process (protein synthesis) after 15 days of BCAAs is indicated by the positive nitrogen balance tendentially higher than in placebo group.

Interestingly, plasma tryptophan concentrations increased when patients were on a standard nutritional regimen, that is, without V-BCAA supplementation. Likely, the intake of tryptophan with food without essential amino acid supplementation, in the presence of less pronounced protein synthesis than in the BCAA groups, is sufficient to increase the plasma tryptophan concentration.

For clinical purposes, this study shows that BCAA supplementation does not further impair the plasma levels of precursors of brain neurotransmitters.

Limitations

For technical reasons, we could not determine or monitor the plasma insulin levels, which would have offered us a better understanding of the mechanisms underlying the effects of BCAAs on cognitive recovery. Future studies are needed to investigate this pathophysiologic aspect during BCAA supplementation.

CONCLUSIONS

BCAA supplementation is useful for enhancing the recovery of cognitive functions in patients with TBI who have normal protein and calorie intake. This supplementation restores plasma levels of BCAAs without having a negative effect on the precursors of brain catecholamines and serotonin.

Clinical Implications

Given that they are essential amino acids, supplemental BCAAs should be given routinely to all rehabilitation patients with TBI who have reduced plasma BCAA levels, regardless of any other considerations of their possible effects on the CNS. This is our starting point for the rehabilitation treatment of patients with TBI. Our study does, however, raise some doubts about the best way to supply the BCAAs. In fact, the intravenous route induces an excess of circulating BCAAs, and we presently do not know what, if any, effect this may have on body tissues and organs. In addition, intravenous BCAAs are costly. A future study is planned to determine whether oral administration of BCAAs or doses of intravenous BCAAs lower than that used in this investigation may conjugate a normalization of plasma BCAAs with a significant improvement of cognitive function in patients with TBI. Whatever the route by which the BCAAs are supplied, it should not be forgotten in clinical practice that BCAAs exert their global positive effects in the presence of an adequate protein and calorie intake. Our study indicates that the provision of 32.3kcal of energy/kg of actual body weight and 1.2g of protein/kg of body weight is adequate to meet the nutritional needs of rehabilitation patients with TBI.

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Suppliers

a. Bieffe Medital SpA, via Lorenzini 4, 20139, Milano, Italy.
b. Hewlett Packard, 3000 Hanover St, Palo Alto, CA 94304.