 Symposium on Branched-Chain Amino Acids: Conference Summary

Luc Cynober* and Robert A. Harris

*Biochemistry Laboratory, Hôtel-Dieu Hospital, AP-HP and Biological Nutrition Laboratory, Paris, France and
†Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN

Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application

BCAAs, especially leucine, were shown in the mid-1970s to be potent regulators of protein turnover (1–3). In the mid-1980s BCAAs were also shown to compete with other large neutral amino acids (LNAA) (4), suggesting that raising the blood concentration of BCAA would limit the formation of false neurotransmitters within the brain. Although these findings would seem to have provided an attractive basis for BCAA supplementation in various physiological or pathological situations, they were not sufficient at that time because our understanding of in vivo metabolism and cell signaling were limited and no clear biomarkers of BCAA status were available (5). As a result, the idea that BCAA supplementation might have merit received limited attention but is now experiencing a major revival thanks to recent progress, as discussed by an enthusiastic group of participants at a conference held last May in Versailles, France.

The conference was opened with the interesting question: Why is it that nature chose to have three BCAAs? Why not one, two, or some other number of BCAAs? Is something special obtained from the metabolism of each of the BCAAs? A strong case was presented for the importance of BCAAs in determining protein structure (6). Indeed, there is growing evidence that BCAAs play significantly different structural roles in proteins, even though only slight differences exist in their side chains. The fact that the catabolism of all three BCAAs is controlled at the common step catalyzed by the branched-chain ketoacid dehydrogenase complex (BCKDC) implies that the individual end products of BCAA catabolism do not have important functions.

Understanding the mechanism of how BCAAs regulate protein synthesis and degradation requires an in-depth appreciation for how other factors affect these processes, particularly insulin, insulin-like growth factor-I (IGF-I), and growth hormone (GH) (7). Although often overlooked, vascular actions of these hormones impact the delivery of amino acids to skeletal muscle cells. Resting blood flow to skeletal muscle is slow but can increase greatly in response to exercise, insulin, IGF-I, and GH. These effects are due to vasodilation induced by nitric oxide stimulation of guanylyl cyclase in endothelial cells of the capillaries of the vasculature, an important component of the mechanisms by which GH, IGF-I, insulin, and amino acids promote muscle protein accretion.

The brain content of BCAA is regulated by the transport properties of the capillary endothelial cells that form the blood–brain barrier (8). The polar distribution of transport proteins in the luminal (blood-facing) and abluminal (brain-facing) plasma membranes of the endothelium mediates amino acid homeostasis in the brain. The blood–brain barrier restricts the entry of glutamine and glutamate into the brain and actively exports these amino acids to the circulation to protect against glutamate and ammonia toxicity.

The mechanism by which leucine regulates muscle-protein synthesis was a major theme of the conference. Upregulation of the initiation of mRNA translation via activation of the mammalian target of rapamycin (mTOR) signaling pathway is clearly involved (9). Several new signaling molecules have been identified recently that may be involved in mediating the effect of leucine on mTOR. Insulin-dependent and independent mechanisms are clearly involved in the mechanism of leucine action. Whether a receptor for leucine exists and whether a leucine metabolite is involved in the signaling mechanism are two of the many questions that remain to be answered.

Major advances have been made recently in understanding the control of skeletal muscle protein degradation by the ubiquitin-proteosome pathway, the most important degradation system for proteins in mammalian cells (10). Most proteins in mammalian cells are degraded by this system. Genes that are expressed at a higher level in atrophying muscle are called atrogens. These include genes encoding enzymes that tag protein substrates with ubiquitin for degradation by the proteosome. Glucocorticoids or lack of amino acids induce atrogens and stimulate protein degradation. IGF-I and insulin suppress the expression of atrogens and inhibit muscle proteolysis. The
signalizing mechanism responsible for the inhibitory effects of leucine on protein degradation is not understood.

Kobayashi et al. (11) measured the rates of protein synthesis and breakdown simultaneously during BCAA infusion in rat models of muscle atrophy. BCAAs stimulate muscle protein synthesis in normal rats independently of insulin. The effectiveness of BCAAs is transient, however, unless the other essential amino acids are provided to sustain protein synthesis. Different types of muscle injury induce different effects upon protein synthesis and protein degradation. The effectiveness of administering BCAA supplements is likely to depend upon the effect that a particular stress situation has upon protein synthesis and protein degradation.

Research into the metabolism and physiological roles of BCAAs in cancer was presented by Baracos (12). BCAA deprivation might limit tumor growth, but BCAA supplementation might prevent loss of host lean tissue mass. Which would be better, if either, is unknown. Better animal models than currently available are necessary to determine whether BCAA deprivation or supplementation should have any place in cancer therapy. Nevertheless, there is evidence to suggest that tumors may be more sensitive to amino acid deprivation than host tissues, suggesting that the manipulation of BCAA levels should be further explored in animal models of cancer.

Knowledge gained by studying the biochemical basis for the thiamin-responsive maple syrup urine disease (MSUD) phenotype was reviewed by Chaung et al. (13). Although thiamin diphosphate is the prosthetic group for the E1 component of the BCKDc, the E2 protein rather than the E1 protein is abnormal in patients with thiamin-responsive MSUD. Chaung’s work indicates that an increase in thiamin diphosphate caused by dietary thiamin consumption leads to inhibition of the kinase that phosphorylates and inactivates the E1 component of the complex. This promotes interaction of the E1 component with the E2 component to give greater decarboxylase activity and lower BCAAs in MSUD patients.

The central role of the BCKDC in regulation of BCAA catabolism was discussed by Shimomura (14). Recent findings suggest that the kinase for BCKDC exists in a free form and a complex bound form. Only the bound form of the kinase is catalytically active and therefore capable of inactivation of BCKDC by phosphorylation. α-Ketoisocaproate (KIC) releases the kinase from BCKDC and promotes activation of the complex. Exercise and chronic liver disease likewise activate BCKDC by promoting release of the kinase from the complex. The findings provide a rationale for the use of BCAA supplements for patients with liver cirrhosis.

Since BCAAs share common cell membrane transport systems and the two first metabolic enzymes, it is likely that these amino acids can influence each others kinetics and requirements (as discussed below). Hence, enriching diets with varying amounts of each of the BCAAs does not necessarily produce the same results. Also, it is well known that leucine, but not valine or isoleucine, has a unique ability to modulate protein synthesis.

The definition of BCAA requirements in healthy subjects remains a matter of debate, despite the use of accurate modern methods (i.e., 24-h direct amino acid oxidation and balance technique, providing a total of 84 mg kg−1 d, which is lower than the 110 mg kg−1 d obtained using the short-term indicator amino acid oxidation method [15]). Another important question is how the requirements of a given BCAA influences the requirements of the other two. Karpad et al. (15) provided convincing data that varying the intake of one BCAA in the range supplied by normal diets has no effect on the oxidation of the two others. However, these authors also show that this is not the case when a large nonphysiological BCAA intake is given. This is a key factor in the effects (including side effects, as discussed in a recent meeting held in Kobe (16)) of pharmacological administration of a given BCAA or a mixture of BCAAs in physiological (e.g., exercise) or pathological (e.g., injury) situations (see below).

The rationale behind consuming a BCAA in addition to diet before, during, and/or after exercise is that exercise leads to a considerable increase in BCAA oxidation, especially during endurance exercise, and most of the oxidized BCAA appears to be released into the system by depression of protein synthesis. Thus, the theoretical utility of providing a BCAA supplement is 1) to replace oxidized molecules and 2) to upregulate protein synthesis (see various contributions in this issue of the journal and Kimball and Jefferson (17) for a recent review). However, this nice story is hampered by a series of facts (18). First, recent evidence shows that endurance training does not really increase leucine oxidation and that protein metabolism becomes more efficient (i.e., increased nitrogen balance achieved at a lower rate of oxidation). Second, meeting energy requirements by eating a normal diet limits BCAA oxidation, making BCAA supplementation unwarranted. Nevertheless, a series of elegant studies suggest BCAA supplementation may increase protein synthesis during or after exercise (19). The molecular targets have been identified: mTOR, p70 S6 kinase, and S6 phosphorylation (19). Whether the mechanisms underlying the action of BCAAs in resistance and endurance exercise are similar or different remains unclear.

Another action of BCAAs in an exercise context is their action on central fatigue syndrome (20). Central fatigue is defined as an inability to maintain power output due to events within the central nervous system. One of the various mechanisms that have been proposed to explain central fatigue is an increase in the amount of tryptophan (Trp) taken up by the brain leading to excessive amounts of the neurotransmitter S-hydroxytryptamine in some neurons. The transport of Trp into the brain is regulated by blood concentrations of other LNAAs, in particular the BCAAs that compete with Trp for this transport. Hence, it makes sense to provide extra BCAAs to limit brain uptake of Trp and consequently central fatigue. The concept has been supported by data obtained during standardized cycle ergometer exercise and in a competitive 30-km cross-country race.

Another situation where protein synthesis is depressed is with aging, which leads to sarcopenia, the progressive loss of muscle mass. Given demographic evolution in western countries, this is certainly a major public health concern. The decrease in protein synthesis in the elderly is multifactorial, but amino acids, and especially leucine, could play a major role in this process: first, splanchnic sequestration of leucine following a meal is almost doubled in the elderly compared with young adults; and second, the ability of leucine to stimulate muscle protein synthesis becomes blunted with aging. In other words, more leucine is needed to induce protein synthesis in the elderly to the same extent as in young adults. Resistance to the anabolic action of insulin, energy-dependent–related abnormalities (as gauged by the low stimulation of protein synthesis in the presence of carbohydrates in the meal), blunted leucine transduction signal (at S6K1), and low-grade chronic inflammation could be involved (21). However, to date, and to the best of our knowledge, there is no available clinical intervention study on the effects of BCAA supplementation in preventing or treating sarcopenia. This research is urgently warranted.

Of current interest is whether protein-rich diets are effective for the treatment of obesity, type 2 diabetes, and the metabolic syndrome. There is evidence in the literature that protein-rich diets may help preserve lean-body tissue, promote loss of body...
fat, and enhance glycemic control during dieting. The hypothesis was advanced by Layman (22) that the effectiveness of such diets may be mediated in part by the stimulatory effect of leucine on protein synthesis during restricted energy intake. Leucine also stimulates glucose use by skeletal muscle by serving as a source of amino groups for alanine synthesis in the glucose-alanine cycle.

Since most chronic or acute malnutrition states are associated with impaired immunological status, it seems logical to search for an association between immunological status and BCAA supply, but as pointed out by Calder (23), there is a lack of studies in this direction. What has been proven by in vitro and experimental studies is that BCAAs are absolutely essential in lymphocyte responsiveness, and that the essentiality of BCAAs for immune cells is related to protein synthesis rather than their role as a cellular fuel, even if immune cells express BCKDC (23). Several animal studies have shown that insufficient availability of BCAA impairs some aspects of immune function, but conversely, there are few studies showing that BCAA supplementation upregulates immune function. At the end of the day, the available human studies are inconclusive (23). The impact of BCAAs on immunological functions certainly deserves further studies.

In newborns, the question of BCAA metabolism and action is of particular importance because a high-protein synthesis rate is essential to support growth. In healthy-term newborns and growing infants, leucine transamination is negatively related to urea synthesis, suggesting a redirection of amino nitrogen toward protein accretion (24). Hence, the effects of BCAA supplementation in this population warrants study.

The decrease in BCAA levels in liver diseases is certainly multifactorial, but the underlying mechanisms remain poorly understood (see Blonde-Cynober, Aussel, and Cynober (25) for a review on the subject). The rationale for BCAA supplementation in this population warrants study.

In this context, chronic renal failure displays several common characteristics with liver failure: a decrease in BCAA pools (especially valine), protein wasting, and altered brain function (27). Specific to renal failure is metabolic acidosis, which is a major factor behind alterations in amino acid homeostasis. From the standpoint of a nutritional therapy, branched-chain keto acids (BCKA) are the target of research because of their ability to trap amino groups and regenerate BCAA. In addition, BCKAs have been suggested to reduce muscle protein degradation (27). In dialysis patients, oral administering of BCAA supplements induces an improvement in appetite and nutritional status. BCAA and BCKA supplementation is certainly of interest in patients receiving a very low protein diet, but their ability to retard the progression of renal failure remains to be demonstrated.

Two lectures (28,29) discussed BCAA intake against a background of injury (postoperative, cancer, trauma, burn, and sepsis). In these cases, the rationale for providing extra BCAAs is to improve protein synthesis and/or decrease protein catabolism. Of note, BCAA content in muscle increases following injury; why this is accompanied by net protein catabolism remains unknown.

Indeed, a number of studies were unable to identify any advantage of providing BCAA-enriched diets in these situations. This may be due to small sample sizes, heterogeneous patients with varying degrees of metabolic stress, poor study designs, including excessive BCAA intake (up to 100% of nitrogen intake), and/or inadequate nonprotein calories, inappropriate endpoints, unbalanced enrichment of valine, and isoleucine vs. leucine. Hence, BCAA-enriched nutrition in injured patients should still be considered, but further studies are required to achieve a firm recommendation for this therapeutic strategy.

In conclusion, in terms of supplementation, very few amino acids deserve as much research as BCAA. However, despite hundreds of papers dealing with BCAA supplementation in various physiological or pathological situations, there is still no consensus on the use of BCAA-enriched diets in most of these situations. Nevertheless, recent large trials with adequate methodology, together with the relatively recent confirmation that leucine specifically modulates protein synthesis, open the door to the promising use of BCAA-enriched diets, provided that we are able to define clear rationales and timely administration at the optimal dosage.

LITERATURE CITED


