Overview of the Molecular and Biochemical Basis of Branched-Chain Amino Acid Catabolism

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ABSTRACT The branched-chain amino acids (BCAAs) are required for protein synthesis and neurotransmitter synthesis. The branched-chain α-ketoacid dehydrogenase complex (BCKDC) is the most important regulatory enzyme in the catabolic pathways of the BCAAs. Activity of the complex is controlled by covalent modification with phosphorylation of its branched-chain α-ketoacid dehydrogenase subunits by a specific kinase [branched-chain kinase (BDK)] causing inactivation and dephosphorylation by a specific phosphatase [branched-chain phosphatase (BDP)] causing activation. Tight control of BCKDC activity is important for conserving as well as disposing of BCAAs. Phosphorylation of the complex occurs when there is a need to conserve BCAAs for protein synthesis; dephosphorylation occurs when BCAAs are present in excess. The relative activities of BDK and BDP set the activity state of BCKDC. BDK activity is regulated by α-ketoisocaprate inhibition and altered level of expression. Less is known about BDP but a novel mitochondrial phosphatase was identified recently that may contribute to the regulation of BCKDC. Reduced capacity to oxidize BCAAs, as in maple syrup urine disease, results in excess BCAAs in the blood and profound neurological dysfunction and brain damage. In contrast, loss of control of BCAA oxidation results in growth impairment and epileptic-like seizures. These findings emphasize the importance of control of BCAA catabolism for normal neurological function. It is proposed that the safe upper limit of dietary BCAA intake could be established with a BCAA tolerance test and clamp protocol. J. Nutr. 135: 1527S–1530S, 2005.

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This paper attempts to relate the regulation of branched-chain amino acid (BCAA) catabolism to the focus of this workshop, the question of the safe upper limit of BCAA intake. BCAAs cannot be synthesized de novo and must therefore be obtained from the diet for protein synthesis. Leucine is of special interest because it promotes protein synthesis (1), inhibits protein degradation (2), and stimulates insulin release (3). As a result of these actions of leucine, BCAAs have therapeutic potential, because they spare lean body mass during weight loss (4), promote wound healing (5), and decrease muscle wasting with aging (6). These positive effects of BCAAs are self-limiting, however, because BCAAs promote their own disposal by oxidative degradation. This is necessary because excess BCAAs are toxic. Furthermore, the high capacity of the body for oxidative degradation of BCAAs should mean that the safe upper limit for BCAA intake will be quite high.

The clinical experience with gene defects in the catabolic pathways for BCAAs fully documents the toxicity of BCAAs. Maple syrup urine disease (MSUD), caused by defects in the genes encoding the subunits of branched-chain α-ketoacid dehydrogenase complex (BCKDC) (7), is best known among these inherited disorders. The neurological damage that occurs in MSUD establishes the risk associated with elevated concentrations of BCAAs or their keto acids. The branched-chain organic acidurias likewise establish the importance of being able to completely catabolize the BCAAs.

Cells of the body do not make a storage molecule to control the concentrations of the BCAAs. The toxicity of glucose and fatty acids, which is evidenced by the long-term consequences of diabetes mellitus, is largely prevented by rapid synthesis of glycogen and triacylglycerol. Amino acids are not known to be converted to protein for later use. Catabolic pathways therefore provide the only way to deal with excess BCAAs. And this has to occur regardless of whether the ATP generated by the catabolism of BCAAs is needed by the organism. The
catabolic pathways for BCAAs, their overall enzymatic capacity, and the factors that regulate flux through such pathways are therefore of central importance to the question of the upper safe limit of BCAA intake.

The degradation pathways for BCAAs

Transfer of the amino group to \( \alpha \)-ketoglutarate to form the corresponding branched-chain keto acids (BCKAs) and glutamate is the first step in the degradation of the BCAAs:

\[
\text{BCKA} + \text{glutamate} \rightarrow \text{BCKDC} + \text{ammonium}
\]

Catalyzed by BCAA aminotransferase, the reaction is fully reversible and therefore does not commit BCAAs to degradation.

Decarboxylation of the carboxyl groups of the BCKAs to form the corresponding branched-chain acyl-CoA esters is the second step:

\[
\text{BCKA} + \text{NAD}^+ + \text{CoA} \rightarrow \text{branched-chain acyl-CoA} + \text{NADH} + \text{CO}_2
\]

Catalyzed by BCKDC, this reaction is irreversible and therefore commits the BCAAs to degradation. This is believed to be the most important site of regulation.

Beyond oxidative decarboxylation, the pathways for degradation of the 3 BCAAs diverge into separate enzyme-catalyzed steps that eventually lead into the citric acid cycle. Carbon originating from leucine enters as acetyl-CoA for complete disposal as \( \text{CO}_2 \), whereas isoleucine and valine provide carbon for the anaplerotic conversion of propionyl-CoA to succinyl-CoA.

Regulation of BCKDC

The activity of BCKDC is regulated by end-product inhibition (NADH and branched-chain acyl-CoA esters), covalent modification by phosphorylation, and altered expression of the amounts of its component enzymes, as reviewed in (8, 9). The complex consists of 12 branched-chain \( \alpha \)-ketoacid dehydrogenase (E1) and 6 dihydrolipoyl dehydrogenase (E3) components noncovalently associated with a core of 24 dihydrolipoyl transacylase (E2) components. A BCKDC kinase [branched-chain kinase (BDK)] also binds to the E2 complex. BDK inactivates the complex by phosphorylation of \( \alpha \) subunits of the heterotetrameric (\( \alpha_2\beta_2 \)) E1 component. Phosphates are removed from E1 to activate the complex by a BCKDC phosphatase (BDP). Regulation of BCKDC activity by BDK and BDP is required for conservation of BCAAs during deficiency and for disposal of BCAAs during surplus, as reviewed in (8,9).

Regulation of BDK and BDP

BCKDC is similar in many ways to the pyruvate dehydrogenase complex (PDC). Both are large multienzyme complexes located in the mitochondrial matrix space. Their E1 and E2 components catalyze similar reactions and require the same coenzymes. The same E3 component serves both complexes, and they are regulated by similar mechanisms, including covalent modification. More is known about the regulation of PDC because of its central role in carbohydrate metabolism and the efforts of many investigators. Much of what is known about PDC is relevant to what remains to be learned about BCKDC. Four pyruvate dehydrogenase kinase (PDK) isoforms are expressed in a tissue-specific manner in mammalian tissues, each encoded by separate nuclear genes, as reviewed in (10). They are related to prokaryotic histidine protein kinases by amino acid sequence but to eukaryotic serine protein kinases by catalytic mechanism. Only one BDK is expressed in eukaryotic cells, in contrast to multiple isoforms of the PDKs. Two pyruvate dehydrogenase phosphatase (PDP) isoforms are expressed in mammalian tissues, each encoded by a separate nuclear gene. PDPs belong to the PP2C family of phosphatases. A BDP has been purified and partially characterized (11), but it has not been cloned nor proven to be a mitochondrial protein. It is not a PP2C phosphatase, like the phosphatases responsible for regulation of PDC. A recent search of the human DNA database revealed a novel PP2C with a mitochondrial leader sequence (Joshi, M., Jeoung, N. H., Popov, K. M. & Harris, R. A., unpublished results). Preliminary evidence indicates that the enzyme is a mitochondrial protein and that it may be a BCKDC phosphatase. This is far from certain at this time, however, because of its relatively low specific activity.

High BCAA intake should be well tolerated because . . .

Considerable “reserve” BCKDC activity exists in mammalian cells. Because of inactivation by phosphorylation, much more potential enzyme activity exists in tissues than necessary for the catabolism of the normal dietary intake of BCAAs. This serves 2 purposes. First, it makes the reaction catalyzed by BCKDC the rate-limiting step of the pathway, thereby ensuring that the activity of the committed step of the pathway can be rapidly shut down to conserve BCAAs for protein synthesis. Second, it provides a reserve of enzyme activity that can be called upon to dispose of BCAAs in the event of a surplus. This is therefore a safety mechanism. This is all made possible because most BCKDC exists in tissues in its phosphorylated, inactive form. The complex present in rat liver is an exception. It is nearly completely dephosphorylated and active, thereby functioning as a sink for the clearance of BCKAs from the portal blood. However, this is not the case in human liver, where measurements suggest that the actual and the total enzyme activity are much less than in rat liver (12). Regulation of the BCKDC activity state (percentage of the enzyme in its dephosphorylated, active form) is rapidly achieved in all tissues through inhibition of BDK activity by \( \alpha \)-ketoisocaproate, the transamination product of leucine. Inhibition of BDK by \( \alpha \)-ketoisocaproate allows activation of BCKDC by the action of BDP. In this way, high dietary BCAA intake mobilizes the reserve of BCKDC for rapid disposal of BCAAs and their keto acids.

Inhibition of BDK by \( \alpha \)-ketoisocaproate provides short-term control of BCKDC activity, whereas altered expression of BDK protein provides long-term control. The combined effects of these mechanisms can be observed in a number of conditions. As reviewed in (8,9), the activity of hepatic BCKDC is reduced markedly in rats fed a low-protein diet or treated with thyroid hormone but increased in starvation, diabetes, sepsis, cancer, uremia, infections, and inflammatory disease caused by endotoxin and cytokines. Starvation for protein upregulates BDK expression (8,9), which in turn activates the BCKDC and conserves BCAAs for protein synthesis. The activity state of hepatic BCKDC is reduced to a minimum level (<10% active) in rats fed a low-protein diet (<8% protein), because BDK expression is greatly increased and the \( \alpha \)-ketoisocaproate concentration is too low to inhibit BDK. In contrast, the activity state of the complex is increased to 100% in rats fed a high protein diet (>25% protein), because BDK expression is greatly reduced and the \( \alpha \)-ketoisocaproate concentration is high enough to inhibit residual BDK activity. Altered BDK expression has been proposed to occur at the level of transcription (13), but recent evidence indicates
regulation at the level of translation may be more important (14).

Although there is much evidence in the literature to support the hypothesis that adjustments of the phosphorylation state of BCKDC by short-term and long-term changes in BDK activity determine BCAA concentrations in the body, we have sought additional evidence for the importance of BDK regulation of BCKDC through studies with a BDK knockout mouse model.

Preliminary findings with a BDK knockout mouse

Weanling BDK knockout mice are smaller than wild-type mice, and half of them die upon weaning at 21 d unless they receive special care (Joshi, M., Jeoung, N. H. & Harris, R. A., unpublished results). BDK knockout mice grow slower than wild-type mice, with significantly reduced actual and percentage of whole body weight of brain, muscle, and epididymal fat pad. Kidneys, on the other hand, are significantly enlarged in knockout mice fed a nonpurified diet and even larger in mice fed a high protein diet. This may be because of the extra load on the kidneys to clear the excess metabolites produced from protein oxidation. BDK knockout mice also develop epileptiform seizures that significantly reduce their life span. The mechanism responsible for neurological abnormalities is not known but may involve the role of leucine in glutamate synthesis. BCAA levels are markedly reduced in the blood and organs of the mice. Based on the role of leucine in the synthesis of brain glutamate (15), our working hypothesis is that maintenance of the "right" brain glutamate concentration depends upon buffering of glutamate by transamination with BCKA. The high BCKDC activity present in the brain of BDK knockout mice may yield low brain BCKA with a concomitant elevation of the excitatory amino acid glutamate.

Poor growth proves that regulation of BCKDC by phosphorylation is required for conservation of BCAAs. Loss of control of BCKDC by knocking out BDK inhibits growth, presumably by limiting availability of BCAAs for protein synthesis and perhaps also because less leucine is available to stimulate translation via the mammalian target of rapamycin (mTOR) pathway (1). An unexpected benefit of this study has been the finding that regulation of BCKDC by phosphorylation is required for normal neurological function.

Thus, it appears that neurological dysfunction can occur when BCAAs are too low, as evidenced by our preliminary findings with the BDK knockout mouse. When BCAAs are too high, neurological dysfunction also occurs, as evidenced by patients with MSUD. Clearly, maintaining BCAAs in a "normal" range of concentrations is important for normal neurological function. Whether dietary deficiency or voluntary control over the consumption of BCAAs can alter the body levels of BCAAs enough to cause neurological dysfunction is not known.

High dietary BCAAs should be well tolerated but . . .

Leucine is toxic when consumed out of proportion to valine and isoleucine. Downregulation of BDK and inhibition of BDK by α-ketosidroacparte activates BCKDC (16). Activated BCKDC depletes α-ketosidrovalerate and α-keto-β-methylvalerate and therefore also depletes valine and isoleucine. Lack of valine and isoleucine inhibits protein synthesis. The consequence is that leucine should not be consumed in large amounts without valine and isoleucine, even though only leucine promotes protein synthesis.

High dietary BCAAs should be well tolerated because of the reserve of BCKDC activity present in tissues of the body. Nevertheless, it is theoretically possible that the safety margin of maximally activated BCKDC may be exceeded at high BCAA intake. There is a need, therefore, to know the maximum capacity for BCAA disposal. Not enough information is currently available to make such calculations. A caveat to keep in mind would be that some medical conditions oppose downregulation of the expression of BDK, e.g., hyperthyroidism (17), which would be required for maximum activation of BCKDC in the face of a maximum load of the BCAAs.

Finally, it should be noted that high dietary BCAAs should be well tolerated as long as downstream enzymes of the BCAA catabolic pathways do not become rate limiting.

Maximum BCKDC activity may exceed maximum activity of downstream enzymes

Little is known about the relative activities and kinetics of enzymes involved in the catabolic pathways for BCAAs in human tissues. Whether enzymes downstream of BCKDC can become rate limiting is not known. This may be particularly important in the elderly, who may benefit from consumption of BCAA supplements, because loss of muscle mass may decrease their capacity for BCAA catabolism. The elderly are also prone to vitamin B-12 deficiency, which induces a defect in the conversion of methylmalonyl-CoA to succinyl-CoA at the distal end of the valine and isoleucine pathways. Accumulation of methylmalonyl-CoA in vitamin B-12 deficiency may promote the accumulation of 3-hydroxyisobutyryl-CoA (an intermediate in valine catabolism) by inhibiting 3-hydroxyisobutyryl-CoA hydrolase (18). Accumulation of 3-hydroxyisobutyryl-CoA may in turn promote the accumulation of methacryl-CoA, which is toxic, because it depletes glutathione (18).

Relevance of this work to the purpose of the workshop

How to determine the safe upper limit of dietary BCAA intake, and what needs to be done to determine the safe upper limit, were the objectives of the workshop. Clearly, BCAA values might be too great when concentrations exceed the capacity of the catabolic pathways for their disposal. Although not yet demonstrated in individuals with normal capacity for BCAA catabolism, the consequence could be negative effects upon neurological function. We propose that a BCAA tolerance test be developed to obtain data on the upper limit of dietary BCAA intake. The data generated by a BCAA tolerance test would allow design of a BCAA clamp protocol in which BCAAs are clamped at various concentrations in the blood to examine their effects on neurological function. Different populations, but especially the elderly and individuals with chronic metabolic diseases, need to be tested to establish maximum blood BCAA responses, duration of elevated BCAA concentrations, and whether there are individuals who exhibit BCAA intolerance or suffer neurological effects. The data obtained in such studies could be compared with the BCAA concentrations that are known to cause neurological damage in MSUD patients. BCAA metabolites should be measured in the urine as markers for overloading of the enzymes of BCAA catabolism. We predict that the safe upper limit for BCAAs will be quite high in most of the population but that such tests will identify a few individuals with BCAA intolerance.

LITERATURE CITED


