The Role of Skeletal Muscle in The Pathogenesis of Altered Concentrations of Branched-Chain Amino Acids (Valine, Leucine, and Isoleucine) in Liver Cirrhosis, Diabetes, and Other Diseases

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Summary
The article shows that skeletal muscle plays a dominant role in the catabolism of branched-chain amino acids (BCAAs; valine, leucine, and isoleucine) and the pathogenesis of their decreased concentrations in liver cirrhosis, increased concentrations in diabetes, and nonspecific alterations in disorders with signs of systemic inflammatory response syndrome (SIRS), such as burn injury and sepsis. The main role of skeletal muscle in BCAA catabolism is due to its mass and high activity of BCAA aminotransferase, which is absent in the liver. Decreased BCAA levels in liver cirrhosis are due to increased use of the BCAA as a donor of amino group to α-ketoglutarate for synthesis of glutamate, which in muscles acts as a substrate for ammonia detoxification to glutamine. Increased BCAA levels in diabetes are due to alterations in glycolysis, citric acid cycle, and fatty acid oxidation. Decreased glycolysis and citric cycle activity impair BCAA transamination to branched-chain keto acids (BCKAs) due to decreased supply of amino group acceptors (α-ketoglutarate, pyruvate, and oxaloacetate); increased fatty acid oxidation inhibits flux of BCKA through BCKA dehydrogenase due to increased supply of NADH and acyl-CoAs. Alterations in BCAA levels in disorders with SIRS are inconsistent due to contradictory effects of SIRS on muscles. Specifically, increased proteolysis and insulin resistance tend to increase BCAA levels, whereas activation of BCKA dehydrogenase and glutamine synthesis tend to decrease BCAA levels. The studies are needed to elucidate the role of alterations in BCAA metabolism and the effects of BCAA supplementation on the outcomes of specific diseases.

Key words
Ammonia • Insulin • Glutamate • Glutamine • Alanine • Pyruvate • Insulin resistance • Systemic inflammatory response syndrome • Cataplerosis • Ketoglutarate

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Introduction

Branched-chain amino acids (BCAAs; valine, leucine, and isoleucine) are nutritionally essential amino acids that serve as substrates for protein synthesis and energy production, perform several signaling functions, mainly via the mammalian target of rapamycin (mTOR) pathway, and act as a nitrogen source for the synthesis of dispensable amino acids, particularly glutamate (GLU), glutamine (GLN), alanine (ALA), and aspartate (ASP). The use of BCAAs as a nutritional supplement, especially to promote anabolic pathways, has been investigated in a wide range of diseases, including cancer, liver cirrhosis, trauma, burn injury, sepsis, and renal failure, for several
decades (Holeček 2018, De Bandt and Cynober 2006, Mascarenhas and Mobaran 2004). However, to date, there are uncertainties regarding the metabolism and pathogenesis of alterations in BCAA levels in these circumstances.

BCAA concentrations decrease uniquely in hyperammonaemic states, such as liver cirrhosis and urea cycle disorders (UCD) (Holecek et al. 2000 and Holecek et al. 2011, Rodney and Boneh 2013, Hayashi et al. 1981, Leweling et al. 1996), and increase markedly in diabetes mellitus – in both type 1 (T1DM, insulin-dependent) and type 2 (T2DM, insulin-independent) (Brosnan et al. 1983, Afring et al. 1988, Wijekoon et al. 2004, Rodriguez et al. 1997, Borghi et al. 1985, She et al. 2013). In liver cirrhosis, BCAAs are recommended as a nutritional supplement mainly due to the adverse role of decreased BCAA levels in the development of fatigue, muscle wasting, and hepatic encephalopathy (Fischer et al. 1975, Marchesini et al. 2003, Nakaya et al. 2007, Urata et al. 2007). On the other hand, increased BCAA concentrations in diabetes are considered to be a factor contributing to the development of insulin resistance and increasing the risk of a number of complications of diabetes (Newgard 2012, Shah et al. 2011).

In addition to hyperammonaemic states and diabetes, significant alterations in BCAA metabolism occur frequently in other diseases, such as burn, trauma, cancer, and sepsis, whose common feature is the presence of signs of systemic inflammatory response syndrome (SIRS) (Beutler and Cerami 1986, Kaukonen et al. 2015, Rangel-Frausto et al. 1995). It is well documented that in these circumstances, BCAAs are used to a greater extent as an energy substrate (Beutler and Cerami 1986, Yang et al. 1997, Tischler and Fagan 1983, Ryan 1976, Holeček 1996, Holecek et al. 1997). However, the alterations in BCAA concentrations are not consistent, and increased, decreased, and unchanged levels have been reported (Askanazi et al. 1980, Vente et al. 1989, Cynober et al. 1982, Hirose et al. 2014, Mierzchala-Pasierb et al. 2020, Druml et al. 2001, Liu et al. 2016, Su et al. 2015).

There is accumulating evidence that skeletal muscle, the main site of BCAA metabolism (Harper et al. 1984, Suryawan et al. 1998), is a crucial player in the pathogenesis of altered BCAA concentrations in liver cirrhosis, diabetes, and disorders associated with SIRS. In the first part of this article, I will give a brief overview of the role of skeletal muscle in the metabolism of BCAAs. In the second part, I will try to explain its role in the pathogenesis of decreased BCAA concentrations in hyperammonaemic states and increased BCAA concentrations in diabetes, and why alterations in BCAA levels are inconsistent in most other disorders, particularly those with marked signs of SIRS.

**BCAA catabolism in skeletal muscle**

Skeletal muscle plays a dominant role in BCAA metabolism because it is the largest tissue in the body and has the highest activity of BCAA amino-transferase (BCAAT), the first enzyme of BCAA catabolism, which is almost absent in the liver (Harper et al. 1984, Suryawan et al. 1998). Hence, whereas most of the other amino acids provided by food are metabolized in the liver, most BCAAs are extracted by muscles. After amino acid infusion via the peripheral vein, skeletal muscle is responsible for the extraction of up to 30% of all amino acids and up to 70% of infused BCAAs (Gelfand et al. 1986).

The pathways of BCAA metabolism have been described in detail in several reviews (Harper et al. 1984, Harris et al. 2004, Harris et al. 2005, Shimomura et al. 2001, Mattick et al. 2013, Holeček 2018). Here, I will provide only information essential for understanding the role of skeletal muscle in the catabolism of BCAAs (Fig. 1).

**BCAA transamination**

The first step in BCAA catabolism is reversible transamination by BCAAT in mitochondria to their respective branched-chain keto acids (BCKA), α-ketoisocaproate (ketoleucine), α-keto-β-methylvalerate (ketoisoleucine), and α-ketoisovalerate (ketovaline). The flux of BCAAs through BCAAT is sensitive to the supply of BCAAs and acceptors of amino nitrogen, i.e. α-ketoglutarate (α-KG), pyruvate (PYR), and oxaloacetate (OA) and removal of products of transamination, glutamate (GLU) and BCKA. Hence, BCAA transamination might be influenced by:

- **BCAA supply** – BCAA transamination is activated by food intake and increased breakdown of muscle proteins (Ruderman and Berger 1974, Durschlag and Smith 1985, Holecek et al. 2016).
- The activity of the citric acid cycle – determines the supply of α-KG. The citric acid cycle might be activated by increased glycolysis and inhibited by increased levels of NADH (Beatty et al. 1958, Spydevold et al. 1976).
• Activity of ALT and AST reactions – ALT and AST enable the regeneration of α-KG from GLU to be available for BCAAT and to attenuate the drain of α-KG (cataplerosis) from the citric acid cycle. Both ALT and AST reactions are reversible and sensitive to the availability of reactants, such as BCAAT reaction.
• GLU level – depends on GLU input from the blood, breakdown of muscle proteins, and removal of GLU by GLN synthetase, ALT, and AST.
• The disappearance of BCKA – depends on the activity of BCKAD and the release of BCKA into circulation by multiple monocarboxylate transporters, which also control the transport of lactate, pyruvate, and ketone bodies (Bonen et al. 2006).

**Fig. 1. Pathways of BCAA catabolism in skeletal muscle.** Three aminotransferases, BCAAT, ALT, and AST, catalyze the transport of amino groups between BCAA/BCKA, PYR/ALA, and OA/ASP with the partner pair GLU/α-KG to form BCKA and avoid the drainage of α-KG (cataplerosis) from the citric acid cycle. The supply of amino group acceptors (α-KG, OA, and PYR) is determined by glycolysis, citric acid cycle activity, and GLN synthesis. The BCKAs produced in the BCAAT reaction are released into the circulation or undergo oxidative decarboxylation catalyzed by BCKAD regulated by multiple mechanisms, including NAD+/NADH, CoA-SH/acyl-CoA, and ATP. There are multiple ways to regulate the activity of BCKAD. Long-term regulation occurs through changes in the expression of the enzyme, and short-term regulation by reversible phosphorylation. Phosphorylation mediated by a specific kinase results in inactivation, and dephosphorylation by a specific phosphatase activates the enzyme. Therefore,

**BCKA decarboxylation**

The second step in BCAA catabolism is irreversible decarboxylation of BCKA by the branched-chain keto acid dehydrogenase (BCKAD) complex at the inner mitochondrial membrane to branched-chain acyl-CoA esters. Enzymatic activity levels in humans are high in the liver, kidneys, and brain and low in skeletal muscle, gut, and adipose tissue (Harper et al. 1984, Suryawan et al. 1998).

Catabolism of branched-chain acyl-CoA esters

Beyond the BCKAD reaction, BCAA metabolism diverges into separate pathways. Catabolism of leucine leads to acetyl-CoA and acetoacetate (leucine is ketogenic), valine is catabolized to succinyl-CoA (valine is glucogenic), and isoleucine is catabolized to acetyl-CoA and succinyl-CoA (isoleucine is both glycogenic and ketogenic). Since leucine degradation gives rise to acetoacetate and acetyl-CoA, unlike valine and isoleucine, this amino acid cannot act as an anaplerotic agent.

Skeletal muscle and decreased BCAA levels in liver cirrhosis and UCD

The decrease in BCAA concentration, as occurs in liver cirrhosis and UCD, is a characteristic feature of hyperammonemia (Holecek et al. 2000, Holecek et al. 2011, Rodney and Boneh 2013, Hayashi et al. 1981, Leweling et al. 1996, Holecek 2020). In liver cirrhosis, hyperammonemia is mainly due to portal systemic shunts, as indicated by increased levels of ammonia following transjugular intrahepatic portal-systemic shunts (He et al. 2020). Although ammonia may increase markedly in acute liver damage, decrease in BCAAs is not observed due to the leakage of amino acids from injured hepatocytes (Rosen et al. 1977, Shi et al. 1984, Holeček et al. 1996 and 1999). In UCD, a marked decrease in BCAAs is observed in acute metabolic decompensation and in subjects treated with phenylbutyrate (Rodney and Boneh 2013, Scaglia et al. 2004, Holecek et al. 2017).

Decreased BCAA levels are believed to play a role in the pathogenesis of hepatic encephalopathy, muscle wasting, and impaired liver regeneration (Fischer et al. 1975, Holecek 1999, Marchesini et al. 2003). Hence, although the pathogenesis of decreased BCAA levels is not entirely clear, BCAAs and BCAA-enriched supplements have been extensively investigated and frequently recommended as therapies for liver disease (Fischer et al. 1975, Marchesini et al. 2003, Nakaya et al. 2007, Urata et al. 2007). In patients with UCD, attempts to use BCAA supplements are rare and have inconclusive results (Adam et al. 2013).

Pathogenesis of decreased BCAA levels

It is supposed that the main role in the pathogenesis of decreased BCAA levels in hyperammonaemic states is increased ammonia detoxification to GLN in muscles (Fig. 2). BCAAs act as the main donor of amino nitrogen to α-KG to form GLU, which is used for ammonia detoxification to GLN in the GLN synthetase reaction (Clemmesen et al. 2000, Girard and Butterworth 1992, Holecek et al. 2011). Hence, in hyperammonemia, increased removal of GLU for ammonia detoxification to GLN enhances the flux of the BCAA through BCAAT and GLN synthetase:

\[ \text{BCAA + } \alpha\text{-KG} \rightarrow \text{BCKA + GLU} \]
\[ \text{GLU + NH}_3 + \text{ATP} \rightarrow \text{GLN + ADP} \]

Several studies have shown a stimulatory influence of ammonia on GLN synthesis in muscles (Smith et al. 1984, Clemmesen et al. 2000, Girard and Butterworth 1992, Holeček et al. 2011) and increased GLN concentration in blood plasma in subjects with liver cirrhosis and UCD (Maestri et al. 1992, Holeček and Vodeničarovová 2018). Apparently, a negligible role in ammonia detoxification in muscles is GLU synthesis from α-KG (α-KG + NH_3 → GLU) because of extremely low glutamate dehydrogenase expression (Mavrothalassitis et al. 1988).

Enhanced use of GLU for GLN synthesis results in the drain of GLU from ALT and AST reactions and decreased synthesis of ALA and ASP, as demonstrated in hyperammonaemic states by increased GLN and decreased ALA concentrations in blood plasma and muscles (Rodney and Boneh 2013, Holecek et al. 2000, Holeček and Vodeničarovová 2018). A detrimental consequence of the preferential use of GLU for GLN synthesis is the loss of α-KG from the citric acid cycle (cataplerosis), which might impair the activity of the citric acid cycle and subsequently decrease ATP production by mitochondria. Marked decreases in α-KG and ATP were found in the muscles of cirrhotic rats (Holeček and Vodeničarovová 2018). ATP and the total level of adenine nucleotides were markedly reduced in skeletal muscle biopsy specimens of patients with liver
cirrhosis (Moller et al. 1984, Davuluri et al. 2016). Hyperammonemiac decreased mitochondrial respiration, the NAD+/NADH ratio, the concentrations of citric acid cycle intermediates, and ATP content in C2C12 myotubes (Davuluri et al. 2016). In addition to cathaplerosis of α-KG, a role in ATP depletion may be malfunction of mitochondria due to the toxic properties of ammonia and the activated GLN synthetase reaction, which is ATP-consuming (Jayakumar and Norenberg 2016).

Fig. 2. Role of skeletal muscle in the pathogenesis of decreased blood plasma BCAA concentrations in hyperammonemic states. Under hyperammonemiac conditions, such as liver cirrhosis and UCD, ammonia detoxification to GLN in muscles is activated. The needs of GLU for the GLN synthetase reaction are covered by GLU synthesis from BCAA and α-KG. The results are depletion of BCAAs, diversion of GLU from ALT and AST reactions to GLN synthesis, and the drainage of α-KG from the citric acid cycle. Most of GLN produced in visceral tissues results in increased ammonia production, which, when its detoxification to urea in the liver is insufficient, is detoxified to GLN in reactions consuming BCAA and α-KG in muscles again. 1, BCAAT; 2, BCKAD; 3, ALT; 4, AST; 5, GLN synthetase. ALA, alanine; ASP, aspartic acid; FA, fatty acids; GLU, glutamic acid; GLN, glutamine; OA, oxaloacetate; PYR, pyruvate.

Increased formation of BCKA due to increased flux of BCAAs through BCAAT, an increased ratio of NAD⁺ to NADH, and decreased ATP levels should increase the activity of BCKAD, resulting in irreversible loss of essential BCAAs. Increased oxidation of the BCAA has been demonstrated in rats after ammonia infusion and muscles incubated in medium with high ammonia concentration (Holeček et al. 2000, Holeček et al. 2011). The leucine-oxidized fraction (ratio between leucine oxidation and leucine appearance from protein breakdown) was higher in rats with liver cirrhosis than in controls (Holeček et al. 1996).

The last step towards a decline in BCAAs and a rise in GLN concentration in blood plasma in cirrhosis and UCD is increased influx of BCAAs from extracellular fluid to muscles (attenuates BCAA depletion in the cytosol) by exchange with GLN via the L-transporter system (Meier et al. 2002). Increased expression of the leucine/glutamine exchanger SLC7A5 (LAT1) mRNA has been reported in cirrhotic patients and C2C12 cells during hyperammonemia (Davuluri et al. 2016).

Consequences of increased GLN release from muscles

Ammonia detoxification to GLN in muscles can potentially reduce ammonia concentration in the blood and its detrimental influence on the brain. Unfortunately, this method of ammonia detoxification is only temporary. It has been shown that enhanced GLN concentrations activate glutaminase in visceral tissues, particularly in enterocytes and kidneys, and subsequently increase the release of ammonia into circulation (Souba et al. 1990, Tietze et al. 1992). If ammonia detoxification to urea in the liver is impaired, as occurs in liver injury and UCD, ammonia is returned to muscles for GLN synthesis, resulting in a further decrease in plasma BCAAs. Hence, a vicious cycle, characterized by increased GLN synthesis in muscles and its breakdown in visceral tissues, has been suggested to play a role in the pathogenesis of hyperammonemia and BCAA depletion in liver cirrhosis (Holeček 2014).
Skeletal muscle and increased BCAA levels in diabetes

Skeletal muscle is not only the main site of BCAA catabolism but also the predominant tissue for insulin-mediated glucose disposal (De Fronzo et al. 1985). In muscles, BCAA concentrations are increased markedly in both T1DM and T2DM (Brosnan et al. 1983, Affring et al. 1988, Wijekoon et al. 2004, Rodriguez et al. 1997, Borghi et al. 1985, She et al. 2013, Holecek et al. 2020). Hence, the role of muscle tissue in the pathogenesis of increased BCAA concentrations in diabetes is obvious.

In Figure 3, it is envisaged that metabolic alterations induced by decreased glycolysis and increased fatty acid oxidation, characteristic features of both types of diabetes, might decrease BCAA transamination and BCKA decarboxylation and subsequently increase BCAA levels in muscles. Impaired glycolysis may decrease BCAA transamination by decreasing the supply of PYR and OA, which limits the supply of α-KG for BCAAT via decreased citric acid cycle activity and decreased regeneration of α-KG from GLU in ALT and AST reactions. Increased oxidation of fatty acids may decrease BCAA catabolism in three ways. First, the surplus of NADH produced during fatty acid oxidation inhibits NADH-producing enzymes of the citric acid cycle and subsequently decreases the supply of α-KG for BCAAT. Second, increased NADH/NAD⁺ inhibits BCKAD. Third, BCKAD should also be inhibited by increased production of acyl-CoA derivatives from increased but, due to impaired entrance of acetyl-CoA into the citric acid cycle, incomplete fatty acid oxidation. Increased BCAA concentrations in muscles can lead to increased BCAA concentrations in blood plasma by their decreased disappearance from the blood after food intake or by increased release from muscles in the postabsorptive state (Felig 1975).

In summary, it is hypothesized that decreased flux of BCAAs through BCAAT and BCKAD in muscles due to decreased glycolysis and increased fatty acid oxidation plays a main role in the pathogenesis of increased BCAA levels in diabetes. The role of decreased BCAA transamination is supported by findings of increased BCAA and decreased ALA concentrations in muscles of subjects with diabetes (Brosnan et al. 1983, Rodriguez et al. 1997, Affring et al. 1988, Jensen-Waern et al. 2009, Borghi et al. 1985). The role of impaired flux through BCKAD indicates increased BCKA concentrations in blood plasma and muscles in animals with T1DM induced by alloxan and in obese Zucker rats (Hutson and Harper 1981, She et al. 2013).
A detailed review focused on the differences in the pathogenesis of increased BCAA levels in T1DM and T2DM and early starvation, also characterized by decreased glycolysis, increased fatty acid oxidation and increased plasma BCAA levels (Holeček and Mičuda 2017), has been published recently (Holeček 2020).

Skeletal muscle and BCAA levels in other diseases


It is the consensus that a wide range of neurohumoral abnormalities associated with the exaggerated response of the whole body to an infectious or noninfectious insult, referred to as SIRS, have a central role in the pathogenesis of metabolic alterations in many serious diseases. Among the most important are the activation of the sympathetic nervous system and cortisol and cytokine production leading to anorexia, rise in body temperature, insulin resistance, increased use of lipids as an energy source, activated protein breakdown in muscles, and increased protein synthesis in visceral tissues (Beutler and Cerami 1986, Kaukonen et al. 2015, Rangel-Frausto et al. 1995). Several studies have shown that TNF-α, IL-6, glucagon, and cortisol activate BCKAD and that increased BCAA catabolism in muscle tissue is associated with the release of high amounts of GLN to the systemic circulation to be used primarily by enterocytes and lymphatic tissue (Holeček 1996, Nawabi et al. 1990, Pacy et al. 1990, Harper et al. 1984). Enhanced GLN availability can help fight infections and increase the ability to survive in seriously ill patients (Hardy and Hardy 2008).

Figure 4 suggests that alterations in BCAA levels in stress disorders are inconsistent due to the contradictory influence of alterations induced by SIRS in muscles. Specifically, activated proteolysis and insulin resistance tend to increase BCAA concentrations, whereas increased activity of BCKAD and increased synthesis of GLN tend to decrease BCAA concentrations. Roles should also be played by altered food intake, muscle mass, and nutritional status, among other factors.

Although increased BCAA oxidation provides a rationale for the use of BCAA-enriched supplements for the therapy of serious stress disorders, such as sepsis, trauma, and cancer, reports on clinical outcomes are controversial (Teasley and Buss 1989, Okada et al. 1988,
Vente et al. 1991, Scholten et al. 1990, Sandstedt et al. 1992). A surprising shortcoming of most studies is the lack of information about the influence of illness and therapy on BCAA concentrations. Hence, the differences in study results might be partly due to differences in BCAA levels and their metabolism depending on the type, extent, and stage of illness.

**Conclusions**

Skeletal muscle plays a central role in BCAA metabolism, and there are several pathways by which muscles can affect BCAA concentrations in blood and tissues. It is suggested that:

(i) Decreased BCAA levels in liver cirrhosis and UCD are due to increased use of the BCAA as a source of amino group for the synthesis of GLU, which is an immediate substrate for ammonia detoxification to GLN by GLN synthetase reaction in muscles. The decrease in BCAA levels is associated with detrimental alterations in mitochondrial function and the catabolism of GLN released from muscles to ammonia in visceral tissues.

(ii) Increased BCAA levels in diabetes are due to decreased glycolysis and increased fatty acid oxidation, which decrease BCAA transamination and BCKA decarboxylation via decreased supply of acceptors of amino group (α-KG, PYR, and OA) and inhibitory influence of NADH and acyl-CoA derivatives on BCKAD.

(iii) Alterations in BCAA levels in disorders characterized by a significant influence of SIRS, such as burn injury, trauma injury, cancer, and sepsis, are inconsistent due to contradictory effects of SIRS on muscles. Specifically, increased proteolysis and insulin resistance tend to increase BCAA levels, whereas activations of BCKAD and GLN synthesis tend to decrease BCAA levels.

In conclusion, altered profiles of plasma BCAAs reflect alterations in their metabolism, particularly in skeletal muscle, and may be used as diagnostic and prognostic tools. Careful studies are needed to elucidate the role of alterations in BCAA metabolism in the development of specific illnesses and to determine how providing extra amounts of BCAAs affects the outcome of patients in most indications.

**Conflict of Interest**

There is no conflict of interest.

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**Abbreviations**


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