The role and metabolic functions of the branched-chain amino acids: a review

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Abstract. In recent years, a number of new functions of branched-chain amino acids (BCAA) - leucine, valine and isoleucine - have been revealed in various states of the body in animals and humans. BCAA are involved in the regulation of the metabolism of not only proteins, but also lipids and carbohydrates, maintain the health of the mammary glands and intestines, and help in early implantation and development of embryos. BCAA increase protein synthesis and are currently considered as feed additives to improve meat productivity in pigs. New aspects of metabolic and regulatory functions of BCAA include a number of regularities: 1) insufficient or excessive levels of them in the diet enhances lipolysis; 2) BCAA, especially isoleucine, play an important role in glucose utilization by activating glucose transporters in the intestines and muscles; 3) BCAA enhance the development of the intestine, the transport of amino acids and the production of mucin; 4) BCAA are involved in the regulation of innate and adaptive immune responses. In the near future, the use of high-performance functional genomics, metabolomics, and proteomics will make it possible to more fully reveal the functions of BCAA in gene expression, protein synthesis, and metabolism regulation.

1 Introduction

Free amino acids and their derivatives are included in a number of universal natural regulators and endogenous modifiers of biological reactions, since their levels are the most important regulatory factors in the biosynthesis of proteins and highly active biological substances (mediators, hormones), in the formation of basic metabolic flows and the functional state of organs and systems. There are various ways of using free amino acids, but basically they take part in three main metabolic reactions: 1) part of the free amino acids is included in tissue proteins, and due to their breakdown, these amino acids return to the pool of free amino acids and thus become suitable for reuse in synthesis squirrel; 2) part of the free amino acids undergoes catabolic reactions, which leads to the loss of the carbon

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skeleton or its deposition in the form of glycogen and fat, while nitrogen is excreted in the urine; 3) some amino acids are used to synthesize new nitrogen-containing compounds, such as purine bases, creatine, adrenaline, etc. Basically, they are gradually broken down without returning end products to the pool of free amino acids (for example, purines decompose to uric acid, creatine to creatinine and etc.).

In recent years, researchers have been of great interest in studying the metabolic and regulatory functions of essential amino acids with branched side chains (BCCA) under various conditions of the body in animals and humans. These amino acids account for up to 60% of all circulating amino acids, and among them a special role belongs to leucine. Unlike isoleucine and valine, leucine is involved in the regulation of protein biosynthesis and catabolism in skeletal muscles, and has a pronounced hepatoprotective and immunomodulatory effect. It should be noted that, despite the widespread use of individual amino acids or their compositions to compensate for their deficiency, the direct pharmacological effects of metabolite therapy, as well as the regulatory effect of these compounds on metabolism, are poorly understood. Branched-chain amino acids (BCAA) are compounds that are physiologically and biochemically unique for a macroorganism, in comparison with other amino acids, and they belong to the essential amino acids for mammals. Although these amino acids are essential, they are not metabolized in the liver, as is the case for other proteinogenic amino acids. The main catabolism of these amino acids occurs in extrahepatic tissues, mainly in skeletal muscles. Skeletal muscles make up to 40% of the body mass, therefore, despite the relatively low activity of leucine, isoleucine, and valine transaminase in skeletal muscles, the ability of these tissues to deaminate this group of amino acids is probably the most significant.

According to recent studies, BCAA affect several synthetic and catabolic cell signaling cascades leading to changes in phenotypes in mammals [1, 2, 3]. In addition, BCAA have unique properties, performing various physiological and metabolic functions. In particular, it has been shown in various in vitro and in vivo models that they increase protein synthesis, inhibit their breakdown, and participate in the regulation of energy metabolism. The use of BCAA and their metabolites open up great prospects for improving the growth and health of animals and humans [4, 5]. In particular, in piglet diets, leucine, isoleucine and valine are typically the next limiting amino acids after lysine, methionine, threonine and tryptophan. The latest amino acids are available in the feed supplement market and allow for the reduction of crude protein in the diet while maintaining the levels of essential amino acids. The development of diets with a reduced content of crude protein requires an accurate knowledge of the need for limiting amino acids, including BCAA. In particular, an excess of leucine and valine can increase the breakdown of isoleucine and vice versa, which can lead to an overestimation of their need, since they are the only amino acids that have common catabolism pathways. The interest of researchers in the physiological significance and metabolic functions of BCAA and the possibilities of their practical use is realized in the way of establishing the body's need for them under various conditions and clarifying their level and ratio, in creating a fairly wide range of drugs, feed additives, indications for the use of which are metabolic and pharmacological effects of leucine, isoleucine and valine.

2 Purpose of the study

The aim of this study was to systematize modern ideas about the physiological significance and metabolic function of branched amino acids.

3 Metabolic functions of leucine, isoleucine and valine

Amino acids perform many functions in the body, being substrates for protein biosynthesis, glucose precursors, and taking part in the synthesis of urea and other metabolic processes. Amino acids and their metabolites are involved in the regulation of the synthesis of phospholipids, glycogen, as well as in the processes of protein breakdown. Amino acids circulating in blood plasma are mainly classified by their ability to form glucose or ketone bodies (glycogenic or ketogenic), as well as by the presence of enzymes for their synthesis in mammalian tissues (essential or essential). The metabolism of amino acids is directly controlled by processes occurring mainly in mitochondria. In addition, amino acids are involved in the regulation and implementation of many other cellular functions. Many amino acids are indispensable ingredients for the synthesis of compounds that act as nitrogen and carbon suppliers [6, 7]. BCAA are key hydrophobic amino acids and are used by cells to synthesize steroids and ketone bodies [6, 7]. In the body of mammals, L-Leucine is practically not synthesized, with the exception of a slight formation from the corresponding α-keto acid.

The daily norm of leucine for a person (1.1-1.2 g) is provided by its intake as part of protein foods of plant and animal origin. Its content in human serum albumin is 11-12%, in ovalbumin - 9.2%, in γ-lactoglobulin - 15.5%, in hemoglobin - 15-16%, in myoglobin - 16-18%, in pepsin - 10.4, β-globulins - 9.3%, in human fibrin - 7.1%, in lysozyme - 6.9%. 100 ml of human blood plasma normally contains about 2 mg of L-Leucine, and depending on the composition of food, its daily excretion in the urine is about 14 mg. It is well absorbed when administered orally, penetrates the blood-brain barrier and is rapidly incorporated into proteins. Advantageously, the incorporation of ^{14}C-Leucine determines the rate of protein synthesis in vivo.

Among BCAA, only leucine has a particularly pronounced ability to stimulate protein biosynthesis, cell metabolism, and cell growth by influencing the signaling pathway involving mTOR [8]. The composition of many tissue proteins in mammals contains a large amount of these amino acids, in connection with which BCAA account for up to 35% of the total requirement of mammals for essential amino acids. Leucine, isoleucine, and valine are the most hydrophobic of the 20 amino acids present in the structure of the protein molecule. BCAA account for 35-40% of all essential amino acids in dietary proteins and 14-18% of the total amino acids in skeletal muscle proteins. Human muscle mass is about 40% of body weight, therefore, the pool of muscle proteins is the largest reservoir of BCAA in the body. On the other hand, animals have a pool of free amino acids that appears to be constant and skeletal muscle BCCA levels are only about 0.6-1.2 mmol/kg). This pool is extremely small compared to the amount of total free amino acids in muscle. The total concentration of BCAA in human blood (0.3-0.4 mM) is relatively high compared to other amino acids (with the exception of glutamine). However, the amount of these amino acids in human blood is very small compared to their content in skeletal muscles [8].

As noted above, leucine, isoleucine and valine are not only the structural unit for tissue protein (which accounts for 35% of the essential amino acids in muscle), but also perform other metabolic functions. Among BCAA, leucine occupies a special place due to its specific function in the activation of the mTOR signaling pathway. Since the 1970s a special role of leucine in increasing protein synthesis both in vitro and in vivo has been reported. BCAA stimulate insulin secretion. It has been shown that their elevated plasma levels lead to insulin resistance and type 2 diabetes mellitus. One possible mechanism for this is that persistent activation of the mTOR signaling pathway uncouples the insulin receptor from insulin receptor substrate-1 [9]. Another possible mechanism is the accumulation of toxic metabolites of BCAA (caused by impaired metabolism), which can provoke mitochondrial dysfunction associated with insulin resistance [9].

Recent studies have shown that BCAA are involved in lipolysis, lipogenesis, glucose metabolism, glucose transport, intestinal absorption, milk quality, breast health, embryonic development and immunity [10, 11]. Leucine contributes to the maintenance of energy metabolism, glucose uptake, mitochondrial biogenesis and fatty acid oxidation, inhibition of protein breakdown. In addition, BCAA in the body can act as a biomarker for the early detection of chronic diseases [10, 11].

4 Metabolism of branched-chain amino acids

The liver is considered the main organ that performs oxidative decarboxylation of branched-chain keto acids captured from the blood. In the catabolism of BCAA, there are common enzymes for the first 2 stages: transamination and subsequent decarboxylation of branched keto acids. Unlike other amino acids, which are mainly metabolized by the liver, BCAA are utilized in significant amounts in skeletal muscles and kidneys. It should be noted that BCAA are the only amino acids that have common pathways of catabolism. These essential amino acids are also unique in that their catabolism begins in skeletal muscle. After ingestion of protein food, more than 50% of BCAA formed as a result of protein hydrolysis pass through the plasma membranes of enterocytes and blood vessels and enter the bloodstream. Since skeletal muscle and other non-parenchymal organs are the main site of uptake of these amino acids, it is easy to surmise that BCAA should be a useful marker for peripheral amino acid availability. In addition, it is likely that one or more amino acids of this group may have the functions of a cellular signal indicating to tissues the need to increase protein biosynthesis or reduce intracellular protein hydrolysis in order to maintain the amino acid pool in plasma and tissues. Glutamate, glutamine, and alanine formed during the catabolism of BCAA are the main precursors of glucose in gluconeogenesis reactions [6].

As mentioned above, BCAA are not directly metabolized in the liver, and most of them are available for transformation in skeletal muscle and other tissues. However, the liver can oxidize BCAA after they are converted to α-keto acids in other tissues [12]. In the first step, BCAA convert these amino acids to branched chain α-keto acids (leucine to α-ketoisocaproate, valine to α-ketoisovalerate, and isoleucine to α-keto-β-methylvalerate) by removing their amino group. Subsequently, branched chain α-keto acids are decarboxylated by branched chain α-keto acid dehydrogenase (BCKD). The main steps of BCAA catabolism are listed below (Fig. 1) [9]. Firstly, with the participation of branched-chain amino-transferase (BCAT). Finally, these BCCA metabolites are catabolized in a series of enzymatic reactions to form end products (acetyl-CoA from leucine, succinyl-CoA from valine, and both acetyl-CoA and succinyl-CoA from isoleucine) that enter the tricarboxylic acid cycle (TCA) [9]. Due to the fact that questions on the regulation of the catabolism of these amino acids still remain unresolved, recent studies consider new mechanisms for controlling the enzymes involved in the regulation of the catabolism of leucine, isoleucine, and valine. Such mechanisms include regulation of their abundance by miRNAs and post-translational modifications such as phosphorylation, acetylation, and ubiquitination. The influence of circadian rhythm, age, and mTORC1 on these enzymes was also analyzed. These studies may be useful for developing methods to prevent disorders associated with the metabolism of BCAA [12].

Approximately 80% of leucine is typically used for protein synthesis, with the remainder converted to α-ketoisocaproate and β-hydroxy-β-methylbutyrate in skeletal muscle. Therefore, it has been hypothesized that some functions of leucine are modulated by its metabolites, and α-ketoisocaproate and β-hydroxy-β-methylbutyrate have received much attention recently as dietary supplements used to increase protein synthesis, inhibit protein degradation, and regulate energy metabolism [12].

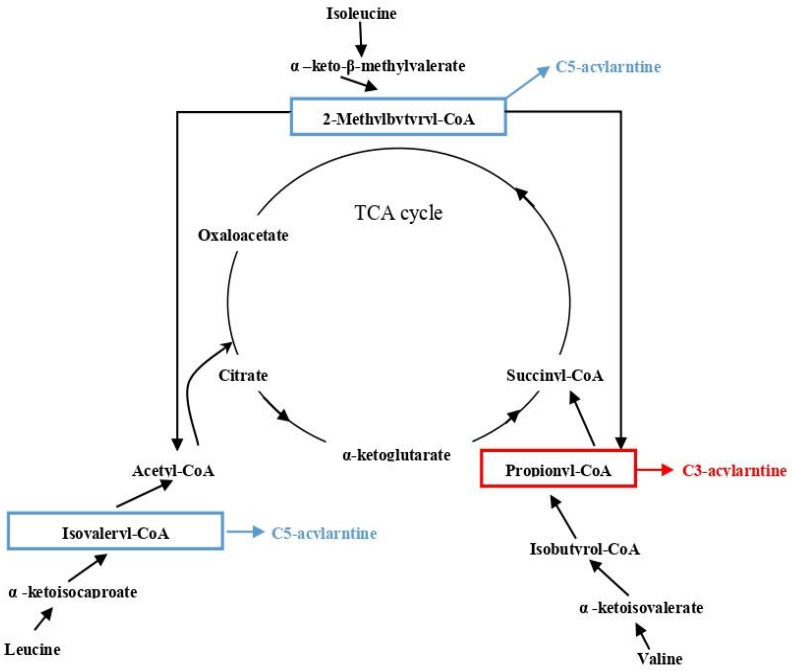

Fig. 1. Pathway of branched chain amino acid catabolism. BCAA are catabolized to acetyl-CoA and/or succinate-CoA and subsequently enter the TCA cycle. The main steps of the catabolic reactions (transamination by BCAT and decarboxylation by BCKD) are shown. With the help of BCAT, BCAA are catabolized into branched-chain α-ketoacids which are subsequently decarboxylated by BCKD. Finally, all the BCAA metabolites are catabolized by a series of enzyme reactions to final products and enter the TCA cycle.

5 Branched-chain amino acids as signaling molecules

BCAA are known to be critical for skeletal muscle and whole body anabolism and for maintaining energy homeostasis. They are also signaling molecules, for example capable of activating the mammalian target of rapamycin complex 1 (mTORC1). This matters for metabolic processes; in addition, elevated blood levels of BCAA and their keto acids, as well as impaired catabolism of these amino acids, have been implicated in the development of insulin resistance and its consequences, including cardiovascular disease and some types of cancer. Supplementation of these amino acids has also been shown to help treat some chronic diseases [12].

BCAA play a critical role in regulating metabolism, nutrition, gut health, and immunity in animals. As the most abundant essential amino acids, they are not only substrates for the synthesis of nitrogenous compounds, but also signaling molecules that regulate glucose metabolism, lipid metabolism, protein synthesis, gut health, and immunity through a special signaling network, especially phosphoinositide-3-kinase and protein kinase, which is the target rapamycin signaling pathway (PI3K/AKT/mTOR) [12]. Recent studies confirm that BCAA and their derivatives are potential biomarkers for diseases such as insulin resistance, type 2 diabetes, cancer, and cardiovascular disease. These diseases are closely related to the catabolism and balance of these amino acids. Therefore, optimizing their dietary levels should positively impact health and disease-related parameters.

It is known that BCAA increase protein synthesis by modulating translation, which explains their attractiveness for athletes for muscle hypertrophy, accelerated recovery and preservation of muscle mass. In addition to their anabolic effects, these amino acids may increase mitochondrial content in skeletal muscle and adipocytes, possibly increasing oxidative capacity. However, elevated levels of circulating α-BCAA correlate with the severity of insulin resistance. It is assumed that an increased level of circulating BCAA in the blood, detected in insulin resistance, may be the result of a dysregulation of their breakdown [1].

Thus, in recent years we have witnessed the emergence of new functions of BCAA: (1) their insufficient or excessive levels in the diet increase lipolysis; (2) these amino acids, especially isoleucine, play an important role in increasing glucose uptake and utilization by activating glucose transporters in the gut and muscles; (3) adding leucine to the diet improves the meat quality of finishing pigs; (4) leucine, valine, and isoleucine are beneficial for breast health, milk quality, and embryo growth; (5) these amino acids enhance gut development, amino acid transport in the gut, and mucin production; (6) leucine, isoleucine and valine are involved in the regulation of innate and adaptive immune responses. In addition, an abnormally elevated blood level of BCAA (decrease in their catabolism) is a good biomarker for the early detection of liver cirrhosis, obesity, diabetes mellitus, and other metabolic diseases [9, 13, 14].

6 Importance of branched-chain amino acids in animal nutrition

Stimulation of muscle protein synthesis by nutritional factors is especially pronounced in the early periods of growth, and the efficiency of the translational mechanism decreases with age. Bolus feeding in newborns has been shown to be more effective in stimulating muscle protein synthesis than continuous feeding. Catabolic stimuli, such as infection, inflammation, intestinal malabsorption, and aging, blunt or block nutrition-induced stimulation of muscle protein synthesis at the level of translation initiation and reduce formation of the triple initiation complex and/or eIF4 complex [9].

Approximately 10% of babies born in the US have low birth weight. Growth failure in the neonatal period is common due to infant intolerance to wholesome nutrition, problems with increased protein intake, and high nutrient requirements for growth and development. A better understanding of nutritional regulation during this critical postpartum period is vital to developing strategies for improved growth and development. Early animal studies have demonstrated that muscle protein synthesis is significantly increased after a meal, and this increase is associated with a postprandial increase in amino acid levels as well as insulin. BCAA are potent activators of mTORC1 in muscle independently of insulin/IGF-I-Akt [9, 11]. Similarly, growth factors can stimulate mTORC1 signaling in skeletal muscle regardless of amino acid levels. Further research has shown that leucine in particular, and its metabolites, α-ketoisocaproic acid and β-hydroxy-β-methylbutyrate, have unique anabolic properties. Leucine supplementation, given parenterally or orally, increases muscle protein synthesis in neonatal piglets, making it an ideal candidate for growth promotion in low birth weight infants [13].

The rapid growth of skeletal muscle in the newborn requires coordination of protein deposition and the number of nuclei in mions. At this stage of development, muscle protein synthesis is very sensitive to the intake of amino acids, especially leucine, while whether this statement is true for satellite cells, as a source of muscle fiber nuclei, remains unknown. In the studies of foreign colleagues, it has been shown that the multiplication of satellite cells and the number of nuclei in mions in newborn pigs worsen when the protein intake with protein diet is reduced from 22.5 to 11.2 g protein/(kg/day), and is not restored with the help of supplements. leucine. The level of protein in the diet in newborns affects

the expression of insulin-like growth factor (IGF). Decreased dietary protein levels reduced IGF2 expression in the longissimus dorsi muscle by 60% but did not reduce IGF1 or IGF1R expression ($P<0.05$). Leucine did not decrease IGF2 expression [9].

In studies on BCAA as a feed additive for growing pigs, there is a problem of determining the body's need for them when using various diets. Recent studies have shown that 100%, 150%, 200%, 250%, 300% excess of leucine in the diet from requirements in piglets with an initial body weight of 30 kg reduces growth rates and the rate of nitrogen deposition, which is probably the result of increased isoleucine catabolism and valine and their reduced availability [14]. One approach to prevent the negative effects of high levels of leucine in traditional diets of growing pigs is to supplement them with valine and isoleucine, which will increase the efficiency of amino acid utilization for protein synthesis and animal growth [9].

Studies have shown that high concentrations of BCAA, especially leucine, can reduce the intake of large neutral amino acids, such as tryptophan, which is a precursor of serotonin and can have a significant impact on the regulation of feed intake. Finally, high concentrations of leucine have the ability to over-stimulate the mTORC1 signaling pathway, resulting in an inhibitory effect on feed intake. Most of the studies done to evaluate the effect of BCAA on pig growth performance seem to agree that high levels of leucine reduce body weight gain, mainly due to reduced feed intake. However, some studies, mostly in piglets, have found no evidence of an effect on growth performance even at extremely high dietary levels of leucine. It can be assumed that these discrepancies are due to the entire amino acid profile in the diet, and not just the level of leucine. Growth diets tend to contain high levels of leucine, but other BCAA are also well above requirements and can potentially mitigate the negative effects of leucine on their catabolism. Indeed, some research suggests that when diets are high in leucine, more isoleucine and valine are needed to optimize growth performance. However, the exact relationship between the level of BCAA and their optimal ratio in the diet of pigs has not been fully studied. More research is needed to understand and quantify the relationship between large neutral amino acids and BCAA [9].

The development of diets low in crude protein while maintaining an adequate supply of amino acids through the addition of crystalline amino acids is an established practice in pig nutrition. The use of crystalline amino acids reduces feed and feeding costs and nitrogen excretion while maintaining growth performance. Currently, lysine, threonine, methionine, tryptophan and valine are widely used in pig breeding. After valine, isoleucine is probably the next limiting amino acid in corn-soybean meal diets [15]. However, while valine and isoleucine limit the intake of low-protein corn-soybean meal, leucine is usually in excess due to its high concentration in corn and corn by-products [4]. In this situation, the use of low-protein diets makes it possible to solve the problem of excess content of amino acids in diets and balance them in terms of amino acid composition [15]. The addition of BCAA to a reduced protein diet increases post-feed protein synthesis and inhibits fasting protein degradation, which ultimately contributes to increased skeletal muscle mass in piglets [15]. Another study showed that mTOR and S6K1 phosphorylation in muscle was increased after the addition of BCAA to the low protein diet of piglets, independent of changes in feed intake. Fortification of low-protein diets with BCAA increases feed intake and lean mass, and improves piglet growth [15].

A growing body of evidence suggests that food has specific direct and indirect effects on intestinal receptor activation, similar to a cocktail of "hormones" [14]. This activation can increase the secretion of gastrointestinal hormones such as peptide YY (PYY), glucagon-like peptide 1 (GLP-1), and cholecystokinin (CCK) [15]. There are many amino acid receptors in the gastrointestinal tract that have been found, such as T1R1/T1R3, CaSR, GCR6A and mGluR [15]. Activation of these receptors can provide regulation of food

intake, proliferation of cells in the gastrointestinal tract, motility of the small intestine, and nervous reflexes. The study of the structure and function of BCAA receptors is vital for a better understanding of their physiological role.

7 Conclusion

Leucine, isoleucine and valine, like BCAA, are essential amino acids for animals and humans, not only because they cannot be synthesized in the body, but also due to their significant metabolic and regulatory role. These amino acids (especially leucine) increase protein synthesis and are currently being considered as feed additives to improve meat production and meat quality in rearing and fattening pigs. Recent studies have identified new metabolic functions of BCAA. These amino acids are regulators of the metabolism of not only proteins, but also lipids and carbohydrates. They support the functioning of the mammary glands, improve the quality of milk and help in the early implantation and development of embryos. Leucine, valine, and isoleucine improve gut health and its ability to transport amino acids, increase immunity by increasing the expression of β-defensin, which increases the regulatory function of pro-inflammatory cytokines and reduces the effect of anti-inflammatory cytokines. Finally, they are biomarkers for the early detection of chronic diseases such as diabetes, insulin resistance, and obesity in humans. In the future, using high-throughput functional genomics, metabolomics, and proteomics, it will be possible to better reveal the functions of BCAA in gene expression, protein synthesis, and metabolic regulation.

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