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Forum Minireview

New Therapeutic Strategy for Amino Acid Medicine: Notable Functions of Branched Chain Amino Acids as Biological Regulators

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Abstract. The branched chain amino acids (BCAAs) leucine, isoleucine, and valine are the most abundant of the essential amino acids. BCAAs have recently been recognized as having functions other than simple nutrition. The importance of BCAAs as nutrient regulators in protein synthesis was recognized over 20 years ago. Leucine is the most potent of the BCAAs in stimulating muscle protein synthesis, while isoleucine and valine are much less effective. The signaling action of leucine in protein synthesis has been well studied, and the mechanisms are currently under investigation. However, the pharmacological effects of isoleucine and valine have not been clarified. It has recently been reported that, among the BCAAs, leucine and isoleucine act as signals in glucose metabolism. We revealed that isoleucine stimulates both glucose uptake in the muscle and whole body glucose oxidation, in addition to depressing gluconeogenesis in the liver, thereby leading to a hypoglycemic effect in rats. Based on these results, we speculate that isoleucine signaling accelerates catabolism of incorporated glucose for energy production and consumption.

Keywords: branched chain amino acid, leucine, isoleucine, protein metabolism, glucose metabolism

1. Introduction

The branched chain amino acids (BCAAs) leucine, isoleucine, and valine are the most abundant of the essential amino acids. In addition to their critical role as substrates for protein synthesis, these amino acids play a variety of roles in the body. It is believed that BCAAs contribute to energy metabolism during exercise as energy sources and substrates to expand the pool of citric acid–cycle intermediates (anaplerosis) and for gluconeogenesis. Moreover, BCAAs serve as regulatory (signaling) molecules that modulate numerous cellular functions (Table 1). BCAAs, acting as nutrient signals, regulate protein synthesis and degradation, and insulin secretion, and have been implicated in central nervous system control of food intake and energy balance (1 – 4). In particular, the signaling action of BCAAs in protein synthesis has been well studied, and the mechanism is currently under investigation (5). Of the BCAAs, leucine appears to be the specific effector on protein synthesis in several tissues including skeletal muscle, liver, and adipose tissue (6 – 8). However, the pharmacological effects of the other BCAAs, isoleucine and valine, have not been well clarified. We recently found that among the BCAAs, isoleucine acts as a nutrient regulator of glucose metabolism (9, 10).

The major focus of this review is on the role of BCAAs in regulating glucose metabolism in rats.

2. Effects of amino acids on glucose metabolism

BCAAs, particularly leucine, play essential roles in hormonal secretion and action, as well as in intracellular signaling. In glucose metabolism, despite the fact that amino acids can stimulate the release of insulin, it has been shown that amino acid infusion actually inhibits glucose utilization. Previous studies have also shown that amino acids, particularly leucine, inhibit insulin-stimu-

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Table 1. Functions of BCAAs

	Substrate for protein synthesis	Source of energy	Action of regulatory molecules			
			Protein metabolism		Glucose metabolism	Lipid metabolism
			synthesis	degradation		
Leu	◎	◎	◎	◎	○	—
Ile	◎	◎	△	—	○	—
Val	◎	◎	—	—	—	△

◎: well-documented function, ○: some studies have demonstrated this function, △: a few studies have demonstrated this function, —: unknown at present.

lated glucose uptake (11 – 14). One mechanism through which this could occur is the preferential oxidation of amino acids leading to glucose sparing. As an alternative to glucose oxidation, amino acids may serve as fuel, and amino acids, including the glucogenic amino acids (alanine, valine, or glutamine), are considered to be able to increase glucose production and blood glucose levels. More recent work has begun to identify intracellular mechanisms through which amino acids appear to control glucose metabolism. This inhibitory action is mediated through the attenuated tyrosine phosphorylation of insulin receptor substrates (IRS)-1 and -2 and subsequent interaction with the regulatory subunit of phosphoinositide 3-kinase (PI-3 kinase), leading to decreased activity of PI-3 kinase, protein kinase B (PKB/Akt), and mammalian target of rapamycin (mTOR) (14 – 16).

Of the amino acids, leucine is involved in glucose uptake in isolated muscle (17), in glycogen synthesis via the inactivation of glycogen synthase kinase-3 (18), and in the insulin-secretion effect in the pancreas (19). On the other hand, leucine, but not isoleucine or valine, also inhibits insulin-stimulated glucose uptake in L6 cells by degrading IRS-1 via activation of the mTOR/S6K1 signaling pathway, leading to desensitization of insulin signaling (14, 16, 20). In addition, leucine reduces the duration of insulin-induced IRS-1–associated PI-3 kinase activity in rat skeletal muscle (21). Given these results, it is to be expected that amino acids will decrease glucose oxidation and lead to amino acid–induced insulin resistance. However, it has been reported that amino acid infusion causes a decrease in blood glucose levels and an increase in glucose oxidation in humans (22, 23), although there have been few investigations of this hypoglycemic effect to date. These changes appear to occur via the action of insulin, as leucine, but not isoleucine or valine, stimulates insulin release from the pancreas, thereby decreasing blood glucose (24, 25). Thus, this contradicts the amino acid-induced insulin resistance described above, and this issue therefore remains controversial.

3. Hypoglycemic effect of isoleucine

Recent studies have demonstrated that a BCAAs mixture decreases plasma glucose levels in vivo. Oral administration of a BCAAs mixture has been shown to ameliorate hyperglycemia in a virus-induced non-insulin-dependent diabetes mellitus mouse model (26, 27). In streptozotocin-induced rats, oral administration of a BCAAs mixture (0.75 – 1.5 g/kg body weight) significantly decreased plasma glucose levels (28). However, it is unknown whether this reflects glucose metabolism caused by leucine, isoleucine, or valine, and the mechanism of action of the individual BCAAs is not understood in vivo or in vitro.

Our collaborators reported that isoleucine prevents a rise in plasma glucose concentration and that the effect of isoleucine is greater than that of leucine or valine on oral glucose tolerance tests in normal rats (29). The hypoglycemic effect of isoleucine was recently confirmed in a human study of oral administration of isoleucine (30). In contrast, valine caused an increase in plasma glucose levels, which suggests that valine, a glycogenic amino acid, is used as a substrate for glyconeogenesis in the liver. In C_2C_{12} myotubes, leucine and isoleucine stimulate glucose uptake in an insulin-independent manner, and the effect of isoleucine is greater than that of leucine (29). In such cells, signaling pathway analysis using a phosphatidylinositol 3-kinase (PI3K) inhibitor (LY294002), a protein kinase C (PKC) inhibitor (GF109203X), and an mTOR inhibitor (rapamycin) suggests that PI3K and PKC, but not mTOR, are involved in the enhancement of glucose uptake by isoleucine. These data suggest that isoleucine assumes the role of a signal for glucose metabolism, thereby stimulating insulin- and mTOR-independent glucose transport in cultured skeletal muscle cells.

We focused on the blood glucose–lowering effects of isoleucine and examined whether isoleucine decreased the plasma glucose concentration in food-deprived rats and whether isoleucine increased glucose uptake in

skeletal muscles in vivo. Valine was excluded from the scope of this research, as valine caused an increase in plasma glucose levels. Oral administration of isoleucine, but not leucine, significantly decreased plasma glucose concentration in food-deprived rats (9). Glucose uptake in the skeletal muscle did not differ after leucine administration, but glucose uptake in the muscles of rats administered isoleucine was 73% greater than that in food-deprived controls, thus suggesting that isoleucine increases skeletal muscle glucose uptake in vivo. These results indicate a relationship between the reduction in blood glucose and the increase in skeletal muscle glucose uptake that occur with isoleucine administration in rats.

Furthermore, we investigated the possible involvement of the energy sensor 5′-AMP-activated protein kinase (AMPK) in the modulation of glucose uptake in skeletal muscle, which is independent of insulin, and also in isoleucine-stimulated glucose uptake (9). AMPK is a serine/threonine kinase consisting of a catalytic subunit (α) and 2 regulatory subunits (β and γ). The catalytic α subunit occurs in 2 distinct isoforms in mammals. AMPK α1 is widely expressed, whereas the α2 isoform is expressed predominantly in the liver, heart, and skeletal muscle (31). AMPK α1 activity in skeletal muscle was not affected by leucine or isoleucine administration. However, isoleucine, but not leucine, significantly decreased AMPK α2 activity. These results indicate that isoleucine-stimulated glucose uptake increases in the absence of increased AMPK α1 and α2 activity in skeletal muscle in food-deprived rats.

4. Effects of isoleucine on glycogen synthesis and glucose oxidation

As mentioned above, administration of isoleucine leads to an increase in glucose uptake in skeletal muscle in vivo (9). However, it remains unknown how the glucose incorporated into the tissues by isoleucine is metabolized. Leucine stimulates glycogen synthesis through the inactivation of glycogen synthase kinase-3 in L6 muscle cells in a manner that is dependent on mTOR and independent of insulin (18). Our collaborators reported that leucine causes a significant increase in D-[U-^{14}C] glucose incorporation into the intracellular glycogen in the myotube cells in vitro, whereas isoleucine does not affect glycogen synthesis when compared with that in controls (29). Therefore, we first examined the effects of isoleucine on glycogen synthesis in skeletal muscle.

Muscle glycogen synthesis, as determined by [U-^{14}C] glucose incorporation into glycogen, was significantly increased by administration of leucine, but not isoleucine, in rat skeletal muscles in vivo when compared with food-deprived control rats. Although leucine has less effect on glucose uptake in skeletal muscle, it stimulates glycogen synthesis in skeletal muscle. In contrast, isoleucine stimulates glucose uptake, although it has less effect on glycogen synthesis (9). We also measured the contents of high-energy phosphate metabolites (AMP, ADP, and ATP) in the skeletal muscles of rats administered leucine and isoleucine to evaluate the cellular energy state (9). Oral administration of leucine or isoleucine did not alter the ADP or ATP contents in the skeletal muscle when compared with control rats. Although isoleucine caused a decrease in AMP content in skeletal muscle when compared with the control and leucine groups, the AMP content was not affected after administration of leucine when compared with the control group. Furthermore, although leucine did not change the AMP:ATP ratio, isoleucine caused a significant decrease in this ratio in the skeletal muscle when compared with control groups. We assume that a depletion of cellular AMP would result in a decrease in the AMP:ATP ratio and improve the availability of ATP in the skeletal muscle without any marked increase in ATP concentration, thereby resulting in an improvement in the cellular energy state.

In order to determine whether the hypoglycemic effect of isoleucine affects whole body glucose oxidation, we examined the effects of isoleucine on expiratory excretion of $^{14}CO_2$ from [U-^{14}C]glucose in vivo at a dose where the hypoglycemic effect was greatest (10). The expiratory excretion of $^{14}CO_2$ of rats treated with isoleucine was significantly elevated between 60 and 90 min after administration when compared with controls. Based on the above results, muscle glucose uptake in isoleucine-administered rats was elevated at 60 min. As the time to achieve maximum plasma isoleucine levels was 60 min (9, 29), this indicates a strong correlation among the increase in muscle glucose uptake, the decrease in blood glucose, and the subsequent increase in glucose oxidation. In contrast, it has been reported that isoleucine suppresses $^{14}CO_2$ production from [1-^{14}C]pyruvate in isolated skeletal muscle (32). As a potential explanation, the suppression might have been caused by an increase in total pyruvate content in the skeletal muscle (33) due to increased glucose uptake by isoleucine (9, 29), which results in a diluting effect for [1-^{14}C]pyruvate and an increase in total CO_2 production. Meanwhile, glycogen synthesis in isoleucine-administered rats was not altered in skeletal muscle (9), thus suggesting that when there is a lowering of blood glucose levels, isoleucine increases glucose uptake in skeletal muscle with the incorporated glucose mainly oxidized in the muscle immediately after uptake.

5. Effects of isoleucine on glucose uptake in peripheral tissues and hepatic glucose production

Generally, the maintenance of blood glucose levels is due to an optimal balance between glucose uptake by peripheral tissues and glucose production occurring mainly in the liver. Therefore, we examined the effects of isoleucine administration on glucose uptake in peripheral tissues and hepatic glucose production.

Glucose uptake (Rg) was significantly increased in the muscle of isoleucine-administered rats when compared with that in controls at the most effective dose of isoleucine. In contrast, there were no significant differences in the Rg in liver or adipose tissue in isoleucine-administered rats when compared with controls. These results suggest that skeletal muscle is the major organ contributing to the hypoglycemic effects of isoleucine on glucose uptake. Leucine has a stimulatory effect on insulin secretion (34). As a temporal increase in plasma insulin levels after oral administration of leucine was observed in this study, the effect of leucine on glucose metabolism may primarily be insulin dependent. On the other hand, the hypoglycemic effect of isoleucine was more potent than that of leucine, although significant changes in plasma insulin and glucagon levels were not observed after isoleucine administration. Furthermore, isoleucine had an additive effect on insulin-stimulated glucose uptake via PI3K (29), in contrast to leucine, which inhibited insulin-stimulated glucose uptake in skeletal muscle cells (35). Although the molecular basis of increased glucose uptake by isoleucine remains unclear, a previous study by another group revealed that glucose uptake by isoleucine is involved in increased glucose transporter GLUT1 and GLUT4 translocation in the skeletal muscle of rats with liver cirrhosis (36). These data suggest that isoleucine improves insulin sensitivity in skeletal muscle via an intracellular signal pathway.

Hepatic gluconeogenesis, as well as glucose utilization by peripheral and hepatic tissues, may be a possible mechanism by which amino acids lower blood glucose levels. During the fasting state, glucose production is largely a result of gluconeogenesis as opposed to hepatic glycogenolysis (37). Therefore, we examined the effects of isoleucine on the gluconeogenic rate-limiting enzymes in vivo. The mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK), which is a well-researched key gluconeogenic enzyme, parallel both PEPCK activity and the rate of gluconeogenesis (38, 39). The data showed that expression levels of hepatic PEPCK mRNA were lower in isoleucine-administered rats than in controls, thus suggesting that PEPCK activity was lower in isoleucine-administered rats and that the inhibitory effects of isoleucine on PEPCK are regulated at the transcriptional level. Furthermore, we demonstrated that expression levels of hepatic glucose-6-phosphatase (G6Pase) mRNA and G6Pase activity were also lower in isoleucine-administered rats. Enzyme activity is regulated by controlling both protein expression and existing enzyme. Although whether isoleucine regulates existing enzyme is unknown, we believe that isoleucine inhibits G6Pase activity by decreasing G6Pase mRNA expression. These findings suggest that isoleucine also downregulates G6Pase activity and associated mRNA, in addition to inhibiting gluconeogenesis in the liver in vivo.

Under in vitro conditions, there is an inhibitory effect of isoleucine on the expression of PEPCK and G6Pase in isolated hepatocytes. It was also demonstrated that the G6Pase activity was lower in isoleucine-added cells when compared with that in the controls. These findings suggest that isoleucine downregulates the transcription of gluconeogenic enzymes and inhibits glucose production in the liver under insulin-free conditions. This suggests that the inhibitory effects of gluconeogenesis by isoleucine involve an insulin-independent signal pathway, as well as the insulinotropic effects of isoleucine in vivo.

Although we can calculate the Rg and endogenous glucose production (EGP) values at the same time through the use of a tracer, the value of EGP may be underestimated in experiments in which Rg values are markedly elevated. Therefore, we employed measurement of glucose production by using isolated hepatocytes in order to determine the mechanism underlying the hypoglycemic effects of isoleucine. As a glucogenic substrate, we examined alanine, because plasma alanine levels were found to be significantly higher in the 0.45 g/kg body weight isoleucine group, which was also the most effective dose for decreasing plasma glucose when compared with controls. Isoleucine significantly inhibited glucose production when alanine was used as a glucogenic substrate in isolated hepatocytes. In addition, phenylalanine, which is a neutral amino acid transported via by the same neutral amino acid transport system as alanine, leucine, and isoleucine (40, 41), also significantly reduced glucose production. These results indicate that the inhibitory effects of isoleucine on glucose production with alanine may be due to a competitive inhibitory effect with alanine for transport via the neutral amino acid transporter.

In conclusion, isoleucine administration stimulates both glucose uptake in the muscle and whole body glucose oxidation, in addition to depressing gluconeogenesis in the liver, thereby leading to a hypoglycemic effect in rats (Fig. 1).

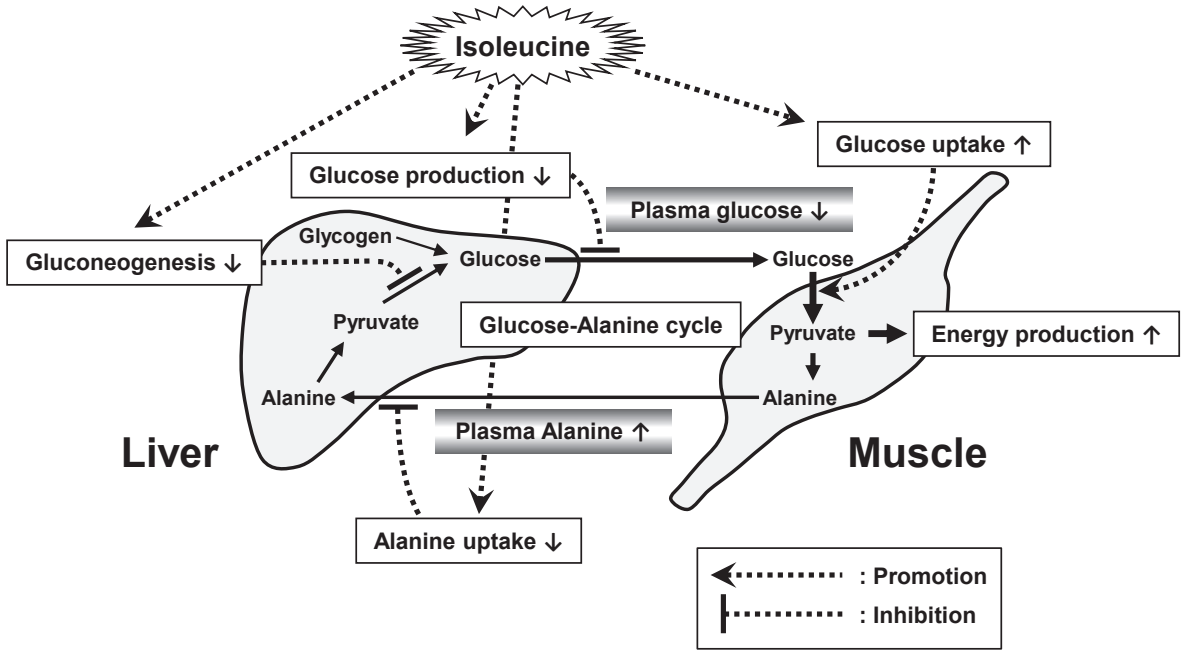

Fig. 1. Schematic diagram of the effects of isoleucine on glucose metabolism. Isoleucine stimulates glucose uptake in skeletal muscle and the incorporated glucose is oxidized without significant elevation of plasma insulin levels. In the liver, isoleucine decreased hepatic gluconeogenic enzyme activity and glucose production. These mechanisms are responsible for the hypoglycemic effect of isoleucine that improves the energy state of the muscle and liver and that may improve insulin resistance in vivo. This figure is partially modified from the original reported by Doi et al. (Ref. 10).

6. Potential action of leucine or isoleucine as a nutritional signal

Based on the results of our study, we speculate that leucine signaling promotes anabolism of protein and glycogen in skeletal muscle. In contrast, isoleucine signaling accelerates catabolism of incorporated glucose for energy production and consumption without affecting protein and glycogen synthesis. The individual BCAAs appear to act as different nutritional signals in the body. To further evaluate the characteristics of leucine or isoleucine as a nutritional signal, we performed a cluster analysis of the transcript profile (unpublished study). We used Affymetrix GeneChip arrays to analyze the expression profile. Differentially expressed genes were classified according to Gene Ontology function using Affymetrix annotation (NetAffx, http://affymetrix.com/index.affx). After appropriate data transformation, hierarchical cluster analysis was performed using the R package "pvclust".

In skeletal muscle, the expression profiles of rats at 3 h after administration of leucine did not form a different cluster from those in food-deprived control group. In contrast, differences in the clustering of food-deprived control rats and rats 3 h after administration of isoleucine were observed using two subsets of genes; glycolysis and protein metabolism. The expression profiles in leucine-administered rats did not form a different cluster from those in food-deprived control rats. In the liver, differences in the clustering of food-deprived control and leucine-administered rats were observed using five subsets of genes; lipid metabolism, glucose metabolism, protein biosynthesis, or proteolysis and protein metabolism. The expression profiles for isoleucine-administered rats did not form a different cluster from those of food-deprived control rats. These results are preliminary, but suggest that leucine and isoleucine have unknown effects on macronutrient metabolism.

7. Conclusion

Numerous hormones play important roles in the metabolic regulation of nutrients. Among these, insulin functions to regulate the metabolism of all macronutrients (e.g., proteins, carbohydrates, and fats), making it a crucial metabolic hormone. Insulin acts anabolically on protein metabolism by stimulating protein synthesis and inhibiting its breakdown. Similarly, leucine functions to stimulate protein synthesis and inhibit its breakdown. The role of isoleucine has been overshadowed by attention on leucine, but it has also been demonstrated to have glucose metabolism-regulating functions similar to insu-

lin, such as stimulation of glucose uptake into cells and inhibition of gluconeogenesis. These findings suggest that leucine and isoleucine share metabolic regulatory functions with insulin. If the remaining BCAA, valine, functions in the regulation of lipid metabolism, it is possible that the metabolic regulation functions of each of these BCAAs can serve as an alternative to the metabolic regulation function of insulin. Although not discussed here, some studies have shown that valine may contribute to lipid metabolism. In particular, a study using a rat model in which 70% of the liver had been resected investigated the effect of valine on lipid dynamics in serum and hepatic tissue during the hepatic regeneration process when the rats were given feed with added valine (42). The results suggested that valine enhanced the uptake of free fatty acids in serum, the accumulation of neutral fats and ATP production in the early stages of liver regeneration following resection, and further stimulated regeneration.

As BCAAs serve to regulate the metabolism of all three major nutrients, similarly to insulin, the value of their use as biological regulators cannot be overestimated. BCAAs are nutrients that should therefore be a focus of investigation as next-generation biological regulators.

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