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REVIEW ARTICLE

Leucine nutrition in animals and humans: mTOR signaling and beyond

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Abstract Macronutrients, such as protein or amino acid, not only supply calories but some components may also play as signaling molecules to affect feeding behavior, energy balance, and fuel efficiency. Leucine, a branched-chain amino acid is a good example. After structural roles are satisfied, the ability of leucine to function as signal and oxidative substrate is based on a sufficient intracellular concentration. Therefore, leucine level must be sufficiently high to play the signaling and metabolic roles. Leucine is not only a substrate for protein synthesis of skeletal muscle, but also plays more roles beyond that. Leucine activates signaling factor of mammalian target of rapamycin (mTOR) to promote protein synthesis in skeletal muscle and in adipose tissue. It is also a major regulator of the mTOR sensitive response of food intake to high protein diet. Meanwhile, leucine regulates blood glucose level by promoting gluconeogenesis and aids in the retention of lean mass in a hypocaloric state. It is beneficial to animal nutrition and clinical application and extrapolation to humans.

Keywords Leucine nutrition · mTOR signaling

Abbreviations

AA	Amino acid
AgRP	Agouti-related protein
AMPK	AMP-activated protein kinase
BCAA	Branched-chain amino acid
BCATm	Mitochondrial branched-chain aminotransferase
BW	Body weight
4E-BP1	Eukaryotic initiation factor 4E binding protein 1
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
NPY	Neuropeptide Y
PI3K	Phosphatidylinositol 3-kinase
S6K1	Ribosomal protein S6 kinase 1
UCP3	Uncoupling protein 3

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Introduction

Amino acids (AA) of leucine, isoleucine, and valine, that named branched-chain amino acids (BCAA), are the most abundant of the essential AA. Leucine is the most abundant one in many dietary proteins, accounting for over 20% of total dietary protein obtained from human diet. Further, among the BCAA, leucine seems to be the most potent one regarding to the effects on protein synthesis and degradation, leptin secretion, energy-balance regulation and so on. Additional metabolic roles for leucine require plasma and intracellular levels above minimum needed for protein synthesis (Harper et al. 1984; Yin et al. 1993; Layman 2003). It displays numerous metabolic roles that function in proportion with cellular concentration, and is dependent on dietary intake and elevating leucine concentration in tissue.

Leucine serves as a substrate for protein synthesis, and increases tissue protein synthesis of weanling piglets fed a low-protein diet (Yin et al. 2010a). In addition, leucine has been correlated with satiety, body weight (BW) control and whole body energy expenditure. First, leucine directly

stimulates mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, in the hypothalamus results in decreased food intake and weight gain (Cota et al. 2006). Second, leucine affects satiety by stimulating of leptin secretion (Lynch et al. 2006). Third, leucine or BCAA have been shown to decrease fat mass, BW and improve glucose metabolism (Bianchi et al. 2005; Donato et al. 2006; Gordon-Elliott and Margolese 2006; Layman and Walker 2006; Zhang et al. 2007). Therefore, it is important to pay attention to the difference between leucine requirements based on minimum levels to prevent deficiencies versus optimum levels for metabolic balance. Metabolic effects of leucine supplementation may be complex, but the effects of leucine on overall body metabolism at multiple levels have received little awareness. This review was performed to gain a better and broader understanding of leucine functions.

Nutritional roles of leucine in animals and humans

Leucine and mTOR pathway in adipose tissue and diabetes

Leucine regulates protein synthesis not only in the skeletal muscle, but also in other tissue including the adipose tissue (Lynch et al. 2002a; Roh et al. 2003). Interestingly, leucine and other BCAA in plasma are markedly raised in both rodent and human with obesity (Rafecas et al. 1991). Moreover, leucine is a direct-acting nutrient signal for stimulation for protein synthesis, but also modulates adipose mTOR (Lynch 2001; Lynch et al. 2002a, b, 2003). In adipose tissue, mTOR signaling has been demonstrated to play tissue-specific roles in preadipocytes differentiation, adipose tissue morphogenesis, hypertrophic growth, and leptin secretion (Lynch 2001). Freshly isolated rat adipocytes treated with leucine/norleucine (an analog to leucine), or adipose tissues of Sprague–Dawley rats orally administered solutions containing 5.4% leucine/norleucine, the mTOR signaling pathway is activated, by depending on both ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1) phosphorylation, two proteins involved in the initiation of protein synthesis. Furthermore, 4E-BP1 appears to be a novel regulator of adipogenesis and metabolism in mammals (Tsukiyama-Kohara et al. 2001). The acute study of 6 h of feeding supplementation of leucine (232 μM/kg/h) in adolescents with type I diabetes promotes the whole-body protein balance (Desikan et al. 2010). In addition, leucine reduces food intake via promoting mTOR signaling pathway in hypothalamus, especially in the region containing orexigenic neurons expressing both neuropeptide Y (NPY) and agouti-related protein (AgRP) (Cota et al. 2006). Except for leucine, no other AA is observed to regulate mTOR signaling in the hypothalamus. Inhibition of mTOR signaling pathway by rapamycin (the inhibitor of mTOR signal pathway) only partially block some effects of leucine or AA (Anthony et al. 2000; Tang et al. 2001; Kimball and Jefferson 2002), showing that there would be two pathways involved in, including rapamycin-sensitive and -insensitive pathways, but the latter is known less.

mTOR functions as part of two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The former is sensitive to rapamycin, but not for the latter. mTORC1 is required for the differentiation and maintainance of adipocytes. Therefore, it is a regulator of adipose metabolism and controls whole body energy homeostasis (Kimball and Jefferson 2006; Polak 2008; Yao et al. 2008).

On another hand, activation of mTOR/S6K1 pathway by AA has been shown to phosphorylate serine (Ser-1101) of insulin receptor substrate 1 (IRS-1) and suppress tyrosine phosphorylation, which results in impaired phosphatidylinositol 3-kinase (PI3K) activity, a critical kinase involved in the mechanism of insulin action on glucose transport and metabolism (Patti et al. 1998; Tremblay and Marette 2001; Tremblay et al. 2005a, 2007). Hence, AA excess may inhibit the first steps of insulin signaling and decrease glucose utilization in skeletal muscle to finally promote insulin resistance (Krebs and Roden 2004; Tremblay et al. 2005b, 2007; Um et al. 2006; Promintzer and Krebs 2006). Furthermore, chronic BCAA, including leucine, contribute to the promotion of insulin resistance in rats fed high fat diet (Newgard et al. 2009). However, leucine supplementation (4.5%) for 5 weeks in adults rats induces a delay in the postprandial stimulation of the early steps of muscle insulin signaling that does not lead to insulin resistance on muscle glucose transport measured in vitro. Meanwhile, perirenal adipose tissue is significantly increased (+27%) in leucine-treated rats and is associated with a decrease in overall oral glucose tolerance (Balage et al. 2011). It is hypothesized that leucine in excess may have been used as energetic substrate, sparing glucose which utilized and stocked as triglycerides by adipose cell, thereby increasing adiposity. It suggests that the role of leucine in insulin resistance remains unclear.

Leucine and leptin and insulin secretion

Leucine modulates the organization of adipocytes into tissue-like structures (Fox et al. 1998), leptin synthesis and secretion from adipose tissue (Roh et al. 2003). Leptin is mainly secreted by adipocytes and regulates food intake and whole body energy balance (Zhang et al. 1994). Its concentrations in plasma are positively correlated with adipose tissue in both obese and lean humans. Leptin expression in adipocytes correlates to energy metabolism

and the level of intracellular ATP (Mueller et al. 1998; Levy et al. 2000). Insulin has been identified as a potential mediator between food intake and leptin production. Mammalian cells have a critical nutrient-sensing pathway that controls protein synthesis at translation level. Phosphatidylinositol kinase-related protein kinase mTOR (Schmelzle and Hall 2000; Raught et al. 2001; Rohde et al. 2001) is the central player in the pathway. Leucine significantly stimulates leptin secretion via nutrient- and insulin- regulated mTOR pathway and subsequent stimulation of leptin mRNA translation in isolated rat adipocyte cells (Roh et al. 2003). Hence, the effect of leucine on leptin expression is likely to be regulated by the mTOR pathway. In vitro, leptin secretion is modulated at mRNA translation level by mTOR and its agonist leucine. However, these effects are blocked by the specific inhibitor rapamycin, suggesting the involvement of mTORC1, which is markedly activated by nutrients and also sensitive to rapamycin (Loewith et al. 2002). The involvement of mTOR in the mediation of leptin expression shows a strong link between insulin resistance and obesity. Furthermore, it provides a feedback device controlling metabolic homeostasis in the body through mTOR-mediated production of leptin, or other similar secreted cytokines. The role of leucine in the regulation of mTOR signal pathway is shown in Fig. 1.

A rise in glucose concentration is necessary for leucine to stimulate significant insulin secretion and in turn decrease the glucose response to ingested glucose. Intravenous administration of 30 g leucine with 30 g glucose synergistically increased the plasma insulin concentration and decreased the glucose rise resulting from the infused glucose alone (Floyd et al. 1970). On the other hand, ingested leucine (1 mmol/kg lean body mass) with 25 g glucose together attenuates the serum glucose response and markedly stimulates additional insulin secretion (Kalogeropoulou et al. 2008). This is likely to be a strong synergistic effect of leucine with glucose on insulin secretion. The ability of leucine to stimulate the rate of muscle protein synthesis is blocked when the increase in insulin release is prevented (Anthony et al. 2002a). It suggests the alteration of protein synthesis is insulin-dependent. However, another investigation indicates that leucine stimulates the rate of muscle protein synthesis in diabetic animals (Anthony et al. 2002b) suggesting that this is as well as an insulin-independent mechanism.

Leucine and glucose homeostasis

Dietary leucine significantly improves glucose metabolism in diet-induced obesity. It is postulated that supplementation of leucine, a natural component of dietary proteins, may play a key role in regulating the metabolic benefits of protein-rich diet (Layman and Walker 2006; Zhang et al. 2007). Leucine not only provides substrates for gluconeogenesis, but also regulates oxidative use of glucose by

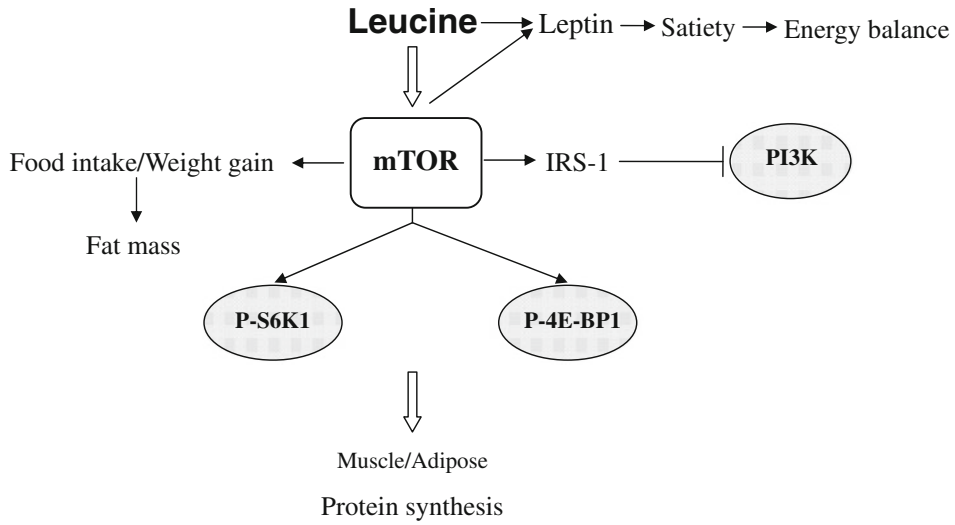

Fig. 1 Role of leucine in the regulation of mTOR signal pathway. Leucine regulates protein synthesis not only in the skeletal muscle, but also in the adipose tissue through mTOR signaling pathway by depending on both S6K1 and 4E-BP1 phosphorylation. It also stimulates mTOR resulting in decreased food intake, weight gain, and fat mass. Meanwhile, leucine affects satiety by stimulating of leptin mRNA translation and secretion, and the effect of leucine on leptin expression is likely to be regulated by the mTOR pathway. Further, activation of mTOR pathway by leucine has been shown to activate IRS-1 and impair PI3 K activity. Abbreviations: mTOR, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase 1; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; IRS-1, insulin receptor substrate 1; PI3K, phosphatidylinositol 3-kinase

skeletal muscle through stimulating of glucose recycling via the glucose-alanine cycle. The potential effects of leucine on metabolic roles are proportional to its dietary intake. For example, daily needs for new proteins on the basis of nitrogen balance are about 1–4 g/day for humans (FAO 1985). When the minimum requirement for protein synthesis is achieved, leucine is turned to contribute to produce alanine and glutamine or to impact the signaling pathway. These functions are dependent on increasing intracellular concentrations (Harper et al. 1984; Anthony et al. 2000; Yin et al. 2010a).

Branched-chain amino acids, specifically leucine, has unique role in metabolic regulation beyond the fundamental role of AA as substrates for synthesis of proteins. Leucine displays a direct link to maintain glucose homeostasis by enhancing recycling of glucose through the glucose–alanine cycle, as shown in Fig. 2. The evidence demonstrates that elevated synthesis of alanine and glutamine requires increased degradation of the BCAA and should be related to a corresponding decrease in leucine (Harper et al. 1984). This enhances the ability of the liver to secrete glucose to regulate blood sugars as required (Layman 2003). During hypocalorism diet period, leucine supplementation serves to promote gluconeogenesis to maintain blood glucose level when dietary glucose is restricted but protein is plentiful. The metabolic route is that leucine or other BCAA donate their amino group to pyruvate, converting it to alanine, which then circulates to liver where it provides substrate for endogeneous glucose production. Leucine not only provides substrate, but also hinders the oxidation of pyruvate by inhibiting its rate limiting enzyme pyruvate dehydrogenase (Chang et al. 1978), and participates in the glucose–alanine cycle. Moreover, leucine significantly stimulates glucose transport under contraction condition in rat epitrochlear muscle, but inhibits insulin-stimulated glucose transport, and these effects are both abolished when rapamycin is employed (Iwanaka et al. 2010). It suggests that leucine regulates contraction- and insulin-stimulated glucose transport in skeletal muscle via mTOR/p70S6K signal pathway.

Chronic leucine treatment improves glycemic control in multiple mouse models of obesity and diabetes with different etiologies. Supplemented 1.5% leucine in drinking water for up to 8 months prevents the development of diabetes in RCS10 mice (Guo et al. 2010), a polygenic model of obesity and type 2 diabetes, and improves glucose-insulin homeostasis in yellow agouti (A^y) mice, a monogenic model for impaired central melanocortin receptor signaling, but independent of BW change. Meanwhile, the expression levels of uncoupling protein 3 (UCP3), carnitine acetyl-transferase, peroxisome proliferators-activated receptor alpha, and nuclear respiratory factor 1, which are demonstrated to regulate mitochondrial

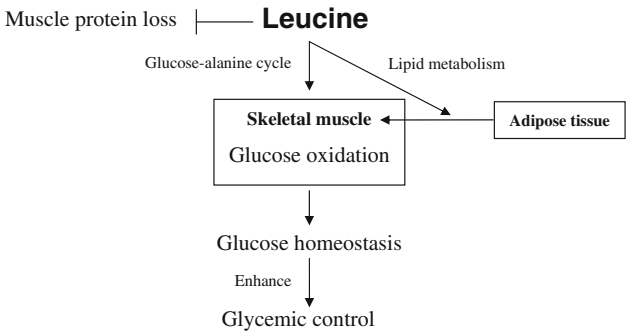

Fig. 2 Potential role of leucine in the regulation of glucose homeostasis. Leucine not only provides substrates for gluconeogenesis, but also regulates oxidative use of glucose by skeletal muscle through stimulating of glucose recycling via the glucose–alanine cycle. It plays critical roles in sparing muscle protein loss and enhancing glycemic control, and regulates adipocytes lipid metabolism to promote partition of lipid to skeletal muscle

oxidative function, are markedly elevated in the soleus muscle of these animals treated with leucine, whereas the expression levels of monocyte chemoattractant protein 1 and tumor necrosis factor alpha and macrophage infiltration in adipose tissue are significantly decreased. The metabolic usefulness of leucine supplementation may be modulated through multiple mechanisms in different target tissues and are likely independent of BW lose.

Leucine and energy metabolism

As we know, metabolic disorders are associated with mitochondrial loss or dysfunction (Kim and Wei 2008; Schrauwen-Hinderling et al. 2007). Leucine regulates adipocytes lipid metabolism to promote partition of lipid to skeletal muscle, and mitochondrial biogenesis is involved in mediating these effects. Leucine plays critical roles in both adipocyte and skeletal muscle energy metabolism (Sun and Zemel 2007). Therefore, lipid metabolism provides the energy substrate of leucine-stimulated skeletal muscle protein synthesis. Mitochondrial biogenesis is also related with skeletal muscle fatty acid oxidation. However, valine, another member of BCAA has no effect in this system, suggesting the effect is specific to leucine (Sun and Zemel 2009). Leucine enhances mitochondrial mass and associated regulatory gene expression in both myocytes and adipocytes, where leucine also stimulates oxygen consumption (Sun and Zemel 2009). It provides further evidence to support the effects of leucine in modulating energy utilization. One or more molecules generated by excess adipose tissue plays a key role in suppressing skeletal muscle fatty acid oxidation via suppressing mitochondrial biogenesis.

On another hand, leucine metabolism can supply TCA cycle with different anaplerotic substrates including α-

ketoisocaproate, which can be further metabolized to acetylcoenzyme A and acetoacetate (MacDonald et al. 2005). Hence, leucine can supply an elevated level of ATP as source of chemical energy. Additionally, β-cell exerts a high level of anaplerotic mitochondrial enzymes, demonstrating increase pyruvate carboxylase and GDH activity, indicates that this process is of particular importance for the insulin secretion metabolism (MacDonald et al. 2005, 2008). Leucine can induce insulin release linked to elevated mitochondrial energy production (Xu et al. 2001). The supplementation of leucine augments the response to glucose in islets of rats fed low protein diets (Filiputti et al. 2010). Reduced glucose oxidation suggests leucine may increase ATP level during insulin secretion (Filiputti et al. 2008, 2010). As we know, nutritional environment plays a critical role in controlling gene expression (Reeves et al. 1993; Jousse et al. 1999, 2000; Xu et al. 1999, 2000). Leucine activating mTOR pathway affects protein translation in β-cells, and the PI3K/mTOR/S6K1 signal pathway is more active in islets (Filiputti et al. 2010). However, blocking the mTOR signaling pathway by rapamycin impairs glucose-induced insulin secretion in the pancreatic β-cells (Fraenkel et al. 2008).

Leucine and body composition change

During weight loss stage, the energy-restricted diet often leads to significant amounts of lean mass loss. High protein diets are beneficial for increasing body fat loss and maintaining lean tissue (Parker et al. 2002; Layman et al. 2003). It links to the critical roles of leucine in sparing muscle protein loss and enhancing glycemic control (Layman 2003; Layman and Baum 2004; Halton et al. 2004; Zemel 2004; Layman et al. 2005). Leucine also displays an anabolic action in terms of protein metabolism and favorably affects body composition in catabolic situations just like arginine metabolism (Tan et al. 2009, 2010, 2011).

Protein intake and metabolism positively influences energy expenditure. Over a 4 h period, about 3.5 g of leucine is infused, resulting in reduction of 24 h nitrogen losses. Long term supplementation of leucine for 12 days to rats showed, except for gastrocnemius muscle, protein synthesis rate increases in adipose tissue and liver (Lynch et al. 2002a), and nitrogen retention is noted in carcasses of rats during the phase of nutritional recovery after protein malnutrition (Ventrucci et al. 2004). Long term of L-leucine administration on rats for 6 weeks in a catabolic situation caused by food restriction (50%) increases body fat loss (25%) and improves liver protein status (Donato et al. 2006). In addition, leucine with other BCAA administered to wrestlers for 19 days of caloric restriction shows reduction of body fat than caloric restriction conducted alone (Mourier et al. 1997). This suggests an effect of nutrient partitioning induced by an increased ingestion of leucine. Another explanation for the smaller amount of body fat in leucine treated subjects is related to the function of leucine above minimum levels in protein turnover (Layman 2003; Escobar et al. 2005). In other words, a higher protein turnover rate contributed to the energy expenditure increase and this additional energy requirement can be satisfied by the oxidation of body fat. Leucine supplementation (2%, as drinking water) in female rats during pregnancy decreases the expression of neuropeptides including NPY and AgRP, and shows a tendency of body composition: higher ratio of lean/fat mass content (López et al. 2010).

Leucine and obesity treatment

Dietary approaches are useful for the prevention and treatment of obesity and diabetes. mTOR signaling pathway plays a key role in the brain mechanisms that respond to nutrient availability and modulating energy balance. By activating mTOR and S6K1, leucine feedback inhibits insulin signaling and decrease glucose utilization in skeletal muscle (Patti et al. 1998; Tremblay and Marette 2001; Krebs et al. 2002; Um et al. 2004). For modulating mTOR signaling, leucine is more efficacious than other AA. Infusion of leucine into the third ventricle of the rat brain reduces the food intake and BW through activating the mTOR pathway in the arcuate nucleus of the hypothalamus (Cota et al. 2006).

Leucine supplementation markedly reduced diet-induced obesity when fed with high fat feed. Supplementation with leucine leads to 32% reduction of weight gain and 25% decrease in adiposity in mice fed high-fat diet. And the decrease of adiposity results from elevated resting energy expenditure linked to increased expression level of UCP3 in brown and white adipose tissues and skeletal muscle, but food intake is not influenced. Furthermore, leucine prevents high-fat diet induced hyperglycemia, which is related to improved insulin sensitivity, reduced plasma concentrations of glucagons and glucogenic AA, and down-regulation of hepatic glucose-6-phosphatase. In addition, plasma levels of total and LDL cholesterol are reduced by 27 and 53% respectively in the mice treated with high-fat diet plus leucine (Zhang et al. 2007). Leucine also decreases food intake and BW in normal rats and *ob/ob* mice. It is similar to that achieved by high protein diet, also indicating that leucine is a major component of the effects of a high protein diet. Moreover, leucine and high protein diet reduces AMP-activated protein kinase (AMPK) but promotes mTOR activity in the hypothalamus, resulting in inhibition of NPY and stimulation of pro-opiomelanocortin expression. It suggests that a corss-regulation between AMPK and mTOR exists to regulate feeding in a leucine dependent manner.

The effect of leucine on BW is also associated with a futile cycle. Leucine differs from other essential AA (including other BCAA) because leucine is primarily metabolized in peripheral tissues, such as muscle, but not by the liver. Because the liver expresses low levels of the mitochondrial branched-chain aminotransferase (BCATm), which is the first enzyme in the catabolism of BCAA in most peripheral tissues (Wahren et al. 1976). So the level of dietary BCAA were unchanged when reach in the blood for absence of branched-chain aminotransferase enzyme in liver. Deletion of BCATm blocks the first step in BCAA catabolism and increases circulating leucine and other BCAA. It leads to a futile and energy-consuming cycle of increased protein turnover rate. The circulating BCAA including leucine is increased over tenfold and the protein synthesis is elevated by about 40% in most tissues. In wild-type mice, leucine breakdown generates α-ketoisocaproate that is concerned to slow the rate of protein breakdown, which does not occur in the BCATm mull mice. These mice show increased food intake, leaner body due to increased energy expenditure and insulin sensitive and resistant to high-fat diet (She et al. 2007).

Furthermore, the relationship between obesity and AA metabolism displays important physiological and clinical implications. Patterson et al. (2002) studied the effect of obesity on regional skeletal muscle and adipose AA metabolism using a combination of stable isotope tracer and arteriovenous balance methods. The rate of leucine release from forearm and adipose tissues in obese women is lower than in lean subjects, and obesity is associated with a lower fractional contribution from skeletal muscle to systemic leucine rate of appearance. It helps to illustrate the reasons that obese persons are more effective than lean ones in preserving body protein during fasting.

However, previous research has been conducted mostly on small experimental animals, such as rat or mouse. Long-term leucine supplementation controlled diets, large human subjects and validation by repetition are required to examine the efficacy of leucine as a weight loss strategy for obese humans.

Leucine and intestinal function

Leucine may play crucial roles in intestinal growth, integrity and function (Rhoads and Wu 2009). Leucine is degraded extensively by the epithelial absorptive cell of the small intestine (enterocyte) in both suckling neonates and post-weaning animals (Wu 1998; Chen et al. 2007; Yin et al. 2010b). Leucine and arginine are the two most effective AA for stimulating the phosphorylation of p70 (S6K) and activating mTOR singaling pathway in intestinal cells (Ban et al. 2004; Wu et al. 2009). It is benefit for cytoskeletal remodeling necessary for coverage of the ulcerated surface (Liu et al. 2008). This area is needed for further study.

Summary and perspectives

Leucine acts as nutrient signals to regulate protein synthesis in skeletal muscle and adipose tissue, leptin release, and is involved in central nervous system control of food intake and energy balance (Hay and Sonenberg 2004; Cota et al. 2006; She et al. 2007). Understanding the important relationship among adipose tissue leucine, glucose, and lipid metabolism may provide new strategies for treating conditions related to dysregulated integrated fuel metabolism. Further studies are necessary to clearly define the beneficial effects of dietary leucine supplementation on healthy subjects.

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