

Amino Acids as Pharmaco-Nutrients for the Treatment of Type 2 Diabetes

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Abstract: Evidence is accumulating showing amino acids to play a key regulatory role in numerous metabolic processes. Amino acids, and leucine in particular, can be applied as potent insulin secretagogues. These stimulating properties are not restricted to healthy humans, but are also effective in long-term diagnosed type 2 diabetes patients. Co-ingestion of amino acid/protein with carbohydrate substantially augments endogenous insulin release, accelerates blood glucose disposal, and improves post-prandial glucose homeostasis. Besides their function as precursors for protein synthesis, some amino acids are also able to stimulate protein anabolism in an insulin-independent manner. Branched chain amino acids (BCAA), and leucine in particular, are capable of activating the mRNA translational machinery through the mammalian target of rapamycin (mTOR), which represents an interesting molecular target for the prevention or reduction of elevated muscle proteolysis in uncontrolled type 2 diabetes. Protein and/or specific amino acid supplementation could help to reduce muscle proteolysis and/or to stimulate protein synthesis, leading to an improved muscle protein balance, which augments whole-body blood glucose disposal capacity. Besides the potential benefits of protein and/or amino acid supplementation, there is evidence showing hyperaminoacidemia to impair skeletal muscle insulin signaling. Understanding the mechanisms by which different amino acids can alter metabolic signaling will be of great value for the development of effective nutritional and/or pharmacological interventions to prevent and/or treat insulin resistance and/or type 2 diabetes. Studies investigating the benefits of long-term amino acid and/or protein supplementation in type 2 diabetes patients are warranted.

Key Words: Leucine, insulin, insulin sensitivity, muscle, metabolism, protein, BCAA, muscle anabolism, insulin resistance.

INTRODUCTION

Besides their function as precursors for protein synthesis, evidence is accumulating showing amino acids to play a key regulatory role in numerous metabolic processes. Amino acids can act as potent hormone secretagogues, stimulating the secretion of insulin, glucagon, cortisol, insulin-like growth factor 1 and/or growth hormone. Furthermore, evidence points towards a key regulatory role for amino acids in gene transcription and translation. Hence, amino acids seem to have an important function in intracellular signaling and are able to modulate fat, carbohydrate and protein metabolism in various tissues. Understanding the mechanisms by which different amino acids alter metabolic signaling will be of great value for the development of effective nutritional and/or pharmacological interventions to prevent and/or treat chronic metabolic diseases like obesity, insulin resistance and/or type 2 diabetes. In this review we will touch upon the potential of amino acids, and leucine in particular, to augment endogenous insulin secretion, insulin sensitivity and/or skeletal muscle protein anabolism. These topics will be reviewed within the context of their application in the prevention and/or treatment of type 2 diabetes.

Amino acid induced endogenous insulin secretion has been proposed as an effective strategy to stimulate post-prandial glucose disposal and, as such, to improve blood

glucose homeostasis in insulin resistant and/or type 2 diabetes patients. Furthermore, besides their role as precursors for protein synthesis, and their insulinotropic characteristics, some amino acids also seem to be able to stimulate protein anabolism in an insulin-independent manner. Branched chain amino acids (BCAA), and leucine in particular, are capable of activating the mRNA translational machinery through the mammalian target of rapamycin (mTOR), which represents an interesting molecular target for the prevention or reduction of elevated muscle proteolysis in uncontrolled type 2 diabetes. Interventions that reduce muscle proteolysis and stimulate protein synthesis can effectively improve muscle protein balance, leading to an increase in skeletal muscle mass and strength, improved functional capacity and an increase in whole-body blood glucose disposal capacity. Apart from these potential benefits of protein and/or amino acid supplementation in the treatment of insulin resistance and/or type 2 diabetes, there is evidence showing that hyperaminoacidemia can also impair skeletal muscle insulin signaling. Therefore, based on the available literature, we will also discuss the potential concerns regarding amino acid supplementation. Finally, a short overview on current ideas, speculations and plans for future research on the application of amino acids as pharmaco-nutrients in the treatment of type 2 diabetes will be provided.

AMINO ACID INDUCED INSULIN SECRETION

In a state of impaired glucose tolerance or type 2 diabetes, insulin secretion shows several abnormalities [1]. Secretory defects include: reduced early insulin secretory re-

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sponse to glucose ingestion, reduced ability of the β -cell to compensate for the degree of insulin resistance, decreased glucose sensing ability of the β -cell and shifts to the right in the dose-response curves relating glucose and insulin secretion, which are indicative of a progressive insensitivity of the β -cell to glucose. These defects involve glucose-sensing and signaling pathways in the β -cell [1]. Even though insulin secretion in response to the prevailing glucose concentration may be blunted in type 2 diabetes patients, it has been speculated that insulin secretion in response to other stimuli remains functional [2].

Amino acids can act as potent stimuli for the secretion of insulin from the pancreatic β -cell [3]. *In vitro* studies using incubated primary islet cells or β -cell lines have described strong insulinotropic effects for arginine, leucine, isoleucine, alanine and phenylalanine [4-12]. The various mechanisms by which amino acids promote and/or enhance insulin secretion from the pancreatic β -cell are diverse and have not yet been fully elucidated (for a recent review on this topic [3]). In the presence of glucose, amino acids like arginine seem to be able to stimulate insulin secretion by the direct depolarization of the plasma membrane [5,13]. The latter results in the opening of voltage activated Ca^{2+} channels, resulting in

the influx of Ca^{2+} , which triggers insulin exocytosis [3,8]. Other amino acids tend to induce their insulinotropic properties by activating the Ca^{2+} channels, through their co-transport with Na^+ [14,15]. Furthermore, similar to glucose mediated insulin secretion [16], intracellular catabolism of the metabolizable amino acids will increase the intracellular ATP/ADP ratio, which closes ATP-sensitive K^+ channels, leading to the depolarization of the plasma membrane and the subsequent Ca^{2+} activated insulin exocytosis. [3,17,18]. In addition, leucine induced insulin secretion is mediated both through its oxidative decarboxylation, as well as its ability to allosterically activate glutamate dehydrogenase [3,11,19]. The latter has also been reported for other amino acids, like phenylalanine [20]. A simplified overview on some of the proposed mechanisms by which amino acids are likely to stimulate insulin secretion in the pancreatic β -cell is provided in Fig. (1).

In accordance with the *in vitro* data on incubated β -cells, *in vivo* studies in humans have shown increased plasma insulin concentrations following the intravenous infusion of amino acids in both healthy [21-23] and type 2 diabetic subjects [24]. In line with those findings, nutritional studies in humans already reported the synergistically stimulating ef-

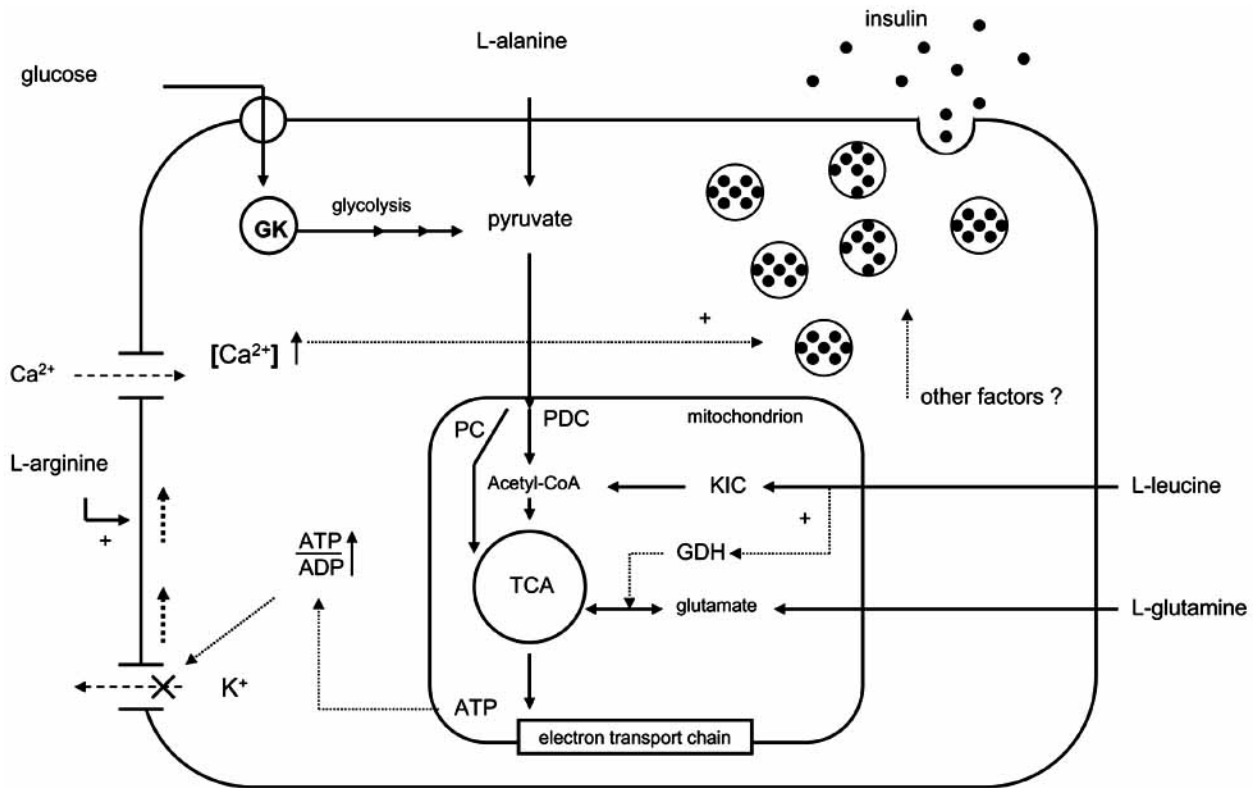


Fig. (1). A simplified overview on some of the proposed mechanisms by which amino acids stimulate insulin secretion in the pancreatic β -cell. Glucose entering the cell is phosphorylated by glucokinase (GK). Pyruvate is formed by glycolysis, after which it becomes a substrate for pyruvate dehydrogenase complex (PDC) or pyruvate carboxylase (PC) before entering the TCA cycle. Increased TCA cycle activity and oxidative phosphorylation will result in an increased ATP/ADP ratio, which will lead to the closing of ATP-sensitive K^+ channels. The latter will lead to the depolarization of the plasma membrane, thereby opening up voltage activated Ca^{2+} channels, resulting in Ca^{2+} activated insulin exocytosis. Arginine has been reported to be able to directly depolarize the plasma membrane. Metabolizable amino acids are catabolized to generate ATP and, as such, to increase intracellular ATP/ADP ratios and activate insulin exocytosis. Numerous interactions and co-factors are evident in the complex regulation of amino acid induced insulin secretion. For example, leucine-induced insulin secretion is mediated by its oxidative decarboxylation as well as by allosterically activating glutamate dehydrogenase (GDH). Figure adapted from Newsholme *et al.* [3].

fect of the combined ingestion of carbohydrate and protein on plasma insulin concentrations in the 1960s [25,26], which were later confirmed in both healthy [27] and type 2 diabetic subjects [28-30]. Over the last few years, such nutritional interventions to optimize post-prandial insulin secretion have regained much attention, as they were proven to represent a practical strategy to accelerate muscle glycogen synthesis [31,32] and/or to increase net muscle protein anabolism [33-35] during recovery from exhaustive exercise.

However, maximizing post-prandial endogenous insulin secretion by co-ingestion of insulinotropic free amino acids and/or protein (hydrolysate) mixtures could be of important clinical relevance in the treatment of type 2 diabetes. Elevating post-prandial endogenous insulin secretion may increase plasma glucose disposal and, as such, improve glucose homeostasis, thereby postponing the patients' dependency on exogenous insulin therapy and/or reducing the risk of developing micro- and macrovascular complications [36,37]. To obtain more background information on this matter, we first performed a series of studies to determine the *in vivo* insulinotropic potential of the ingestion of various free amino acids and protein (hydrolysates) in combination with carbohydrate [31,38,39]. In trying to define an optimal insulinotropic amino acid and/or protein (hydrolysate) mixture, when co-ingested with carbohydrate, we found a mixture containing a protein hydrolysate with the addition of free leucine and phenylalanine to be most potential [38,39]. More recent studies from our lab suggest that the addition of phenylalanine is either less important or that phenylalanine is already abundantly present in the protein hydrolysate, as the administration of a protein hydrolysate with only free leucine seems to augment the insulin response to a similar extent [40,41].

Though it was reported that the *in vivo* insulinotropic potential of the combined ingestion of carbohydrate with protein (hydrolysate) could be enhanced in healthy males by the addition of leucine and phenylalanine [38,39], its clinical relevance to type 2 diabetes was not yet established. During the early stages of type 2 diabetes, hyperglycemia is generally accompanied by a compensatory hyperinsulinaemia. During the progression of the disease this compensatory hyperinsulinaemia gradually disappears. As a consequence, it has been assumed that the absolute insulin secreting capacity of the pancreas is markedly reduced in long-term diagnosed type 2 diabetes patients. From that perspective it would be questionable to assume that nutritional or pharmacological stimuli can substantially augment endogenous insulin secretion in these patients. Therefore, we also investigated the insulinotropic response to the co-ingestion of the mixture containing a protein hydrolysate, leucine and phenylalanine in a population of longstanding diagnosed type 2 diabetes patients in which compensatory hyperinsulinemia was no longer present [2]. The latter showed a massive (+189%) increase in the insulin response when compared to the ingestion of carbohydrate only [2]. The latter implies that the insulin secreting capacity of the compromised β -cell remains functional when responding to stimuli other than glucose.

Various studies have shown that the greater increase in post-prandial insulin secretion following co-ingestion of protein (hydrolysate) and/or free amino acids is associated with lower post-prandial glucose concentrations in both

healthy, normoglycemic and type 2 diabetic subjects [27-30,42,43]. Hence, many studies speculate that increasing post-prandial endogenous insulin secretion represents a clinically relevant strategy to improve blood glucose homeostasis in type 2 diabetes. However, it remains to be established whether the improved blood glucose response is attributed to an increase in insulin mediated glucose uptake, or simply due to impaired gastric emptying as a consequence of protein co-ingestion [41]. The latter also represents an effective strategy in the development of clinical nutrition for diabetes patients in which slowly digestible carbohydrate sources are applied to reduce the glycemic response of a supplement meal. Recently, we determined whether the lower blood glucose response following amino acid/protein co-ingestion is attributed to a measurable increase in insulin stimulated blood glucose uptake [44]. Continuous infusions with labeled [6,6- $^2\text{H}_2$]glucose were applied to determine blood glucose appearance and disappearance rates following carbohydrate ingestion with or without addition of the insulinotropic protein, leucine and phenylalanine mixture in long-term diagnosed type 2 diabetes patients [44]. In accordance to the other studies, a substantially (+299%) greater insulin response was observed following amino acid/protein co-ingestion, accompanied by a 28% lower blood glucose response. The latter was attributed to a 13% greater increase in plasma glucose disposal ($P < 0.05$). Whereas it took the normoglycemic controls about 90 min to match their glucose rate of appearance with an appropriate disappearance rate, the type 2 diabetes patients needed about twice as much time to upregulate glucose uptake to the level of the rate of appearance of glucose from the gut. Protein/amino acid co-ingestion, and the subsequent increase in endogenous insulin release, accelerated this process [44]. From these findings, it is concluded that co-ingestion of an amino acid and/or protein mixture can augment post-prandial insulin release, accelerate glucose disposal, and reduce the blood glucose response in long-term diagnosed type 2 diabetes patients. The applicability of these findings for the treatment of type 2 diabetes needs to be addressed in future studies.

From both *in vivo* and *in vitro* work, it has become clear that leucine forms a particular interesting insulin secretagogue. As described, leucine both induces and enhances insulin secretion in the pancreatic β -cell. The latter tends to be in line with recent *in vivo* observations, which show co-ingestion of relatively small amounts of free leucine to further augment the insulin secreting response to the combined ingestion of carbohydrate and protein [40,41]. Leucine stimulates insulin secretion by its mitochondrial oxidative decarboxylation as well as by allosterically activating glutamate dehydrogenase in the pancreatic β -cell [11,19,45]; Fig. (2). Interestingly, Xu *et al.* [11] reported that both the generation of acetyl-CoA and α -ketoglutarate are needed for leucine to fully stimulate mitochondrial activity in the pancreatic β -cell. Though the subsequent downstream mechanisms that lead to insulin exocytosis remain to be established, they likely include ATP and/or other secondary signals. It has been proposed that the same signals are responsible for the leucine-induced activation of the mammalian target of rapamycin (mTOR) signaling pathway in the pancreatic β -cell (Fig. 2). The potency of leucine to activate protein translation through interacting with the mTOR signaling pathway may contribute to enhanced β -cell function through

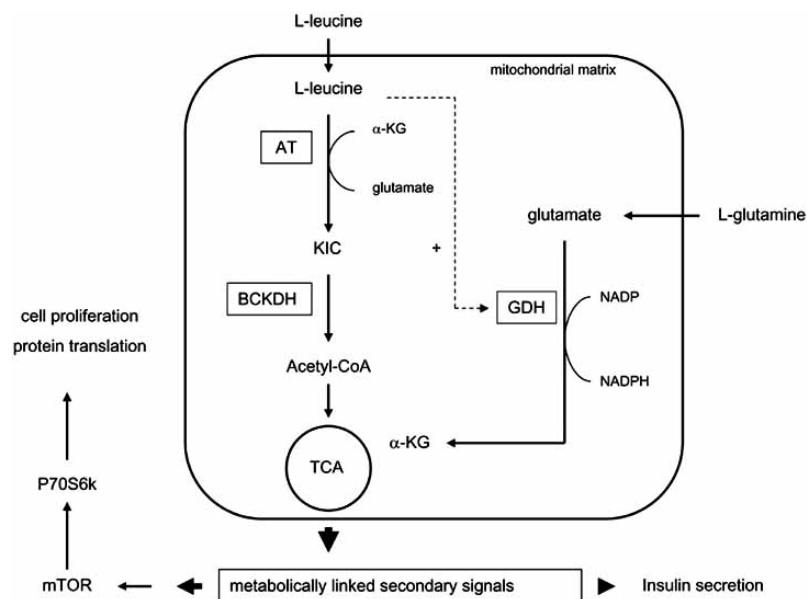


Fig. (2). A simplified overview on the mechanisms by which leucine stimulates insulin secretion in the pancreatic β -cell. Leucine-induced insulin secretion is mediated by its oxidative decarboxylation as well as by allosterically activating glutamate dehydrogenase. Both the generation of acetyl-CoA and α -ketoglutarate are needed for leucine to fully stimulate mitochondrial activity in the pancreatic β -cell. The metabolically linked secondary signals that subsequently lead to insulin exocytosis remain to be established, and seem also responsible for the leucine-induced activation of the mammalian target of rapamycin (mTOR) signaling pathway. AT: aminotransferase; α -KG: α -ketoglutarate; KIC: α -ketoisocaproate; BCKDH: branched-chain α -keto-acid dehydrogenase; GDH: glutamate dehydrogenase; TCA: tricarboxylic acid cycle; P70S6k: P70S6 kinase. Figure adapted from Xu *et al.* [11].

the maintenance of β -cell mass. Research is warranted to investigate the role of leucine in β -cell growth and proliferation and to assess the safety and efficacy of pharmacological and/or nutritional interventions with leucine administration. The capacity of leucine to activate the translational machinery through mTOR signaling has also been observed in other cell models [11,46-48], and represents an important molecular target for modulating muscle protein metabolism. The latter will be discussed in the next section.

In short, amino acid administration, and leucine in particular, effectively stimulate insulin secretion *in vivo* in humans. These stimulating properties are not restricted to the healthy, uncompromised pancreatic β -cell in healthy humans, but are also effective in long-term diagnosed, type 2 diabetes patients. Co-ingestion of an insulinotropic amino acid/protein hydrolysate mixture with carbohydrate, substantially augments insulin release, accelerates blood glucose disposal, and reduces the post-prandial rise in blood glucose levels in type 2 diabetes patients. Further increasing our knowledge on the mechanisms by which amino acids can modulate β -cell function will be of great importance to the discovery of novel drug and nutrient targets to improve glucose homeostasis in type 2 diabetes.

AMINO ACID INDUCED MUSCLE PROTEIN ANABOLISM

In addition to the progressive deterioration of pancreatic β -cell responsiveness to glucose ingestion, skeletal muscle protein breakdown rates are markedly elevated in uncon-

trolled type 2 diabetes [49]. The progressive decrease in (metabolically active) skeletal muscle mass in type 2 diabetes patients further reduces the capacity for glucose disposal and, as such, diminishes insulin sensitivity on a whole-body level. As such, the loss of skeletal muscle mass (due to aging, inactivity and/or an inadequate diet) represents an important contributing factor to the development of insulin resistance and/or type 2 diabetes, but can also be a direct consequence of the disease. The latter further accelerates the loss of skeletal muscle tissue, thereby contributing to the progression of the disease. Therefore, pharmacological and/or nutritional interventions in the elderly, and especially in elderly, type 2 diabetes patients, should aim to reduce muscle proteolysis and stimulate protein synthesis to effectively improve muscle protein balance, leading to a net increase in skeletal muscle mass, improved functional capacity and a greater whole-body glucose disposal capacity.

Administration of carbohydrate, free amino acids and/or protein, leading to hyperaminoacidemia and hyperinsulinaemia, has been shown to stimulate protein synthesis and to inhibit proteolysis [33,50-53]. Insulin has been proposed as an important regulatory factor in protein metabolism, as acute physiologic elevations of plasma insulin levels, especially during conditions of hyperaminoacidemia, result in an additional increase in net muscle protein anabolism *in vivo* in man [54-56]. Therefore, it could be speculated that the reduced insulin response following the ingestion of a carbohydrate-rich meal in type 2 diabetes patients could impair the post-prandial anabolic response. In accordance, Volpi *et al.*

[57] reported hyperaminoacidemia with endogenous hyperinsulinemia to result in an impaired muscle protein synthetic response in healthy elderly men, when compared to a young control group [57]. This could be attributed to the lower insulin response that was reported in the elderly subjects. In line with those observations, it could be speculated that nutritional interventions that combine the provision of ample amino acids with a relatively large insulinotropic response would be effective in promoting post-prandial net muscle protein anabolism in type 2 diabetes patients. Studies to assess the relative importance of the level of free amino acid availability and the accompanying circulating plasma insulin concentrations needed to maximize net protein balance are warranted to improve dietary guidelines and to define effective pharmacological and/or nutritional interventions to counteract the age-associated loss of skeletal muscle mass in elderly and/or type 2 diabetes patients.

Most *in vivo* human studies have investigated protein metabolism during resting conditions. However, daily physical activity can strongly modulate protein metabolism. Physical exercise stimulates muscle protein synthesis [58,59] as well as muscle protein breakdown [58,60], which in the absence of food intake will result in a negative muscle protein balance [58,60,61]. Post-exercise ingestion of carbohydrate and protein/amino acids will result in a potent inhibition of exercise-stimulated protein breakdown and stimulates protein synthesis, resulting in net muscle protein accretion [33,34,50,62]. Though physical activity and exercise training play a crucial role in the prevention and treatment of insulin resistance and/or type 2 diabetes, it would be beyond the scope of this review to elaborate on the health benefits of exercise. However, it should be noted that amino acids, as precursors for protein synthesis, are essential for the skeletal muscle adaptive response to occur. Moreover, Esmarck *et al.* [63] reported that the ingestion of protein immediately after cessation of resistance exercise, as opposed to 2 hours later, is crucial for the development of skeletal muscle hypertrophy in elderly men.

Even though insulin forms an important regulatory factor in muscle protein metabolism [54-56], insulin should not be regarded as a primary regulator. In the absence of elevated amino acid concentrations, insulin levels exert only a modest effect on muscle protein synthesis [52]. Besides their role as precursors for protein synthesis, some amino acids seem to be able to directly stimulate protein anabolism in an insulin-independent manner. Early *in vitro* studies in isolated rat hemidiaphragms by Buse and Reid [64] already showed the potential of leucine to reduce protein breakdown and to stimulate protein synthesis. Since then, numerous studies [65,66] have investigated the proposed anabolic responses to amino acid administration, with a special interest in the administration of BCAA (leucine, isoleucine and valine). In general, most *in vitro* and *in vivo* animal studies support the findings by Buse and Reid [64] and report that leucine inhibits muscle protein breakdown and stimulates muscle protein synthesis [65]. In accordance, Anthony *et al.* [67,68] have reported a direct, stimulating effect of leucine ingestion on muscle protein synthesis in rodents. In line with those data, the same group showed that leucine supplementation enhances protein synthesis in skeletal muscle from diabetic rats through insulin-independent mechanisms [69]. Though most *in vitro* and *in vivo* animal studies report that leucine

administration can inhibit protein breakdown and stimulate protein synthesis, most *in vivo* human studies report that leucine and/or BCAA administration reduces muscle protein breakdown, without stimulating muscle protein synthesis (reviewed by Matthews *et al.* [65]). The apparent discrepancy between observations in humans and rodent studies remains to be elucidated.

Recently, we determined post-exercise muscle protein synthesis and whole-body protein balance following the combined ingestion of carbohydrate with or without protein and/or free leucine in healthy young men [40]. Addition of free leucine resulted in a greater whole-body net protein balance compared to the other trials. Mixed muscle fractional synthetic rate (FSR), as determined by the incorporation rate of continuously infused L-[ring-¹³C₆]phenylalanine into protein in skeletal muscle biopsies, was significantly greater in the leucine trial compared with the trial in which only carbohydrate was ingested. Intermediate FSR values were observed in the carbohydrate and protein ingestion trial, which were not significantly different from the other trials [40]. It was concluded that co-ingestion of protein and leucine stimulates muscle protein synthesis and optimizes whole-body protein balance when compared with the intake of carbohydrate. The proposed surplus value of additional free leucine ingestion to inhibit protein breakdown and/or to stimulate protein synthesis, resulting in a more positive muscle protein balance, needs to be studied in the (type 2 diabetic) elderly population. More studies investigating the proposed potential of leucine and/or other amino acids to stimulate protein anabolism *in vivo* in humans are warranted.

Over the last few years, advances in the field have greatly increased our knowledge on the role of amino acids as nutritional signals in the regulation of numerous metabolic processes [70-73]. Much of this work has been published by Jefferson and Kimball [70-73], showing amino acids, like leucine, to regulate protein metabolism through modulation of the signal transduction pathways that control mRNA translation. Interestingly, these signaling pathways overlap with the classic pathways of insulin and insulin-like growth factor induced stimulation of protein metabolism. Various amino acids, of which leucine seems to be the most potent, have been reported to activate mTOR in an insulin-independent manner. This mechanism integrates the information on amino acid availability and insulin stimulation to modulate protein anabolism by controlling mRNA translation and transcription, leading to the increased synthesis of specific sets of proteins. A simplified overview of the amino acid induced activation of the mTOR signaling pathway is provided in Fig. (3). An excellent review on the mechanisms by which amino acids are likely to regulate gene expression has been published by Kimball and Jefferson [73]. Though much progress has been made in elucidating the role of amino acids as regulators of gene expression, there is still a great lack of knowledge regarding the pathways that determine how the synthesis of specific sets of proteins is regulated by nutritional and/or exercise stimuli. Insight in these mechanisms will provide us with new molecular targets to modulate the expression of specific sets of proteins in both health and disease. This area will provide exciting new information and will open up new possibilities for the prevention and treatment of obesity, insulin resistance and/or type 2 diabetes.

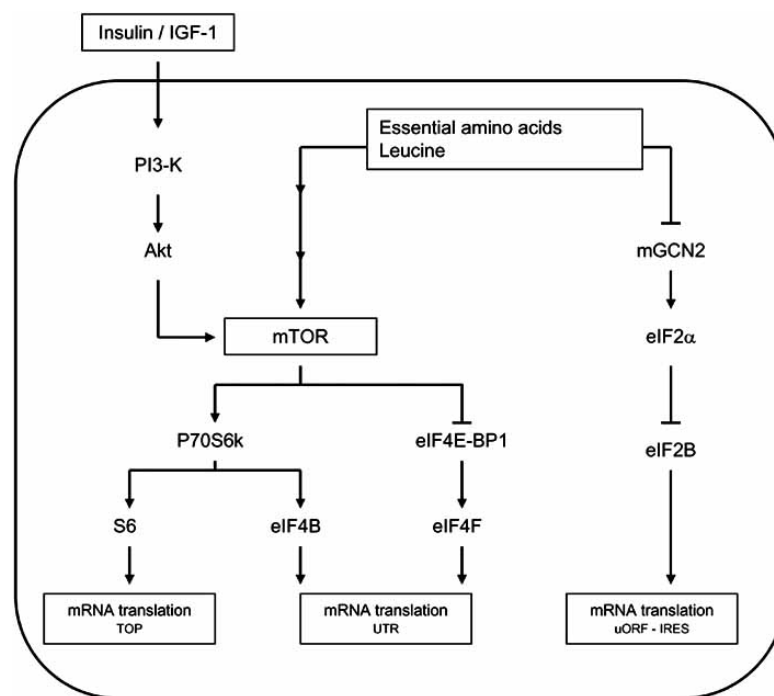


Fig. (3). An overview on the proposed mechanisms by which insulin, insulin-like growth factors and amino acids can regulate protein metabolism by modulating the signal transduction pathways that regulate mRNA translation. Besides the insulin mediated signal cascade that leads to the activation of the mammalian target of rapamycin (mTOR) signal transduction pathway, various amino acids (of which leucine seems to be the most potent) have been reported to activate mTOR in an insulin-independent manner. Activation of mTOR leads to an increase in the phosphorylation status of ribosomal protein S6 kinase (P70S6k) and the eukaryotic initiation factor 4E-binding protein (eIF4E-BP1). Activation of P70S6k leads to the phosphorylation of ribosomal protein S6 and the eukaryotic initiation factor 4B (eIF4B), which promote the preferential translation of those mRNAs containing terminal oligopyrimidine tracts adjacent to the 5' cap (TOP) and those with highly structured 5' untranslated regions (UTR), respectively. Phosphorylation of eIF4E-BP1 prevents binding with eIF4E, thereby enhancing the assembly of the eIF4F complex. In combination with eIF4B phosphorylation, enhanced eIF4F assembly leads to the translation of those mRNA transcripts containing UTRs. All essential amino acids have the potential to globally regulate mRNA translation through the phosphorylation of the eukaryotic initiation factor 2 (eIF2). Phosphorylation of the eIF2 -subunit is mediated by the mammalian ortholog of the yeast general control non-derepressing kinase 2 (mGCN2), which responds to changes in (essential) amino acid availability. Phosphorylation of eIF2 leads to the inhibition of the eukaryotic initiation factor 2B (eIF2B), thereby repressing the translation of most mRNAs, while enhancing the translation of those specific mRNAs containing multiple upstream open reading frames (uORF) and those transcripts with an internal ribosome entry site (IRES). Figure adapted from Kimball and Jefferson [73].

In short, nutritional and/or pharmacological interventions that stimulate endogenous insulin secretion, and provide an ample supply of amino acids combined with a relative large amount of free leucine likely form an optimal strategy to stimulate *in vivo* muscle protein anabolism, thereby preventing, or counteracting the gradual loss of skeletal muscle tissue mass in type 2 diabetes patients. The latter will augment the capacity for whole-body blood glucose disposal and, as such, improve blood glucose homeostasis.

AMINO ACID INDUCED INSULIN RESISTANCE?

Though leucine and/or amino acid co-ingestion has been shown to stimulate endogenous insulin secretion and to enhance the sensitivity of muscle protein synthesis to circulating insulin concentrations, there are also reports showing leucine or amino acid administration to impair insulin-stimulated glucose uptake [74]. Besides the described effects of leucine on the translational control of muscle protein metabolism, there are likely various other downstream effects following the activation of the mTOR pathway. *In vitro* studies have shown that prolonged incubation with leucine

inhibits insulin-stimulated glucose uptake in skeletal muscle tissue preparations [75]. The latter has been verified in human *in vivo* studies, showing amino acid infusion to reduce insulin stimulated glucose uptake in skeletal muscle [74,76-80]. Furthermore, amino acid infusion has also been shown to inhibit the ability of insulin to suppress hepatic glucose production under conditions of hyperinsulinemia [80]. As such, ample evidence has been collected to support the hypothesis that amino acids act as stimulatory signals for protein anabolism through mTOR signaling, while inhibiting insulin action on glucose metabolism [48]. These amino acid induced effects on glucose metabolism are likely attributed to the downstream effects of amino acid induced activation of the mTOR signaling pathway. It has been proposed that overactivation of mTOR/p70S6 kinase can lead to an increased phosphorylation of IRS-I on its serine residues, thereby inhibiting PI3-K and, as such, inducing insulin resistance [48,74,80-82]. A simplified overview of the proposed mTOR/P70S6 kinase - mediated negative feedback loop within the insulin signaling cascade is provided in Fig. (4) [81].

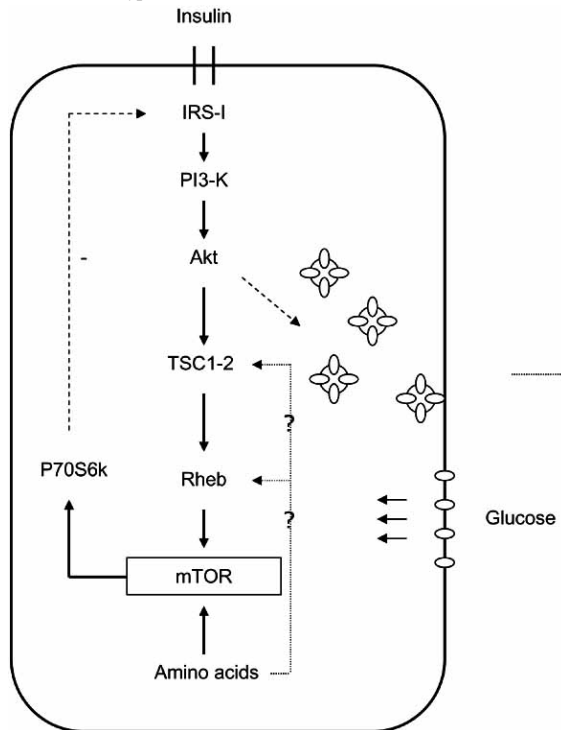


Fig. (4). An overview on the proposed mechanism by which amino acids might impair insulin signaling and reduce insulin stimulated glucose uptake through activating the mTOR signal transduction pathway. Insulin binds to its receptor, leading to tyrosine phosphorylation of the insulin receptor substrate 1 (IRS-I). This activates phospho-inositide 3-kinase (PI3-K). The latter will lead to the activation of Akt (protein kinase B), thereby promoting translocation of the glucose transporter 4 (GLUT-4) to the sarcolemma. A negative feedback inhibition has been proposed through the subsequent phosphorylation of the tuberous sclerosis 1 and 2 complex (TSC1-2), which through the Ras homolog enriched in brain (Rheb) can activate mTOR. As amino acids like leucine can activate mTOR (possibly acting through TSC1-2 or Rheb) this could likely represent the mechanism by which amino acids might interact with the insulin signaling cascade. It has been proposed that overactivation of mTOR/P70S6kinase can lead to an increased phosphorylation of IRS-I on its serine residues, thereby inhibiting PI3-K and inducing insulin resistance. Figure adapted from Tremblay *et al.* [81].

In the context of the application of defined insulinotropic and/or proposed anabolic amino acids as pharmaconutrients in the treatment of type 2 diabetes, it is important to consider these reports on amino acid induced insulin resistance. The global increase in diabetes prevalence [83] is strongly associated with our western lifestyle, in which overfeeding and obesity are instrumental to the development of insulin resistance, leading to type 2 diabetes. With the continuous intravenous infusion of fatty acids [84-86] or amino acids [76,78-80] as a model for overfeeding, it has become clear that there are various mechanisms by which prolonged nutrient oversupply can induce insulin resistance in skeletal muscle, liver and/or adipose tissue. Excess provision of any of the macronutrients (leading to hyperlipidemia, hyperglycemia and/or hyperaminoacidemia) seems to be able to induce insulin resistance. Interestingly, there are cross-sectional studies that have reported a high-protein intake to be associated with glucose intolerance or insulin resistance [87,88]. However,

the latter does not necessarily imply that a relative high protein intake induces insulin resistance. Numerous other co-factors associated with either a low or high relative protein intake (like for example total energy, dietary fat and/or carbohydrate intake, meat, fruit and vegetable consumption, smoking and/or physical activity status) could contribute to the observed correlations between insulin sensitivity and protein intake in the diet. Nonetheless, future studies investigating the potential health benefits of high-protein diets should be attentive to the described bidirectional modulation of insulin action by amino acids. However, it seems fair to assume that, within the scope of a well-balanced diet, merely the supplementation with protein and/or specific amino acids is unlikely to induce insulin resistance.

In line with these considerations, intervention studies in type 2 diabetes patients have actually reported an increase in whole-body insulin sensitivity and/or improved glucose homeostasis following amino acid supplementation [89,90] or by increasing the relative protein content of the diet [91,92]. Gannon *et al.* [92] investigated the effects of an increase in dietary protein intake from 15 to 30% of total energy intake, at the expense of carbohydrate ingestion, over a period of 5 weeks in 8 untreated, male type 2 diabetes patients. In contrast to the low-protein content diet (with a carbohydrate:protein:fat intake ratio of 55%:15%:30%), a significant decrease in blood glycohemoglobin content, a decrease in postprandial glucose concentrations and a modest increase in insulin concentrations were reported when subjects were provided with the high-protein, low-carbohydrate diet (carbohydrate:protein:fat intake ratio of 20%:30%:50%). Solerte *et al.* [90] assessed the metabolic consequences of the supplementation with 8 g of essential amino acids in elderly subjects with poorly controlled type 2 diabetes. Over a period of 16-weeks of amino acid supplementation, a significant reduction in fasting and post-prandial blood glucose concentrations and a lowered blood HbA1c content were observed. The latter was also accompanied by a reduction in fasting insulin levels and an improved whole-body insulin sensitivity, as evaluated by the HOMA index [93]. No adverse side effects were reported during and/or after supplementation. Though potential mechanisms to explain these findings were not studied, the authors speculated that amino acid induced effects on muscle protein anabolism were likely responsible for these observations [89,90]. These long-term intervention studies provide us with promising results on the application of protein and/or specific amino acids as a dietary strategy to improve blood glucose homeostasis in type 2 diabetes patients. However, more long-term intervention studies are warranted to assess the proposed benefits of such nutritional and/or pharmacological interventions.

It has been suggested that amino acid/protein supplementation could impose some concerns for the elderly, due to the potential adverse effects of an increased protein intake on renal function [94]. In addition, other concerns have been raised regarding the speculated stimulatory effect of amino acid or, more specifically the BCAA, supplementation on tumor growth [94]. Though amino acid infusion has been shown to accelerate fractional protein synthetic rates *in vivo* in both muscle and tumor tissue, increasing the proportion of BCAA in the administered amino acid mixture does not seem to further increase protein synthesis [65,95]. Due to the limited amount of information, we can only speculate on

potential safety limits for (free) amino acid supplementation. It seems that large dietary excess intake of an individual BCAA is well tolerated when consumed in a diet containing surfeit levels of protein and the other BCAA [96]. In agreement, based on the work to date, Matthews *et al.* conclude that leucine and the other BCAA can be safely consumed in large amounts relative to the other amino acids in protein [65].

In short, research is warranted to elucidate the mechanisms by which amino acids, and leucine and/or the BCAA in particular, can modulate insulin signaling by their interaction through the mTOR nutrient sensing pathway. The latter could likely lead to the discovery of multiple molecular targets for pharmaceutical and/or nutritional intervention in the prevention and/or treatment of type 2 diabetes. Prolonged intervention studies implementing protein, amino acid and/or leucine supplementation with the intention to improve blood glucose homeostasis either by stimulating insulin secretion and/or increasing muscle protein anabolism in type 2 diabetes patients should be attentive to the possible bidirectional modulation of insulin action by amino acids.

CURRENT IDEAS, SPECULATIONS AND PLANS FOR FUTURE RESEARCH

The previous paragraphs have shown various applications of specific amino acid and/or protein (hydrolysates) in the prevention and/or treatment of type 2 diabetes. The knowledge that some amino acids can have strong insulinotropic properties when co-ingested with carbohydrate has recently been extended with the observation that amino acid induced insulin secretion remains functional in long-term diagnosed type 2 diabetes patients. Moreover, co-ingestion of a protein hydrolysate, phenylalanine and leucine mixture with carbohydrate can substantially augment endogenous insulin secretion, accelerate glucose disposal and improve post-prandial blood glucose homeostasis. For now, these findings are restricted to a laboratory setting, in which standardized beverages with a relatively large carbohydrate content were administered. Therefore, these findings merely provide proof of principle, showing amino acid co-ingestion to represent a promising strategy to improve post-prandial glucose homeostasis in type 2 diabetes patients. However, these findings need to be translated and tested in a more applied setting to determine their practical and/or clinical relevance. Therefore, follow-up studies should focus on the properties of different amino acids and/or designer protein (hydrolysates) to augment insulin release when co-ingested in combination with a normal diet in both healthy and type 2 diabetes patients. Subsequently, studies should assess the impact of co-ingestion of such amino acid/protein mixtures with every main meal on 24 h blood glucose profiles. The latter will determine the relevance of investigating the benefits of more long-term nutritional interventions.

Of course, more mechanistic studies are warranted to elucidate the molecular mechanisms of amino acid induced insulin secretion and to provide more insight in the observed synergistic response of specific amino acids and glucose on insulin release from the pancreatic β -cell. In addition, it has been proposed that leucine-mediated activation of protein translation through the mTOR pathway likely contributes to enhanced pancreatic β -cell function by stimulating growth-

related protein synthesis and β -cell proliferation, which is associated with the maintenance of β -cell mass. Therefore, both *in vitro* and *in vivo* studies are warranted to assess the more long-term effects of leucine administration on pancreatic β -cell mass and function. Increasing our knowledge on the mechanisms by which amino acids can modulate β -cell function will be of importance to the discovery of novel drug targets to augment endogenous insulin secretion and, as such, to improve blood glucose homeostasis in type 2 diabetes.

The capacity of leucine to activate the translational machinery through mTOR signaling has also been observed in muscle cells. An increasing amount of *in vitro* and *in vivo* data show that leucine administration can activate the mTOR signaling pathway leading to an increase in muscle protein synthesis. However, human *in vivo* studies generally seem to report that leucine administration improves muscle protein balance by inhibiting protein breakdown, not by increasing muscle protein synthesis. At this stage, human *in vivo* studies are warranted to investigate the properties of leucine administration, under conditions of non-limiting amino acid availability, to increase muscle protein synthesis rates. As leucine co-ingestion induces a large insulinotropic response *in vivo*, it will be difficult to differentiate between the proposed insulin-dependent and insulin-independent pathways by which leucine is thought to stimulate muscle protein anabolism. Therefore, measurement of the activation/phosphorylation state of key factors within the mTOR signaling pathway are warranted to improve our understanding of the *in vivo* effects of leucine administration on skeletal muscle protein metabolism in humans.

As the combined ingestion of protein (hydrolysate) and leucine with carbohydrate stimulates endogenous insulin secretion, and provides an ample supply of amino acids with a relative large amount of free leucine, this would theoretically form an optimal strategy to stimulate muscle protein anabolism *in vivo*. The latter would be of clinical relevance as a means to prevent, or counteract, the gradual loss of skeletal muscle tissue mass in type 2 diabetes patients. The latter will augment the capacity for whole-body blood glucose disposal and improve blood glucose homeostasis. Combined exercise and nutritional interventions have been shown to improve skeletal muscle mass and function. Future research should focus on maximizing the benefits of exercise interventions through nutritional and/or pharmacological support. The latter seems to be essential in the elderly population in which the benefits of exercise training seem evident only when protein or amino acids are administered immediately after each bout of exercise. Clearly, both content as well as the timing of such nutritional interventions are likely to be essential. Long-term intervention studies are needed to define proper guidelines for more effective lifestyle intervention programs for the elderly and elderly, type 2 diabetes patients.

Though relatively little work has been done in this field, previous studies on amino acid and/or protein supplementation in type 2 diabetes patients have provided promising results. Increasing the protein content of the diet or supplementation with an amino acid mixture have been shown to improve markers of blood glucose homeostasis in type 2 diabetes patients. Long-term intervention studies are war-

ranted to investigate these proposed benefits and possible side-effects of specific amino acid and/or protein supplementation. The outcome of these future studies will determine whether amino acids can be effectively and safely applied as pharmaco-nutrients in the prevention and/or treatment of type 2 diabetes.

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