Role of Dietary Proteins and Amino Acids in the Pathogenesis of Insulin Resistance

Frédéric Tremblay,[1] Charles Lavigne,[2] Hélène Jacques,[2,3] and André Marette[1,2]

[1]Department of Anatomy & Physiology and Lipid Research Unit, Laval University Hospital Research Center, Québec, Canada, [2]Institute of Nutraceuticals and Functional Foods, and [3]Department of Food Science and Nutrition, Laval University, Québec, Canada; email: andre.marette@crchul.ulaval.ca

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Abstract

Dietary proteins and amino acids are important modulators of glucose metabolism and insulin sensitivity. Although high intake of dietary proteins has positive effects on energy homeostasis by inducing satiety and possibly increasing energy expenditure, it has detrimental effects on glucose homeostasis by promoting insulin resistance and increasing gluconeogenesis. Varying the quality rather than the quantity of proteins has been shown to modulate insulin resistance induced by Western diets and has revealed that proteins derived from fish might have the most desirable effects on insulin sensitivity. In vitro and in vivo data also support an important role of amino acids in glucose homeostasis through modulation of insulin action on muscle glucose transport and hepatic glucose production, secretion of insulin and glucagon, as well as gene and protein expression in various tissues. Moreover, amino acid signaling is integrated by mammalian target of rapamycin, a nutrient sensor that operates a negative feedback loop toward insulin receptor substrate 1 signaling, promoting insulin resistance for glucose metabolism. This integration suggests that modulating dietary proteins and the flux of circulating amino acids generated by their consumption and digestion might underlie powerful new approaches to treat various metabolic diseases such as obesity and diabetes.

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INTRODUCTION

Dietary intake of proteins is essential for normal growth and development. In Western societies, protein consumption has increased considerably during the past 50 years and is now thought to exceed by ~80% the recommended dietary intake (43). Although our understanding of fat and carbohydrates as nutrients affecting glucose and energy metabolism has greatly increased in the past two decades, the roles of proteins and amino acids in glucose homeostasis and insulin resistance and the mechanisms behind their effects are still poorly characterized. In this review, we explore the mechanisms by which dietary proteins and amino acids modulate glucose and energy homeostasis in relationship to the pathogenesis of insulin resistance.

DIETARY PROTEINS AND INSULIN RESISTANCE

The popularity of high-protein diets (e.g., Atkins and Montignac) has created a renewed interest in dietary interventions as effective and relatively easy means to achieve significant and sustained body weight loss. Furthermore, it is now well recognized that not only glucose and lipid but also protein metabolism are altered in diabetic states (98). However, the long-term safety and efficacy of high-protein diets are not well documented. This underscores the need for rational and tailored approaches in the clinical management of body weight, as well as insulin resistance and glycemic control in obese prediabetic or diabetic individuals, that involve modulation of both the quantity and quality of proteins from the diet.

High-Protein Diets: Their Effects on Energy Balance

Numerous studies have shown that an increased consumption of dietary proteins results in greater body weight loss (reviewed in 52, 66, 83). What is unclear at this point is whether high-protein diets reduce body weight by reducing energy intake through satiety signal(s) or by increasing energy expenditure. On one hand, some studies support the concept that the consumption of a high-protein diet decreases circulating ghrelin, an orexigenic gut peptide, whereas it increases the concentrations of the anorexic peptides cholecystokinin and glucagon-like peptide 1 (16, 21, 142). On the other hand, however, other studies suggest that the satiety induced by high-protein diets is unrelated to changes in circulating ghrelin (102, 155). Genetic evidence also supports a role for the anorexic peptide YY (Pyy) in protein-mediated reduction in food intake. Indeed, Pyy-null mice are hyperphagic and display obesity in comparison with their wild-type littermates and are insensitive to the satiating effect of dietary proteins (9).

High-protein diets may also promote a negative energy balance by increasing energy expenditure. This has been attributed to the heightened thermal effect of dietary proteins (23%–30%) as compared with that of

carbohydrates (5%–10%) and lipids (2%–3%) (106). Dietary proteins may also increase energy expenditure through up-regulation of uncoupling proteins (UCPs) and facultative thermogenesis. Indeed, high-protein intake was recently found to increase UCP-2 in the liver and UCP-1 in brown adipose tissue (115). Changes in UCP-1 and UCP-2 expression were inversely correlated with feeding efficiency and positively correlated with energy expenditure and oxygen consumption (115).

High-Protein Diets: Their Effects on Insulin Resistance and Glycemic Control

Varying the amount of protein not only affects body weight maintenance and feeding behavior, but also insulin secretion and action, thereby regulating glucose homeostasis. Short-term studies in normal or diabetic humans showed that dietary proteins stimulate insulin secretion (2, 46, 114, 122, 138) and reduce glycemia (75, 138). Importantly, consumption of a high-protein diet for six months in healthy nonobese individuals induced a higher glucose-stimulated insulin secretion, increased fasting glucose level, impaired suppression of hepatic glucose output by insulin, and enhanced gluconeogenesis (92). Furthermore, a one-year study in type 1 diabetic patients revealed that consumption of a high-protein diet decreased overall insulin sensitivity while it increased glucose production by the liver (91). Feeding rats with an increasing amount of protein at the expense of carbohydrates was also shown to increase fasting glycemia and the basal rate of endogenous glucose production (124). Furthermore, the assessment of insulin action in these animals, using the euglycemic-hyperinsulinemic clamp technique, revealed that a higher protein intake promotes a state of insulin resistance in peripheral tissues (124). Another study showed that rats fed a high-protein diet for 12 months had lower glycemia but higher insulin levels (136). Finally, it has been proposed that an accelerated pancreas "fatigue" or "failure" due to β-cell apoptosis might be responsible for the higher incidence of diabetes in prediabetic animals fed a high-protein diet (93, 126).

Dietary Proteins Sources and Insulin Resistance

Although high-protein diets might have beneficial effects on body weight and energy homeostasis, their potential long-term consequences on glycemic control, insulin resistance, and renal function limit their appeal for improving energy balance. Varying the source rather than the amount of proteins might represent a safer approach to the treatment of metabolic disorders.

Soy protein. Soy protein is well known for its hypolipidemic and hypocholesterolemic effects in animals and in humans (3). In addition to its effect on serum lipids, soy protein intake can also positively affect glucose homeostasis. For instance, consumption of soy protein, in comparison with casein, reduces fasting insulin and glucose levels in animals (15, 139). Similarly, human studies revealed that ingestion of a soy protein–based meal induces a lower insulin response than that induced following a casein-based meal (67, 125). However, the differential hormonal response following consumption of casein or soy proteins was not observed in another study (20). Incorporation of dietary soy protein was also shown to improve glucose tolerance and insulin sensitivity in rats fed a high-sucrose diet when compared with those fed with casein (87). Although the exact mechanism behind the beneficial effect of soy proteins on glycemic control is not known, it was proposed that a decrease in serum glucagon levels might be involved (87) since glucagon is known to promote hepatic glucose production. Soy protein may also improve insulin sensitivity by increasing insulin signaling in fat and liver since insulin receptor mRNA expression was reported to be increased in the liver and adipose tissues of soy protein–fed rats (72).

Fish protein. It has been known for years that populations from Alaska and Greenland that consume large amounts of fish have a low incidence of type 2 diabetes (39, 84, 104, 105). The beneficial effect of fish consumption was first attributed to fish oil. Although a meta-analysis revealed that intake of as much as 3 g of fish oil per day has no beneficial effect on glycemia in type 2 diabetes (44), consumption of lean fish (24 g/day; providing only ~140 mg of ω-3 fatty acid) was inversely correlated with the incidence of insulin resistance and type 2 diabetes (38). This latter finding was consistent with the hypothesis that a constituent other than lipid was responsible for the beneficial effect of fish consumption on glucose metabolism. Because protein is the most abundant nutrient in lean fish, it was proposed that fish protein could actually be responsible for the beneficial metabolic effects of lean fish consumption. This hypothesis was first tested in rabbits fed purified diets containing as the sole source of protein either casein, soy, or cod protein (12–14). The results showed that consumption of fish protein improved cholesterol transport via high-density lipoprotein (HDL) while reducing triglyceride-rich very-low-density lipoprotein (12–14). Human studies also demonstrated beneficial effects of cod protein consumption on plasma lipid profile by reducing plasma triglycerides in women and increasing HDL_2 cholesterol in men (47, 73, 86, 118).

In addition to their effect on plasma lipids, proteins derived from fish were also shown to affect glucose metabolism and insulin sensitivity. For instance, cod protein–fed rats in comparison to casein-fed animals are protected against the development of insulin resistance and glucose intolerance induced by diabetogenic diets rich in sucrose (87) or in saturated fat (88). These studies further revealed that soy protein can prevent insulin resistance induced by a diet rich in sucrose (87), but it had no beneficial effect on insulin sensitivity when the animals were fed a high-fat diet (88). Prevention of diet-induced insulin resistance by cod protein was related to enhanced insulin-stimulated glucose uptake by skeletal muscles but not by adipose tissues (88). Investigation of the mechanisms behind the insulin sensitization of skeletal muscles revealed that cod protein restored the activation of the phosphoinositide (PI) 3-kinase/Akt pathway and selectively improved GLUT4 translocation to the T-tubules (148), a cell-surface domain thought to mediate the bulk of glucose transport in response to insulin (145). The beneficial effect of cod protein could be recapitulated by incubating cultured skeletal muscle cells with a mixture of amino acids as found in the plasma of rats fed with the fish proteins, indicating that cod protein–derived amino acids can affect muscle insulin sensitivity in a cell-autonomous fashion (88). Importantly, human studies are compatible with the proposition that cod protein exerts beneficial effects on glycemic control. Indeed, it has been reported that postmeal plasma insulin concentrations increased significantly less in six healthy males when they were consuming a cod fillet meal compared with a beefsteak meal (137), suggesting improved insulin sensitivity in the former group. Moreover, a recent study reported that during a test meal in humans (119), cod protein induced a lower insulin/glucose ratio at 120 min and a lower area under the insulin curve as compared with milk protein. The results from the two studies cited above suggest there are decreased insulin secretion and/or higher insulin clearance and sensitivity with lower insulin/glucose ratio when humans are consuming cod protein as compared with other animal sources.

AMINO ACIDS AND INSULIN RESISTANCE

In pioneering studies published several decades ago, Felig et al. (36, 37) were the first to observe that amino acid levels are elevated in the plasma of obese individuals. These findings raised the possibility that amino acids may be involved in the development of obesity-linked and/or diet-induced insulin resistance. Elevated amino acid concentrations

have also been detected in the skeletal muscle of diet-induced obese animals, but not in the same tissue of genetically induced obese *fa/fa* rats (61), a finding that suggests that dietary modulation of insulin action in skeletal muscle might directly implicate amino acids. Indeed, the role of amino acids on multiple aspects of glucose metabolism and insulin action has become well recognized. (The various effects of amino acids are schematically represented in **Figure 1**; see text below for details and explanations.)

Regulation of Insulin Action and Glucose Metabolism

Studies performed in healthy humans have shown that short-term elevations of plasma amino acids lead to a decreased whole-body glucose disposal under euglycemic and hyperinsulinemic conditions (40, 82, 118, 143). This insulin-resistant state induced by amino acids has been primarily attributed to a reduced peripheral glucose uptake (40, 82, 118). In addition, infusion of the branched-chain amino acid leucine alone was found to impair glucose uptake in humans despite inducing a significant rise in insulin levels (1). The regulatory role of amino acids on skeletal muscle insulin sensitivity in vivo appears to involve a direct action of amino acids on muscle cells. Indeed, the rise of basal and insulin-stimulated glucose transport in ex vivo muscle preparations after prolonged incubation could be prevented by amino acids (50). Furthermore, amino acids acutely inhibit insulin-stimulated glucose transport in muscle cells in vitro (149). The negative effect of amino acids on insulin action in muscle was shown to be associated with inhibitory phosphorylation of insulin receptor substrate-1 (IRS-1) on serine and/or threonine residues and impaired activation of PI 3-kinase (149), a key effector of insulin's metabolic actions that signals to the downstream effectors Akt and atypical protein kinase C ζ/λ (PKC-ζ/λ) (145 and **Figure 2**). The negative modulatory effects of amino acids on insulin action have also been demonstrated in cultured hepatocytes (111) and adipocytes (140), and this was also shown to involve dysregulated IRS-1-mediated signaling. Impaired muscle insulin signaling as a consequence of increased amino acid sufficiency was reported in animal and human studies. For instance, oral administration of leucine was found to reduce the duration of PI 3-kinase activation by insulin in rat skeletal muscle (10). Furthermore, a cross-over study in which human subjects were studied twice showed that infusion of amino acids under physiological hyperinsulinemia markedly increased the phosphorylation of IRS-1 on Ser312 and Ser636/639 and completely blunted the activation of PI 3-kinase associated with IRS-1 in skeletal muscle while leaving intact the stimulation of Akt phosphorylation (147).

Another mechanism by which amino acids can negatively affect insulin-stimulated glucose transport was revealed by Traxinger & Marshall (144), who showed that incubation of adipocytes with amino acids under hyperglycemic and hyperinsulinemic conditions promotes a state of insulin resistance. They further found that among all amino acids, glutamine was the most potent at reducing glucose transport through its ability to activate the hexosamine pathway (99). In agreement with a potential role of hexosamine build-up in amino acid–mediated insulin resistance, direct activation of this pathway by glucosamine infusion in animals also induces peripheral insulin resistance and defective postreceptor insulin signaling in muscle (59, 112).

Although amino acids are generally believed to interfere with insulin's ability to increase peripheral glucose uptake, most notably in skeletal muscle, some studies suggest that amino acids might actually be beneficial for one aspect of glucose metabolism, i.e., glycogen synthesis. Indeed, amino acids stimulate glycogen synthesis in human skeletal muscle cells in vitro via phosphorylation and inactivation of GSK-3 and activation of glycogen synthase (5, 116). However, amino acid infusion in humans did not result in the

phosphorylation of GSK-3 or activation of glycogen synthase (94). Interestingly, it was recently found that under conditions in which Akt is inhibited, S6K1 can phosphorylate and inactivate GSK-3 (161). The branched-chain amino acids leucine and isoleucine were found to improve glucose tolerance and stimulate glucose uptake in skeletal muscle by mechanisms that might involve enhanced glycogen synthase activity and a greater translocation of glucose transporters GLUT1 and GLUT4 at the plasma membrane (33, 34, 108). This reported effect of leucine (108), however, is at odds with other studies showing a negative effect of leucine on insulin action and muscle glucose uptake (1, 10). In line with a positive role of amino acids in glucose metabolism is the observation that insulin-stimulated GLUT4 translocation in 3T3-L1 adipocytes is positively modulated by the addition of amino acids to the incubation medium (18). Finally, amino acids were also shown to slightly enhance insulin-stimulated Akt phosphorylation in the presence of the PI 3-kinase inhibitor, wortmannin in freshly isolated adipocyte, and in untreated adipose tissue of obese *db/db* mice (62, 63). This latter effect of amino acids is still ill defined but was proposed to be mediated by PDK-1 independently from PI 3-kinase and required the presence of glucose in the incubation medium (63).

Amino acids also play a role in the modulation of hepatic glucose production through direct and indirect mechanisms (35, 80, 81). By acting as substrates (35), amino acids can contribute to gluconeogenesis, endogenous glucose production, and the development of hyperglycemia in humans under conditions where insulin secretion is inhibited by somatostatin (81). Amino acids, through their ability to stimulate glucagon and insulin secretion (41, 110) infused in the absence of somatostatin can directly and indirectly affect gluconeogenesis, in which case glycemia might not be affected because of peripheral glucose uptake (81). Infusion of amino acids can also interfere with the ability of insulin to repress endogenous glucose production when hyperinsulinemia is kept constant (17, 40, 137, 147), although this observation remains controversial (82). In fact, it appears that low peripheral, fasting-like insulinemia is not sufficient to decrease amino acid–induced endogenous glucose production (147), whereas prandial-like hyperinsulinemia is generally sufficient to completely blunt hepatic glucose output (82, 147). Importantly, it has been recently shown that the contribution of gluconeogenesis to hepatic glucose output in obese nondiabetic subjects was associated with their increased postprandial protein catabolism (28). This latter study suggests that an increased flux of amino acids in obesity may be involved in the increased gluconeogenesis observed during the development of insulin resistance with respect to protein metabolism (28).

Amino Acids, Nutrient Sensing, and Insulin Resistance

The notion that amino acids not only serve as metabolic substrates but also are involved in activating and/or modulating signaling pathways has refined our understanding of how proteins from the diet can affect insulin action at the cellular and molecular levels. Early evidence linking amino acids to activation of intracellular signals came from in vitro studies showing amino acid–dependent phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4E-BP1) (54, 111, 153, 158), two effectors of mTOR involved in mRNA translation (60). These effects were found to be fully reversible upon amino acid withdrawal or by exposure to the specific mTOR inhibitor rapamycin (54, 111, 153, 158). These studies were later extended to animals and humans (24, 78, 128, 147). It has been suggested that amino acids activate the mTOR pathway through activation of class III phosphatidylinositol 3-kinase, hVps34 (25, 109). Interestingly, amino acids and insulin act in concert to promote phosphorylation of mTOR

effectors (54, 111, 149). Insulin stimulates the mTOR pathway in a PI 3-kinase/Akt-dependent manner, which leads to phosphorylation and destabilization of the tuberous sclerosis 1 and 2 (TSC1/2) complex and enables Rheb-mediated activation of mTOR (reviewed in 60, 90, 133; schematically represented in **Figure 2**). Alternatively, Akt was reported to directly phosphorylate mTOR in vitro (107, 127); however, recent data suggest that S6K1 is responsible for this phosphorylation in cells (29, 64).

Temporal analysis of amino acid–mediated inhibition of glucose transport and activation of the mTOR pathway revealed similar kinetics suggesting that these events might be linked together (149). This idea was first tested in skeletal muscle cells in vitro, where it was observed that amino acid–induced inhibition of insulin-stimulated glucose uptake was completely reversed by the mTOR inhibitor rapamycin (149). It was further revealed that activation of the mTOR pathway by both insulin and amino acids causes insulin resistance by inhibiting PI 3-kinase due to increased serine/threonine phosphorylation of IRS-1 (149), a process known to cause inhibition of downstream signaling (163). Administration of the branched-chain amino acid leucine, a potent activator of mTOR, was shown to decrease the duration of insulin-induced IRS-1-associated PI 3-kinase in rat skeletal muscle (10), whereas mTOR blockade by rapamycin injection had the opposite effect in the mouse (49). This negative feedback loop mediated by the mTOR pathway during amino acid sufficiency (mimicked by leucine alone) was also found to be operative in murine and human adipocytes (58, 140, 147) and in hepatocytes (76).

Increased amino acid availability leads to a rapamycin-sensitive phosphorylation of IRS-1 on Ser307 and Ser636/639 in cultured adipose and muscle cells (27, 146, 147). In addition, overactivation of S6K1 was detected in skeletal muscle biopsies obtained from humans infused with amino acids, and this was associated with increased phosphorylation of IRS-1 on Ser307 and Ser636/639 and impaired PI 3-kinase activity (147). Genetic evidence also supports the role of mTOR and/or S6K1 in mediating inhibitory phosphorylation of IRS-1 on serine and the development of insulin resistance. Deletion of TSC1/2 in mouse embryonic fibroblasts was found to dramatically increase the activation of mTOR/S6K1, causing inhibition of insulin signaling at the level of IRS-1/PI3K/Akt (56, 131). In addition, S6K was found to directly phosphorylate IRS-1 at Ser302, an effect that was proposed to reduce the association of IRS-1 with the insulin receptor (56). The physiological importance of the mTOR/S6K1 pathway in insulin resistance was further highlighted by the findings that its activation was enhanced in obese animals (76, 152) and that S6K1 deficiency in mice protects against diet-induced insulin resistance by preventing, at least in part, phosphorylation of IRS-1 on serine residues and inhibition of Akt phosphorylation (152).

The activation of mTOR by amino acids may also modulate insulin action independently from the negative feedback mechanism toward IRS-1. For instance, modulation of hypothalamic mTOR by leucine was shown to reduce food intake in rats (31). Interestingly, leptin also increased the phosphorylation of S6K1 (31), which suggests that the anorexic effect of leptin might be mediated via the mTOR pathway. Alternatively, the inhibitory effect of leptin on AMPK in the hypothalamus (4, 101) might mediate the activation of S6K1 because AMPK is a negative regulator of mTOR (19, 71, 132). If leptin is able to control the activation of the mTOR pathway in the brain, amino acids can stimulate leptin secretion from white adipocytes by activating the mTOR pathway (96, 123). It should be noted that central sensing of amino acids also occurred in the brain's anterior piriform cortex, where deficiency of essential amino acids affects food selection and feeding behavior (53, 79) in mice by a mechanism that involves the phosphorylation of eukaryotic initiation factor 2-α (eIF2α) by the eIF2α

kinase general control nonderepressible 2 (48, 100).

The role of amino acids as regulators of gene expression and protein synthesis is well documented (reviewed in 74, 78). However, it is unclear how induction of certain genes upon amino acid starvation or increased mRNA translation following amino acid exposure can modulate glucose metabolism and insulin resistance. Of particular interest is the observation that C/EBP homologous protein (CHOP) gene expression is induced in response to amino acid starvation, a process involving the transcription factors ATF2 and ATF4 (6, 23). Indeed, ATF2 and ATF4 can bind to the amino acid response element located in the CHOP promoter (22). CHOP expression has been shown to be downregulated during adipogenesis while ectopic expression of CHOP prevents the adipocytic conversion of 3T3-L1 cells by interfering with C/EBPα/β expression and function (8, 141). Signaling through the mTOR pathway also plays a crucial role in adipogenesis. Indeed, rapamycin treatment blocks clonal expansion and terminal differentiation of 3T3-L1 and human preadipocytes (11, 45, 159). In addition, mice lacking the mTOR effector 4E-BP1 have reduced fat pad mass, which suggests an important role for translational events in adipose tissue development (150). Furthermore, amino acid–induced activation of mTOR was reported to be important for peroxisome proliferator-activated receptor γ (PPARγ) in 3T3-L1 adipocytes; amino acid withdrawal or rapamycin decreased, whereas adding back amino acids restored PPARγ transcriptional activity (77). Interestingly, the thiazolidinedione troglitazone was shown to reverse the effect of rapamycin or amino acid starvation on PPARγ activity (77). mTOR has also been implicated in the phosphorylation of lipin induced by insulin and amino acids in rat adipocytes (68). Mutation of the lipin gene has been shown to cause lipodystrophy in fatty liver dystrophy mice (113), which suggests an important role of lipin in adipogenesis. Indeed, lipin deficiency in mouse embryonic fibroblasts prevents lipid accumulation and induction of PPARγ and C/EBPα (117). Amino acids were also found to participate in the three-dimensional organization of rat adipocytes into clusters in vitro (42). These data suggest another set of mechanisms by which amino acids might modulate insulin sensitivity in vivo through adipose tissue remodeling, brought about at least in part by the modulation of key adipogenic transcription factors.

Amino Acid Sensing via mTOR: Linking Diabetes and Cancer

Much evidence points toward mTOR as an important checkpoint hub involved in diabetes and cancer (reviewed in 97, 121, 133, 151, 157). Multiple signaling branches converge at the level of mTOR, including LKB1/AMPK, TSC1/2, and PI3K/PTEN/Akt (see **Figure 3**). The LKB1/AMPK pathway senses the energy status of the cells and is activated upon elevation of the AMP/ATP ratio (55). Glucose starvation, exercise, and the widely prescribed antidiabetic drug metformin activate LKB1/AMPK, which in turn shuts down ATP-consuming processes such as protein synthesis by inhibiting mTOR activity (71, 132, 134). The LKB1/AMPK signaling pathway is necessary for mediating the glucose-lowering effect of metformin (135, 162) and was recently shown to mediate inhibition of breast cancer cells in vitro (160). These data suggest that inhibition of mTOR via LKB1/AMPK might underlie, at least in part, the beneficial effect of metformin on glucose homeostasis/insulin sensitivity and tumor development. In a similar fashion, the PI3K/PTEN/Akt pathway is involved in both glucose homeostasis and tumor development (32, 51, 89, 133). Mutation of the tumor suppressor PTEN leads to up-regulated Akt and mTOR activities (32, 51, 89, 133). Interestingly, cancer cells in which the PTEN gene is mutated are particularly sensitive to the antiproliferative effect of rapamycin (32, 51, 89, 133). Furthermore,

selective deletion of PTEN in muscle or adipose tissue in mice improves both glucose metabolism and insulin sensitivity (85, 156), whereas it was shown to cause hepatocellular carcinomas when selectively deleted from mouse liver (65). The TSC1/TSC2 complex is also involved in the modulation of cell proliferation as well as in the control of insulin action/sensitivity (57, 69, 129). Analysis of mouse embryonic fibroblasts from TSC2$^{-/-}$ mice showed dysregulated mTOR/S6K1 activity as well as hyperphosphorylation and degradation of IRS-1 (56, 130, 131). As an upstream negative regulator of mTOR, the TSC1/2 complex integrates signals from both the LKB1/AMPK and PI3K/PTEN/Akt pathways through phosphorylation events (70, 71). AMPK-mediated phosphorylation of TSC2 enhances its activity, which leads to an effective repression of Rheb/mTOR (71), whereas phosphorylation of TSC2 by Akt destabilizes the complex, allowing for Rheb-mediated activation of mTOR (70).

Because mTOR is exquisitely sensitive to amino acid availability, exogenous as well as endogenous supplies of amino acids are likely to play an important role not only in glucose homeostasis and insulin resistance as described above, but also in tumor development. Furthermore, the observations that amino acids signal to mTOR independently from TSC2 (25, 109) and in synergy with hormonal stimuli (54) strongly suggest that modulation of the quantity as well as the quality of amino acids derived from dietary proteins might have profound and significant effects on chronic diseases associated with insulin resistance and mTOR overactivation such as diabetes, obesity, and cancer. Of significant importance is the observation that through increased protein catabolism in the postabsorptive state, plasma amino acid concentrations are elevated in obese mice and human subjects (28, 35, 37, 154). Recent studies have shown that the mTOR/S6K1 pathway is overactivated in the liver and muscle of high-fat-fed obese rats despite the occurrence of insulin resistance for PI3K/Akt activation (76). The activation of both mTOR and S6K1 by insulin was found to be accelerated in obese rats, in association with increased inhibitory phosphorylation of IRS-1 on Ser636/639 (76). Interestingly, the activities of both enzymes were higher even in the absence of insulin administration (76). One can hypothesize that mTOR and S6K1 are chronically activated because of the elevated and constant flux of amino acids (which activate mTOR independently from the insulin-signaling cascade), thereby worsening their insulin resistance for glucose metabolism, but also possibly promoting tumor development. Indeed, obesity is associated with various forms of cancer in humans, including breast and prostate cancer (26, 95, 120). Increased muscle wasting/cachexia in cancer patients may also contribute to mTOR activation through increased availability of amino acids upon protein catabolism (7, 103). However, some studies indicate that nutritional support of cancer patients with branched-chain amino acids improves nitrogen balance, increases skeletal muscle protein synthesis, and reduces skeletal muscle catabolism (30). Whether such treatment also promotes mTOR-induced insulin resistance in these patients remains unclear. Thus, more work is needed to determine whether amino acid–mediated modulation of mTOR directly plays a role in tumor growth and development.

CONCLUSION

Dietary proteins and amino acids have emerged in recent years as potent modulators of insulin action and glucose metabolism. Amino acids are thought to play a significant role in the pathogenesis of insulin resistance by modulating the endocrine function of the pancreas, acting as gluconeogenic precursors, stimulating hexosamine biosynthesis, or activating the mTOR-signaling pathway. Although activation of the mTOR pathway is increased in liver of obese animals (76, 152) and in amino acid–induced insulin resistance in humans (147), it remains

to be determined whether overactivation of mTOR is a common feature of human obesity. How varying the amount or the source of dietary proteins affects the activation of mTOR/S6K1 and the phosphorylation status of IRS-1 under acute and chronic settings is clearly an area of investigation that deserves more attention in relationship to both glucose metabolism and tumor development. Finally, identification of mechanistic links between energy-signaling (LKB1/AMPK) and hormonal-signaling (PI3K/PTEN/Akt) pathways in relationship to amino acid sensing via hVps34 might help define novel nutritional and/or pharmacological approaches for the treatment of diabetes, obesity, and cancer.

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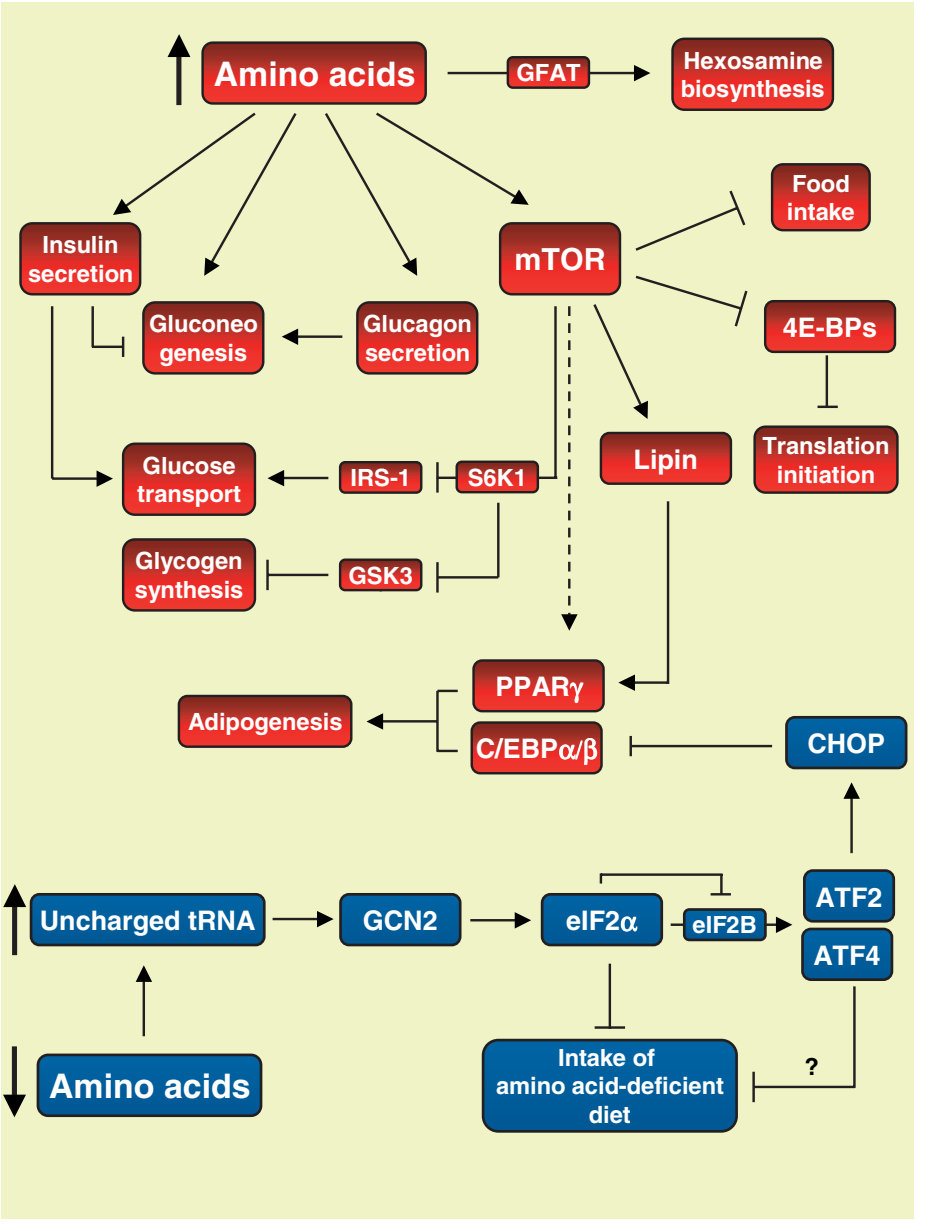

Figure 1

Effects of amino acid sufficiency (*red*) or deficiency (*blue*) on cell signaling and biological processes involved in the pathogenesis of insulin resistance. (See Amino Acids and Insulin Resistance section for details and explanations.)

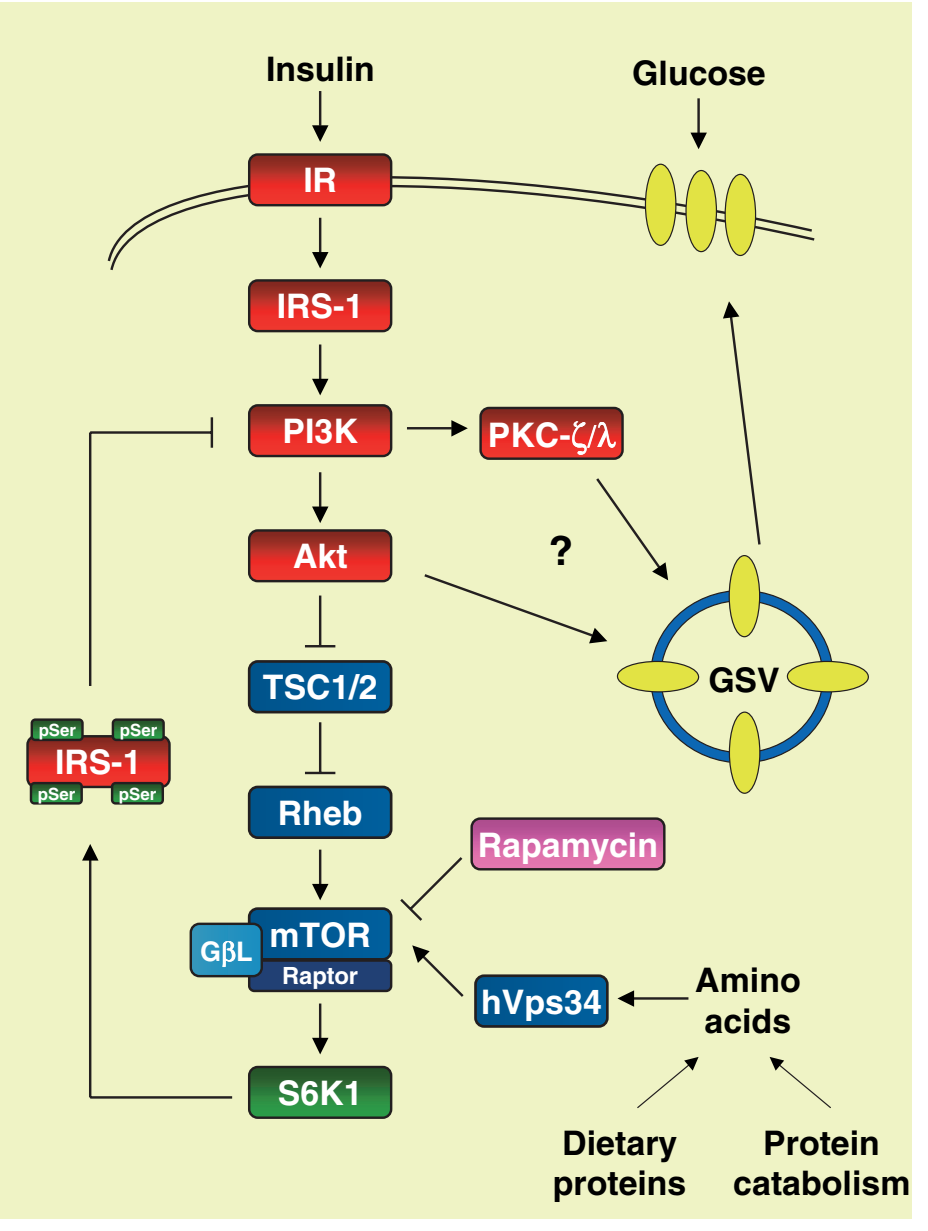

Figure 2

See legend on next page.

Figure 2

Feedback inhibition of IRS-1 signaling mediated by the mammalian target of rapamycin (mTOR). Insulin binds and activates the insulin receptor (IR), which phosphorylates intracellular substrates such as insulin receptor substrate-1 (IRS-1). Upon binding to tyrosyl-phosphorylated IRS-1, the activated phosphatidylinositol 3-kinase (PI3K) relays the signal to both Akt and protein kinase C (PKC)-ζ/λ, which are thought to be involved in glucose transporter 4 translocation and glucose transport stimulated by insulin. Akt can also phosphorylate and destabilize tuberous sclerosis complex (TSC)2, enabling Rheb-mediated mTOR activation. Amino acids can also activate mTOR through hVps34. The rapamycin-sensitive mTORC1 [composed of mTOR, raptor, and G protein β-subunit-like protein (GβL)] requires both insulin and amino acids for mediating full activation of S6K1 and promoting inhibitory phosphorylation of IRS-1 on serine residues. Heavily serine-phosphorylated IRS-1 causes insulin resistance by blocking PI3K signaling.

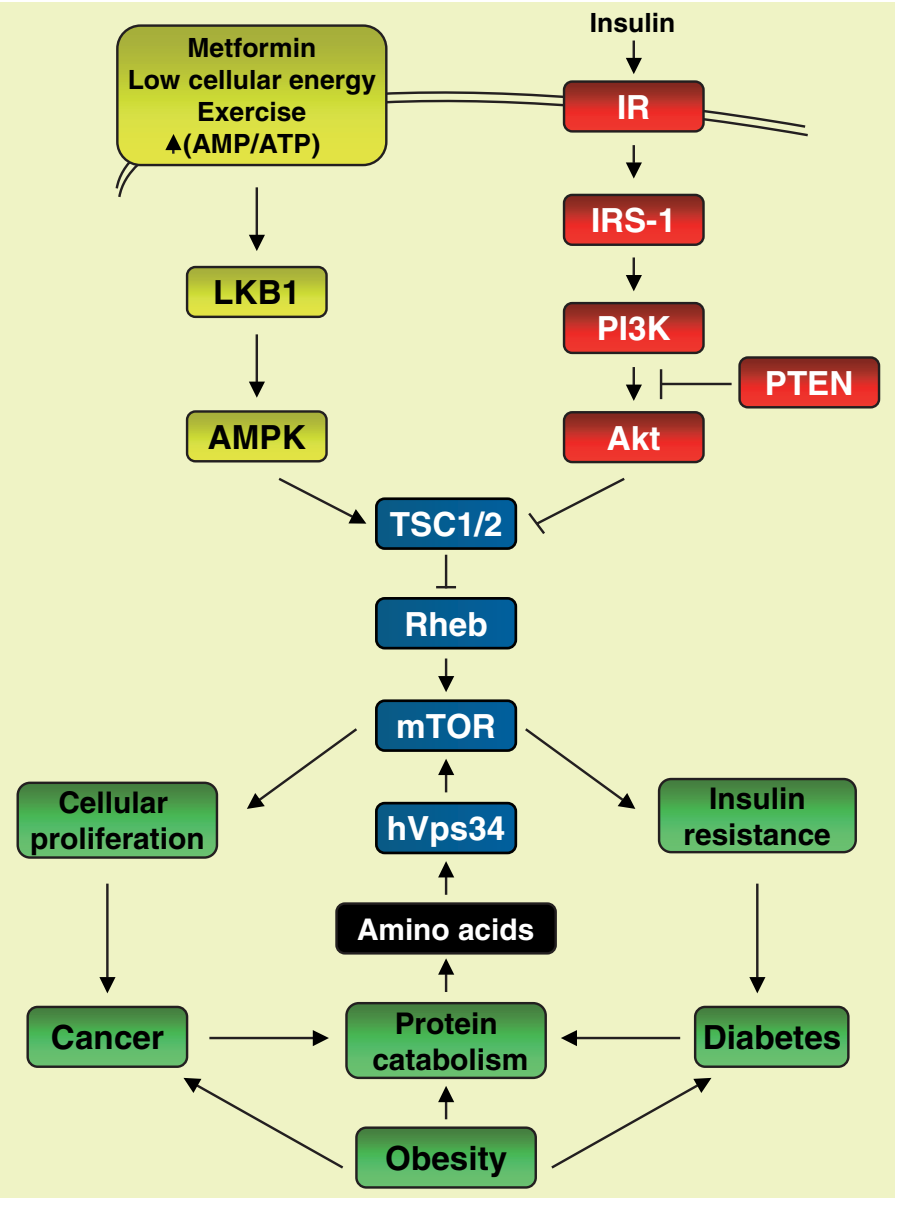

Figure 3

See legend on next page.

Figure 3

Interplay between energy-, hormonal-, and amino acid–sensitive signaling pathways in relationship to the development of cancer and diabetes. LKB1/AMPK activates TSC2, whereas PI3K/PTEN/Akt inhibits TSC2, resulting in the inhibition or activation of Rheb/mTOR, respectively. Amino acids can also activate mTOR via hVps34 independently from the insulin-signaling pathway. Activation of the mTOR pathway increases cellular proliferation and causes insulin resistance, events that are closely linked to the development of cancer and diabetes, respectively. Obesity, which has been shown to be an important risk factor for both cancer and diabetes, can maintain the mTOR pathway activation despite insulin resistance by favoring a constant supply of amino acids via enhanced protein catabolism.

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Errata

An online log of corrections to *Annual Review of Nutrition* chapters (if any, 1997 to the present) may be found at http://nutr.annualreviews.org/errata.shtml