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Tissue-specific Insulin Signaling in the Regulation of Metabolism and Aging

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Abstract

In mammals, insulin signaling regulates glucose homeostasis and plays an essential role in metabolism, organ growth, development, fertility, and lifespan. Defects in this signaling pathway contribute to various metabolic diseases such as type 2 diabetes, polycystic ovarian disease, hypertension, hyperlipidemia, and atherosclerosis. However, reducing the insulin signaling pathway has been found to increase longevity and delay the aging-associated diseases in various animals, ranging from nematodes to mice. These seemly paradoxical findings raise an interesting question as to how modulation of the insulin signaling pathway could be an effective approach to improve metabolism and aging. In this review, we summarize current understanding on tissue-specific functions of insulin signaling in the regulation of metabolism and lifespan. We also discuss potential benefits and limitations in modulating tissue-specific insulin signaling pathway to improve metabolism and healthspan.

Keywords

insulin signaling; tissue-specific; metabolism; aging

I. Introduction

Insulin is synthesized and secreted from pancreatic β cells in response to postprandial nutrient influx [1]. By suppressing glucose production in the liver and stimulating glucose uptake in muscle and fat, insulin reduces blood glucose levels to maintain glucose homeostasis in humans and animals. Insulin also regulates many important anabolic processes such as facilitating protein and glycogen synthesis in muscle and liver, promoting lipid synthesis and storage in liver and fat, as well as inhibiting fatty acid oxidation, glycogenolysis, and gluconeogenesis in insulin responsive tissues.

Great progress has been made during the past 3 decades on the mechanisms regulating the insulin signaling cascade in various species. Pharmacological or genetic manipulations of key components in the insulin signaling pathway have shown that defects in insulin

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signaling result in insulin resistance and contribute to metabolic dysfunctions and cardiovascular diseases [2]. However, recent studies have found that reducing or disrupting insulin signaling improves health-span and longevity in diverse model organisms such as yeast, worms, flies, and mammals [3-5]. These paradoxical findings raise an interesting question as to how the beneficial effects of insulin on energy homeostasis and longevity are achieved. In this review, we summarize recent progress on the mechanisms regulating the insulin signaling pathway. We also discuss tissue-specific functions of the insulin signaling pathway in the regulation of metabolism and/or longevity.

II. Insulin signaling and regulation

Insulin exerts its function by binding to its receptor on the cell membrane. The insulin receptor (IR) is a heterotetrameric transmembrane protein consisting of two extracellular α-subunits and two transmembrane β-subunits. The binding of insulin to the IR leads to the activation of IR tyrosine kinase and subsequent tyrosine phosphorylation of the β-subunits, the latter functions as docking sites for tyrosine phosphorylated adaptor proteins such as insulin receptor substrates 1 and 2 (IRS1/2). While the binding of IRS1/2 to IR is essential for regulating insulin signaling and function, how IRS1/2 is recruited to IR remains unclear. Very recently, Ryu and colleagues found that the interaction between IRS1/2 with IR is mediated by APPL1, an adaptor protein containing multiple function domains including the Bin1/amphiphysin/rvs167 (BAR) domain, pleckstrin homology (PH) domain, phosphotyrosine binding (PTB) domain, and coil coiled motif [6]. Under basal conditions, APPL1 forms a complex with IRS1/2 and Akt in the cytosol. In response to insulin and adiponectin stimulation, APPL1 is phosphorylated at Ser^{401} and this phosphorylation promotes APPL1 binding directly to the IR, allowing APPL1 to piggyback IRS proteins onto the IR. Insulin stimulation induces the dissociation of IRS proteins and Akt from the APPL1-IR complex, facilitating the binding of IRS1/2 to the activated IR near the plasma membrane [6]. The recruitment of IRS1/2 to IR leads to the activation of two major downstream signaling pathways, the phosphatidylinositol-3-kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) pathway (Fig. 1). Activation of the PI3K signaling pathway promotes 3-phosphoinositide-dependent protein kinase 1 (PDK1)-dependent phosphorylation and activation of protein kinase B (PKB or Akt), which in turn phosphorylates a number of important downstream effectors, including glycogen synthase kinase (GSK)-3, Forkhead box protein O (FOXO), and mechanistic target of rapamycin (mTOR). Activation of the MAPK cascade, which consists of Raf, MEK, and ERK1/2, plays a critical role for insulin to regulate mitogenic events such as cell proliferation, differentiation, and survival (Fig. 1 and [7]). Insulin signaling is negatively regulated by a number of mechanisms, such as the binding of the adaptor protein Grb10 to the kinase domain of IR [8, 9], serine phosphorylation of IRS1 [10, 11], and dephosphorylation of IR and IRS1/2 by protein tyrosine phosphatase-1B (PTP-1B) [12].

III. Tissue-specific function of insulin signaling in metabolism

1. Insulin suppresses glucose production in the liver

Insulin activates the insulin receptor in the liver, which phosphorylates IRS1 and IRS2, leading to activation of PI3K and ultimately Akt2. Activation of Akt2 promotes glycogen

synthesis and inhibits gluconeogenesis and glucose production. One of the most important functions of insulin in liver is to suppress hepatic glucose production (HGP) when serum glucose levels are high, such as after a meal. Insulin suppresses HGP by inhibiting gluconeogenic enzymes and activating glycolytic and fatty acid synthetic enzymes, resulting in the switch from fatty acid oxidation to fatty acid synthesis [13]. Insulin action in the liver leads to the reduction in glycogenolysis and gluconeogenesis, increased glycogen synthesis, and enhanced lipid and glycogen storage. The regulation of hepatic glucose metabolism by insulin is mainly through a direct effect, but some evidence suggests that insulin could also indirectly regulates HGP by inhibiting glucagon secretion from pancreatic α cells [13-15], by suppressing free fatty acid production from adipose tissues via inhibition of lipolysis [16-18], and by activating hepatic IL-6-STAT3 signaling via brain insulin action through the brain-liver axis [19]. Suppression of lipolysis by insulin in adipose tissue decreases serum non-esterified fatty acids (NEFA) and glycerol levels, which is responsible for the acute inhibition of hepatic glucose production[18]. Brain insulin signaling has been shown to reduce IL-6 expression and STAT3 phosphorylation in the liver, leading to a decrease in the expression of the gluconeogenic protein Glucose 6-phosphatase (G6Pase) [20].

Defects in insulin signaling and action in the liver lead to increased HGP, impaired glucose disposal, and reduced postprandial VLDL-TG secretion. Since insulin clearance in vivo occurs primarily in the liver, insulin resistance in the liver also results in reduced insulin degradation. Consistent with this, liver-specific insulin receptor knockout mice (LIRKO) are hyperglycemic and hyperinsulinemic, and display reduced liver size compared to control wild-type mice [21]. Liver-specific IRS1 knockout (LIRS1-KO) mice show insulin resistance after refeeding, but fail to exhibit insulin resistance during fasting. Conversely, liver-specific IRS2 knockout (LIRS2-KO) mice display insulin resistance during fasting but not after refeeding, displaying dynamic functional relay [22]. LIRS1-KO mice show significant impairment in hepatic nutrient homeostasis and fatty acid oxidation due to dysregulation of FOXA1, while IRS2 apparently controls gluconeogenesis by inhibiting FOXO1 and CREB-binding protein (CBP). Nutrient-sensitive transcripts are significantly downsregulated in LIRS1-KO mice but are normal in LIRS2-KO mice, indicating that IRS1, but not IRS2, plays a major role in regulating hepatic nutrient homeostasis [23]. However, liver-specific knockout of both IRS1 and IRS2 leads to severe glucose intolerance and impaired lipid metabolism [24].

2. Insulin promotes glucose and fatty acid uptake in skeletal muscle

Skeletal muscle accounts for 60-70% of whole body insulin-stimulated glucose uptake and thus plays an essential role in the regulation of whole body energy homeostasis [27]. Insulin regulates muscle metabolism by promoting glucose uptake, glycogen synthesis, and lipid utilization and storage. Insulin stimulates glucose uptake in skeletal muscle by promoting the membrane translocation of GLUT4, the major glucose transporter in skeletal muscle [28]. Insulin has also been shown to stimulate glucose and free fatty acid (FFA) uptake in skeletal muscle by increasing glucose flux and activating key enzymes involved in muscle glucose or fat oxidation [29]. The effects of insulin, however, are reduced in skeletal muscle of type 2 diabetic subjects [30].

Muscle is one of the major sites of insulin resistance in type 2 diabetic patients [31] and in fact, muscle insulin resistance has been considered to be one of the earliest signs in the pathogenesis of metabolic syndrome [32]. Impaired tyrosine phosphorylation of IRS1 and activation of PI3K have been found in skeletal muscle of type 2 diabetic patients [33]. Defects in glucose transport in skeletal muscle also correlate with impaired whole body glucose uptake in type 2 diabetic patients [34]. Muscle-specific knockout of IR led to increased fat mass, elevated serum triglyceride levels, and muscle insulin resistance in mice, but had no significant effects on global glucose tolerance [30]. A possible explanation for these findings is that glucose is shunted from insulin-resistant muscle to the relatively more insulin sensitive adipose tissue, where it is converted into triglyceride for storage, thus compensating for reduced muscle insulin sensitivity at the whole body level in the mice [35]. Impaired glucose uptake is also found in mice lacking both IRS1 and IRS2 in skeletal and cardiac muscle, although without hyperglycemia or hyperinsulinemia [36].

Insulin does not stimulate lipid storage in muscle under both normal and insulin resistant conditions. In contrast, palmitate, by elevating intracellular synthesis of ceramide and activation of protein kinase PKC_{ζ} [37], suppresses insulin-induced plasma membrane recruitment and phosphorylation of Akt, which is associated with a loss in insulin-stimulated glucose transport [37]. The cellular mechanisms that lead to the initial accumulation of intracellular lipid intermediates have not been completely elucidated yet, but may result from lower rates of fatty acid (FA) oxidation, higher rates of FA uptake, or both, in insulin-resistant skeletal muscle [38].

Recent studies have identified skeletal muscles as a secretory organ that secretes several hundred peptides (myokines) in response to environmental and/or metabolic changes [39]. Among them, the brain-derived neurotrophic factor (BDNF) [40] and irisin [41] have been shown to regulate glucose homeostasis and obesity by cross-talking with other organs (e.g. adipose tissue). Irisin improves energy homeostasis by promoting UCP1 expression and the browning process of white adipose tissue (WAT) [42]. Inhibition of myostatin, a member of the transforming growth factor-β (TGF-β) superfamily predominantly expressed in skeletal muscle, has been found to prevent diabetes and hyperphagia in a mouse model of lipodystrophy [43]. However, whether biosynthesis and secretion of the myokines are regulated by insulin remains to be determined.

3. Insulin inhibits lipolysis and stimulates lipid biosynthesis in adipose tissues

Adipose tissue is an important energy storage and endocrine organ that plays important roles in the maintenance of energy homeostasis, including insulation and protection of tissues and organs from heat and cold, providing protective padding, thermogenesis, adipogenesis, and the production of a variety of hormones/cytokines collectively known as adipokines [44]. The expansion of adipose tissue is known to exert a buffering effect that prevents excess lipids from being ectopically deposited in other metabolically important organs such as liver, muscle and pancreas, which are major causes of insulin resistance [45]. However, under excess nutrition, diet-induced obesity remains to be the leading cause of insulin resistance, unless the lipid storage capacity of adipose tissues is enhanced by other means, such as PPARγ stimulation [46].

Insulin stimulates the uptake of glucose into adipocytes where it converts into lipids as a more efficient form of energy storage (adipogenesis) [47]. Although adipose tissue accounts for a relatively small proportion (<10%) of the peripheral glucose utilization in response to insulin [48], it is not a passive repository for excess energy over the research in the past decades [49, 50]. Adipose tissue is the primary site for triacylglycerol storage [51], while enhancement of glucose uptake increases triacylglycerol and fatty acid synthesis in adipose tissues and might lead to obesity [52]. The major bucket of triacylglycerol in the body is in adipose tissues, which can be mobilized in the form of long-chain fatty acids to other tissues via the bloodstream [53]. Insulin regulates lipid, glucose and protein metabolism primarily in adipose tissue [49, 54]. First of all, insulin effectively decreases the rate of lipolysis in adipose tissues, leading to reduced plasma fatty acid level [55]. Insulin also has the ability to increase triglycerides uptake from the blood into adipose tissue [55]. Secondly, the major effect of insulin on adipose tissue is to increase glycolysis rate and to promote glucose transport across the cell membrane [55]. Insulin stimulates the translocation of the glucose transporter GLUT4 from intracellular pools to the surface of cell membrane to increase glucose uptake in adipose and muscle [56]. Finally, insulin increases the rate of protein synthesis in adipose tissue. The protein synthesis stimulated by insulin causes some proteins to be phosphorylated and dissociate from eIF-4E, the translation initiation factor regulator, thereby relieving the translational inhibition [57].

Insulin promotes the biosynthesis of lipids and inhibits its degradation. The action of insulin in the regulation of lipid metabolism is mediated by two main transcriptional factors: SREBP1c, which determines the transcription of many adipocyte specific genes [58, 59], and FOXO1 [60]. Down-regulation of SREBP-1 blocks the expression of gluconeogenic and lipogenic genes [61]. On the other hand, overexpression of SREBP-1 increases gluconeogenic and lipogenic gene transcription [62]. Insulin-stimulated glucose uptake in adipocytes activates ChREBP, which up-regulates de novo lipogenesis [63]. Recently studies show that the mTORC1 signaling pathway mediates insulin-stimulated processing of SREBP-1c through its substrate protein kinase S6K [64, 65]. Insulin also profoundly inhibits lipolysis in adipocytes via inhibition of phosphodiesterase 3b (PDE3b) via Akt-mediated phosphorylation, leading to reduced intracellular cAMP levels and thus PKA activity [66]. However a noncanonical pathway has been described by which insulin regulates lipid metabolism via an Akt-independent and PKA-mediated phosphorylation of a lipid droplet-associated substrate, perilipin [67]. Secondly, insulin inhibits lipolysis through phosphorylation of adipose-specific phospholipase A2, which via arachidonic acid production increases prostaglandin E2 levels and in a paracrine/autocrine manner reduces cAMP levels through inhibition of adenylate cyclase [68, 69]. Thirdly, insulin represses lipolysis by transcriptionally silencing lipase genes via repression of the transcription factors FOXO1 and IRF4 [70, 71]. FOXO1 is phosphorylated by Akt and the phosphorylation prevents FOXO1 from entering into the nucleus, leading to increased PPARγ activity [72]. Finally, insulin upregulates the transcription of lipid droplet protein FSP27 and dampens thus the downstream target of Akt, lipolysis [73] [74].

Insulin resistance in adipose tissue, which is a characteristic of the obese state [75], has been recognized as one of the leading causes of type 2 diabetes [76, 77]. Key steps in the insulin signaling pathway, such as tyrosine phosphorylation of IRS1 and activation of PI3K, are

greatly reduced in adipose tissue than in muscle in type 2 diabetic patients [78]. However, fat-specific knockout of IR, which led to a 50% decrease in fat pad mass and a 30% decrease in whole body triglyceride content, improves metabolism in mice [79]. There is some evidence showing that the insulin signaling pathway plays a more important role in the development and maintenance of normal triglyceride storage than in the maintenance of euglycemia in mice [52]. Knockout of IR in brown adipose tissue (BAT), which is important for thermal adaptation as well as determining peripheral insulin sensitivity [80], led to age-dependent brown fat atrophy, accompanied with deteriorated β cell function, decreased β cell mass, and hyperglycemia [81]. These findings suggest a functional link between β cell mass/function and BAT. Fat-specific knockout of GLUT4 in mice led to a 53% decrease in insulin-stimulated whole body glucose uptake and a reduction in glycolysis and glycogen synthesis [82]. Interestingly, fat-specific knockout of GLUT4 in mice also led to a 40% reduction in insulin-stimulated glucose transport into skeletal muscle and impairment in insulin-stimulated suppression of HGP, indicating that down-regulation of GLUT4 in adipose tissues can cause whole-body insulin resistance, a hallmark of type 2 diabetes. However, it remains to be determined whether insulin resistance in adipose tissues leads to dysfunction in other targeted organs and whether the effect is mediated via an endocrine or a paracrine mechanism.

4. Insulin positively regulates insulin secretion and β cell function

Pancreatic β cells, which produce and secrete insulin in response to the blood glucose concentration in order to keep glycaemia in a narrow physiological range, are of vital importance in maintaining glucose homeostasis. Glucose, the principal nutrient secretagogue of insulin, is transported into pancreatic β cells via glucose transporter 2 (GLUT2). In β cells, glucose is metabolized via glycolysis and Krebs cycle, leading to an increase in cellular ATP/ADP ratio and subsequent closure of the ATP-sensitive potassium (KATP) channels [83]. The closure of KATP channels results in β cell membrane depolarization that induces opening voltage-dependent Ca^{2+} channels and subsequent influx of Ca^{2+} into β cells. The increase of the intracellular free Ca^{2+} concentration constitutes the indispensable triggering signal to induce exocytosis of insulin-containing secretory granules [83]. Historically, insulin was thought to have a negative or no effect on β cell proliferation, insulin biosynthesis, and secretion [84]. However, many more recent studies have clearly demonstrated that insulin plays a positive role in transcription, translation, ion flux, β cell survival, proliferation and insulin secretion [85].

Targeted overexpression or specific ablation of key components of insulin signaling in mouse pancreatic β cells significantly affects β cell function and survival [86]. Overexpression of IR greatly promoted insulin-stimulated insulin gene expression in mouse β cells [87]. Disruption of insulin signaling in β cells by β cell-specific knocking out IRS2 [88] or the IR [89] reduced pancreatic insulin content, accompanied by the development of a phenotype similar to that of type 2 diabetes mellitus. Mice with a global knockout of IRS1 [90] or IRS2 [91], β cell-specific knockout of IR [92], or pancreas-specific deletion of IRS2 [93] all showed decreased β cell mass and a marked impairment in glucose-stimulated insulin secretion. On the other hand, constitutive activation of Akt1/PKBα increased β cell proliferation (hyperplasia) and increases β cell size (hypertrophia) [94].

While it is now well-established that insulin signaling plays critical positive role in β cell function, the autocrine effect of insulin on β cell function remains unclear [95]. A major argument is that since pancreatic β cells are exposed to such a high level of secreted insulin, the respective insulin signal transduction pathway must be desensitized [96]. Another question is whether the central nervous system (CNS) has a predominant influence on β cell function [97, 98]. While most of the intriguing data on the roles of insulin in β cell function come from knockout mice generated by using the pancreatic and duodenal homeobox 1 (PDX1) gene promoter- or the rat insulin gene promoter (RIP)-driven Cre recombination system [92, 99, 100], recently studies have revealed the caveats of these genetic knockout approaches due to non-specific knockout of the target genes in other tissues in addition to β cell or pancreas [101, 102]. Indeed, the RIP and PDX1 gene promoters have been shown to drive Cre-expression in several regions of the brain, including the hypothalamus [102]. Moreover, the RIP-Cre mice themselves display mild glucose intolerance [103]. Therefore, further studies may still be needed to elucidate the exact mechanisms underlying the phenotypes of the knockout mice generated by using the aforementioned promoter-driven Cre mice. Recently, the mouse insulin promoter (MIP)-driven Cre recombination system has been shown to disrupt gene expression exclusively in β cells [101], providing a critical tool to determine the functional roles of a protein in β cells in vivo.

5. Central insulin signaling promotes neuronal survival and regulates whole-body energy homeostasis

Brain insulin signaling has been shown to be essential for the regulation of various neuronal activities such as learning, memory, food intake, reproduction, and peripheral metabolism [104, 105]. Insulin in the brain, especially in the hippocampus, cortex, hypothalamus, olfactory bulb, and pituitary, can reach a level 10- to 100-fold greater than that in the plasma [106, 107]. The origin of central insulin remains to be a topic of debating, but some evidence suggests that insulin could cross the blood–brain barrier (BBB) by an active and saturable process [105]. Consistent with this finding, circulating insulin levels are positively correlated with the concentration of this peptide hormone in cerebrospinal fluid (CSF) [107].

Brain insulin signaling also regulates whole body energy balance by cross-talking with peripheral tissues, involving complex interactions of various hormones, neuropeptides, and other signaling molecules involved in the regulation of food intake and energy expenditure [1]. Direct administration of insulin into the brain reduces food intake and body weight gain in C. elegans, Drosophila, and baboons [108-110]. Deletion of IR in neurons of mice increases food intake and body weight in conjunction with increased body fat, plasma insulin levels, and hypertriglyceridemia [111]. Deletion of IRS2 specifically in the brain increases lifespan in the presence of peripheral insulin resistance [112]. Surprisingly, increasing neuronal-specific IRS2 expression in mice also increased fat mass, insulin resistance, and glucose intolerance during aging due to decreased locomotor activity in the presence of unaltered exploratory behavior and motor function [113]. CNS inflammation contributes to peripheral tissue insulin resistance, particularly in the liver, via a brain-liver neuronal signal [114]. These observations demonstrate that insulin signaling in the brain can influence glucose homeostasis in response to afferent input from peripheral tissues.

However, the precise mechanisms underlying the cross-talk between central insulin signaling and peripheral signaling pathways remain to be further elucidated.

IV. Insulin signaling in aging and aging-associated diseases

1. Insulin signaling is conserved among species

The insulin/insulin growth factor (IGF) signaling (IIS) cascade is an evolutionarily conserved pathway among diverse species, ranging from yeast to humans ([115] and Fig. 2). While defects in the insulin signaling pathway lead to insulin resistance and diabetes in rodents and humans, disruption of this signaling pathway has been shown to significantly extend lifespan in *C. elegans* [116], flies [117], mice [118], and humans [119, 120]. These long-lived IIS mutants share some important phenotypic characteristics including reduced insulin signaling, enhanced insulin sensitivity, and reduced serum IGF-1 levels, together with reduced oxidative damage of macromolecules and increased stress resistance. The daf-2/IR/IGFR mutant *C. elegans* live twice as long as wild-type, and the longevity phenotype does not need go through the dauer state [116]. PI3K-null adult *C. elegans* are more resistant to oxidative and electrophilic stresses and live remarkably longer under both normal and toxic environments compared to wild-type controls [121]. These effects have been shown to depend on the integrity of the dauer daf-16/FOXO, which has similarity to a family of mammalian forkhead transcriptor factors[109]. The lower level of free radicals in daf-2/IR/IGFR mutants has been shown to be essential for life span extension [122]. Indeed, the gene ctl-1, which encodes a cytosolic catalase, is required for the extension of adult life span by daf-2/IR/IGFR [123]. The expression of mitochondrial Superoxide dismutase 2 (SOD2) is required for the longevity extension caused by mutations decreasing the activity of the Ras/Cyr1/PKA and Sch9 pathways in yeast [124]. Similarly, flies homozygous for chico/IRS null mutant have increased levels of SOD, reduced body size, greatly reduced fecundity, and increased longevity [125]. These findings highlight the central position of oxidative stress in the aging-regulatory machinery and superoxide toxicity plays an important role in aging and death. *C. elegans* proteotoxicity models indicate that the IIS pathway directly links aging to the onset of toxic protein aggregation, and the protective effects are dependent on daf-16/FOXO, as the effects could be abolished by RNAi-mediated depletion of daf-16 [126]. Another strong link between insulin/IGF-1 signaling and life span in animal models comes from dietary restriction. Primates maintained on dietary restriction feeding regimens exhibit increased insulin sensitivity and enhanced glucose tolerance [127]. Calorically restricted rats have lower levels of IGF-1 that contributes to the protective effect against age-related pathology and resistance to p-cristine-induced bladder cancer [128]. Taken together, these results suggest that the IIS pathway is critical for regulating various aging-related disorders and longevity.

2. Impaired neuronal insulin/IGF-1 signaling (IIS) is associated with aging and aging-related neuronal degenerative diseases

Human aging is associated with neurophysiological changes in the brain and variable degrees of cognitive decline. While systemic disruption of the IIS pathway has been shown to extend lifespan in diverse species, abnormality in insulin signaling in peripheral neurons has been shown to contribute to diabetic neuropathy [129]. Noteworthy, aging has been

found to be associated with a decrease in brain IR number and the binding capacity of insulin, especially in hippocampus, cortex, and choroid plexus [130]. Defects in neuronal IIS pathway such as reduced Akt and GSK3 activities are implicated in the development of Alzheimer's disease (AD) [131], one of the most important aging-related neuronal disorders. AD is associated with a decrease in insulin level in the CSF and CSF/plasma insulin ratio [106] and insulin signaling impairment is more severe in individuals with both T2DM and AD [132, 133], suggesting that T2DM may be a risk factor for AD. Consistent with this, brains of T2DM and AD mice show similar pathophysiological changes [134]. In addition, reversing diabetes-induced neuronal mitochondrial dysfunction is beneficial for neuronal survival [135]. These studies clearly suggest a connection between AD and diabetes, and reinforce the idea that AD can be considered as a form of diabetes in the brain (also known as type 3 diabetes or brain-type diabetes) [133]. However, neuron-specific knocking out IRS2 has been found to extend lifespan in mice [112]. Interestingly, knockout of IRS2, but not neuronal IR or IGF-1R, prevents premature death and delays amyloid accumulation in a mouse model of AD [136]. Thus, it remains to be established whether the neuronal IIS pathway has a protective role in aging and aging-associated neuronal degeneration and various other diseases.

3. Disruption of insulin signaling in adipose tissues improves healthy aging and extends lifespan

Insulin lowers plasma fatty acid levels by inhibiting lipolysis and stimulating fatty acid and triacylglycerol synthesis and by promoting triglyceride uptake in adipose tissues [53]. Fat-specific knockout of the IR led to a 50% reduction in adipose tissue mass despite normal food intake [52, 79]. The fat-specific IR knockout mice (FIRKO) have a significantly lower insulin level and extended lifespan compared to control mice [52, 79], demonstrating an essential role of fat-specific insulin signaling in aging.

How disrupting insulin signaling in adipose tissues leads to extended lifespan remains unknown. The FIRKO mice showed increased metabolic rate, suggesting that reducing free radical damage may not be an important factor to extend lifespan. Another possibility is that the extended lifespan of the FIRKO mice is caused by reduced fat mass. Consistent with this, caloric restriction, a well-established strategy to extend lifespan in rodents; reduces fat mass, particularly visceral fat, in mice [79, 137]. However, calorie restriction also greatly extends the maximum lifespan of genetically obese ob/ob mice [138], suggesting that reduced adiposity *per se* may not be the major component in extending longevity of the obese mice. Lastly, the increased longevity in FIRKO mice may be the direct result of altered insulin signaling in adipose tissues, which is in agreement with the findings that reducing IIS increases lifespan in *C. elegans* and *Drosophila* [110, 116, 125, 139, 140]. However, how altering adipose insulin signaling leads extended longevity remains unclear.

4. mTORC1: The downstream target of insulin/IGF-1 signaling

mTOR (mechanistic/mammalian target of rapamycin) is an evolutionarily conserved serine/threonine kinase that acts downstream of the IIS pathway (Fig. 3). mTOR exists in two functionally distinct complexes, mTOR complex 1 (mTORC1) and complex 2 (mTORC2). As an energy sensor, mTORC1 is activated by nutrients such as amino acids and glucose,

cellular energy levels, and many growth factors/hormones such as insulin and IGF-1, and plays critical roles in the regulation of key cellular events, including cell growth, proliferation, differentiation, gene transcription, mRNA translation, autophagy, survival, and metabolism [141-148]. mTORC2 phosphorylates Akt and plays an important role in insulin signaling. Suppression of mTOR signaling extends lifespan in yeast [149, 150], worms [151], *Drosophila* [152], and mice [153]. mTOR signaling suppression has also been found to attenuate various age-related diseases such as obesity, neurodegenerative diseases, osteoporosis, osteoarthritis, and age-related macular degeneration [154].

The activation of the mTORC1 signaling pathway by insulin or IGF-1 is mediated by Akt-mediated phosphorylation and inhibition of Rheb, a negative upstream regulator of mTORC1 (Fig. 3). mTORC1 is inhibited by rapamycin, a small molecular immunosuppressant drug used to prevent rejection in organ transplantation. Although rapamycin is generally considered as a specific inhibitor of mTORC1, extended treatment with rapamycin can also effectively inhibit mTORC2 [155]. There is some evidence that suggests that the negative effects of rapamycin on glucose tolerance and hepatic insulin are mediated by inhibition of mTORC2, whereas lifespan extension is considered to result from mTORC1 inhibition [156]. Very recently, we identified Grb10, a Src-homology 2 (SH2) and pleckstrin homology (PH)-domain containing adaptor protein that interacts with tyrosine phosphorylated IR and inhibits insulin/IGF-1 signaling [8], as a negative feedback regulator of mTORC1 [157]. mTOR-mediated phosphorylation at $Ser^{501/503}$ switches the binding preference of Grb10 from the insulin receptor to raptor, leading to the dissociation of raptor from mTOR and thus down-regulation of mTORC1 signaling [157], demonstrating a feedback mechanism of regulation.

5. Will suppressing mTORC1 in adipose tissues improve healthspan?

Disrupting insulin signaling in adipose tissues improved metabolism and extended lifespan in mice [52, 79], yet the precise mechanism remains unknown. Since mTORC1 is a downstream target of insulin signaling (Fig.1), fat-specific knockout of IR may improve healthspan and longevity by suppressing mTORC1 signaling in adipose tissues. Interestingly, adipose-specific disrupting mTORC1 signaling by knocking out Raptor prevented diet-induced obesity in mice [158]. On the other hand, overactivation of mTORC1 signaling in adipose tissues by fat-specific knockout of Grb10 led to obesity as well as glucose and insulin intolerance [157], suggesting that reducing mTORC1 signaling in adipose tissues has a beneficial effect on metabolism. However, it is currently unknown if suppressing mTORC1 signaling in adipose tissues is sufficient to improve healthspan and lifespan.

V. Conclusion

The insulin-signaling cascade is an evolutionally conserved signaling pathway among diverse species that plays a critical role in the regulation of metabolism and longevity. In mammals, insulin regulates lipid and glucose metabolism and energy homeostasis by initiating its signaling events in target tissues such as liver, skeletal muscle, adipose tissues, and the brain. Defects in the insulin signaling pathway may cause insulin resistance, leading to various metabolic disorders such as type 2 diabetes. However, numerous studies have

demonstrated that suppressing insulin and/or IGF-1 signaling pathway may lead to extended lifespan. An important question is whether targeting the IIS signaling pathway is a valid strategy to improve healthspan and lifespan. Interestingly, disrupting the insulin signaling pathway in adipose tissues improves metabolism and extends lifespan [52, 79], suggesting tissue-specific suppression of insulin signaling may be an effective approach to improve healthspan and longevity. The mechanisms by which altering adipose tissue insulin signaling leads to extended lifespan remain unknown, but reduction of mTORC1 signaling could play a role. Generation and characterization of adipose tissue-specific transgenic and knockout animal models in which the mTORC1 signaling pathway is specifically altered should enable us to test this hypothesis and elucidate the mechanistic links between aging and metabolic disease. Results from these studies should provide insights into the development of new therapeutic approaches aimed at alleviating aging-associated disorders and improving healthspan.

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Abbreviations

AD	Alzheimer's disease
APPL1	adaptor protein, phosphotyrosine interaction
BAR	Bin1/amphiphysin/rvs167
BAT	brown adipose tissue
BBB	blood–brain barrier
BDNF	Brain-derived neurotrophic factor
CBP	CREB-binding protein
ChREBP	carbohydrate response element binding protein
CNS	central nervous system
CREB	cAMP response element-binding
CSF	cerebrospinal fluid
ERK1/2	extracellular signal-regulated kinases1 and 2
FA	fatty acid
FFA	free fatty acid
FIRKO	fat-specific insulin receptor gene knockout
FOXA1	Forkhead box protein A1
FOXO	Forkhead box protein O
FSP27	fat specific protein 27
HGP	hepatic glucose production
G6Pase	Glucose 6-phosphatase
GLUT	Glucose transporter
Grb10	Growth factor receptor-bound protein 10
GSK3	Glycogen synthase kinase 3
IGF-1	Insulin-like growth factor 1
IGFR	Insulin-like growth factorreceptor
IL-6	Interleukin-6
IIS	insulin/insulin-like growth factor signaling

IR	insulin receptor
IRF4	interferon regulatory factor 4
IRS	insulin receptor substrates
KATP	ATP-sensitive potassium
LIRKO	liver-specific insulin receptor gene knockout
LIRS1-KO	liver-specific IRS1 knockout
LIRS2-KO	liver-specific IRS2 knockout
MAPK	Mitogen-activated Protein Kinase
MEK	Mitogen-activated protein/extracellular signal-regulated kinase kinase
MIP	mouse insulin promoter
mTOR	mammalian target of rapamycin
mTORC1/2	mTOR complex 1/2
NEFA	non-esterified fatty acids
PDK1	Phosphoinositide- dependent kinase-1
PDE3b	phosphodiesterase 3b
PDX1	Pancreatic and duodenal homeobox 1
PH	pleckstrin homology
PI3K	Phosphatidylinositol-3-Kinase
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PPARγ	Peroxisome proliferator-activated receptor
PTB	phosphotyrosine binding
PTP-1B	protein tyrosine phosphatase-1B
RIP	Rat insulin gene promoter
Rheb	Ras homolog enriched in brain
S6K	S6-kinase
SH2	Src-homology 2
SOD	Superoxide dismutase
SREBPs	Sterol Regulatory Element-Binding Proteins
STAT3	Signal transducer and activator of transcription 3
T2DM	type 2 diabetes mellitus

TG	triglyceride
TGF-β	transforming growth factor-β
UCP1	uncoupling protein 1
VLDL	Very-low-density lipoprotein
WAT	white adipose tissue

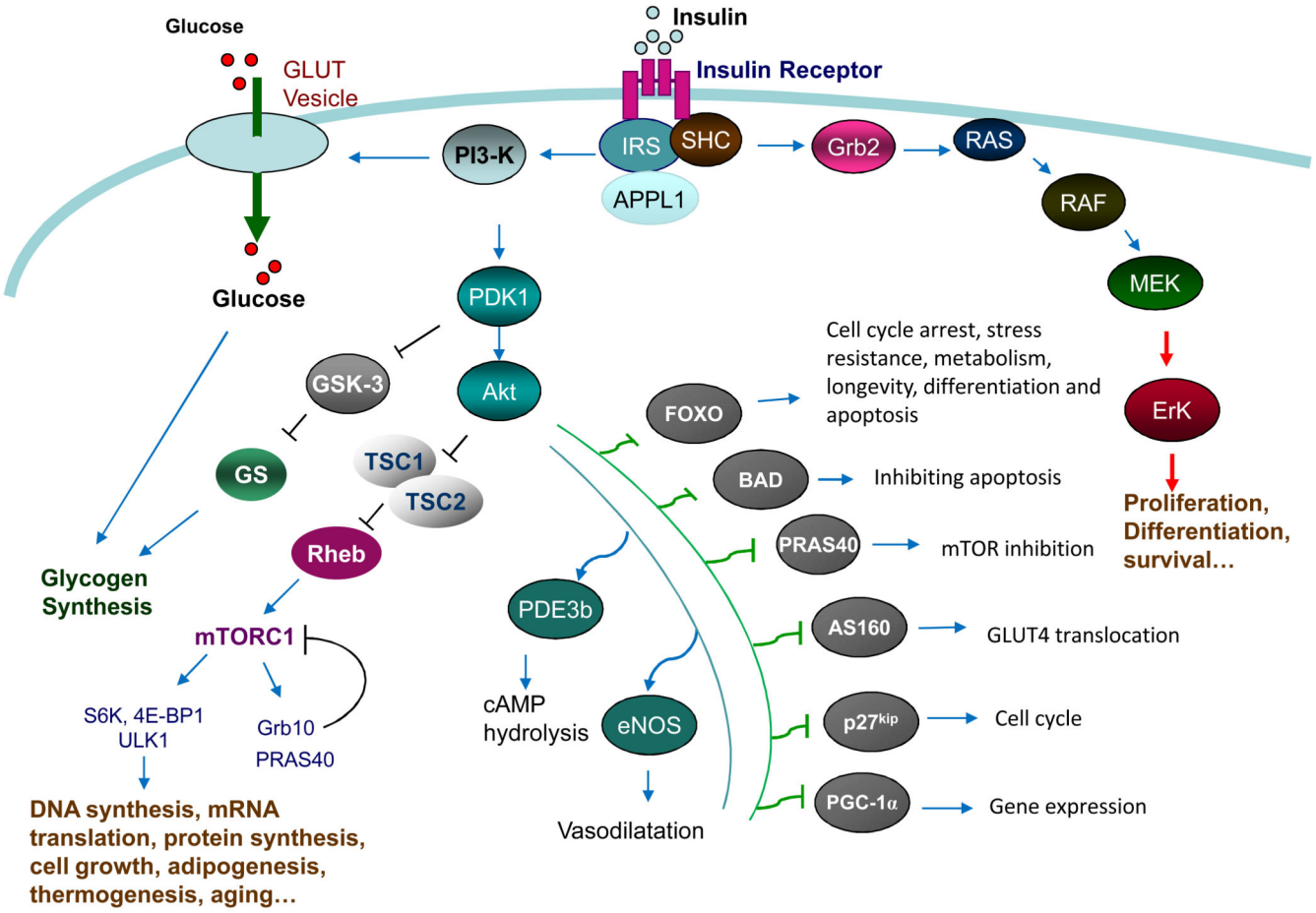

Figure 1. Insulin signaling and function

The binding of insulin to its receptor on cell membrane results in insulin receptor (IR) tyrosine kinase activation and IR tyrosine phosphorylation. APPL1 functions as a piggyback protein that promotes IRS binding to the tyrosine phosphorylated IR, leading to IRS tyrosine phosphorylation and subsequent activation of the PI3K/PDK1/Akt signaling pathway. Activation of Akt promotes the phosphorylation and inhibition of TSC1/2, a negative regulator of the mTORC1 signaling pathway. Akt also phosphorylates many other cellular proteins and plays key roles in various cellular events. Tyrosine phosphorylation of IR promotes the association of the adaptor proteins Shc and Grb2 to the IR, leading to the activation of the RAS-RAF-MEK-ERK1/2 cascade, which is essential for cell growth, differentiation and protein synthesis.

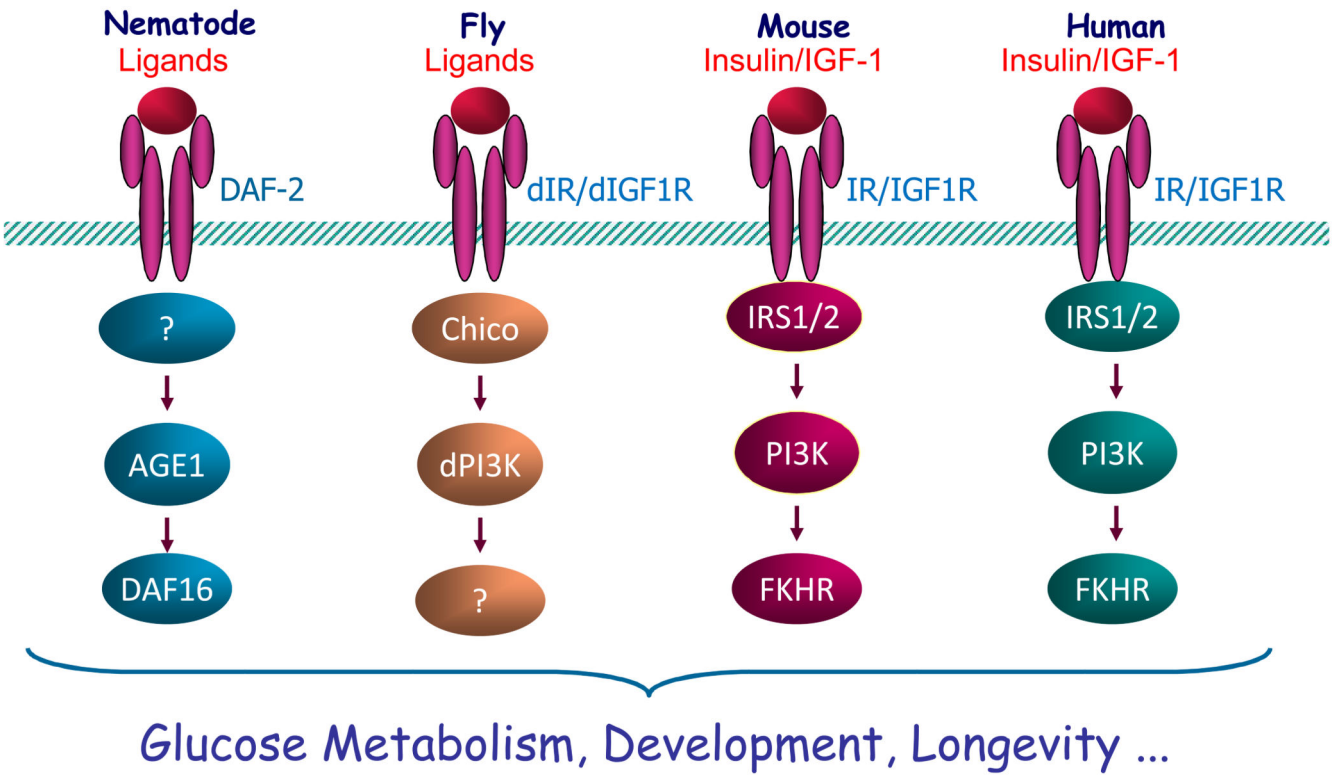

Figure 2. Conserved insulin/IGF-1 regulation in longevity

The insulin/IGF-1-like pathway is conserved among various species and suppressing this pathway extends lifespan in species such as worm, fly, and mouse.

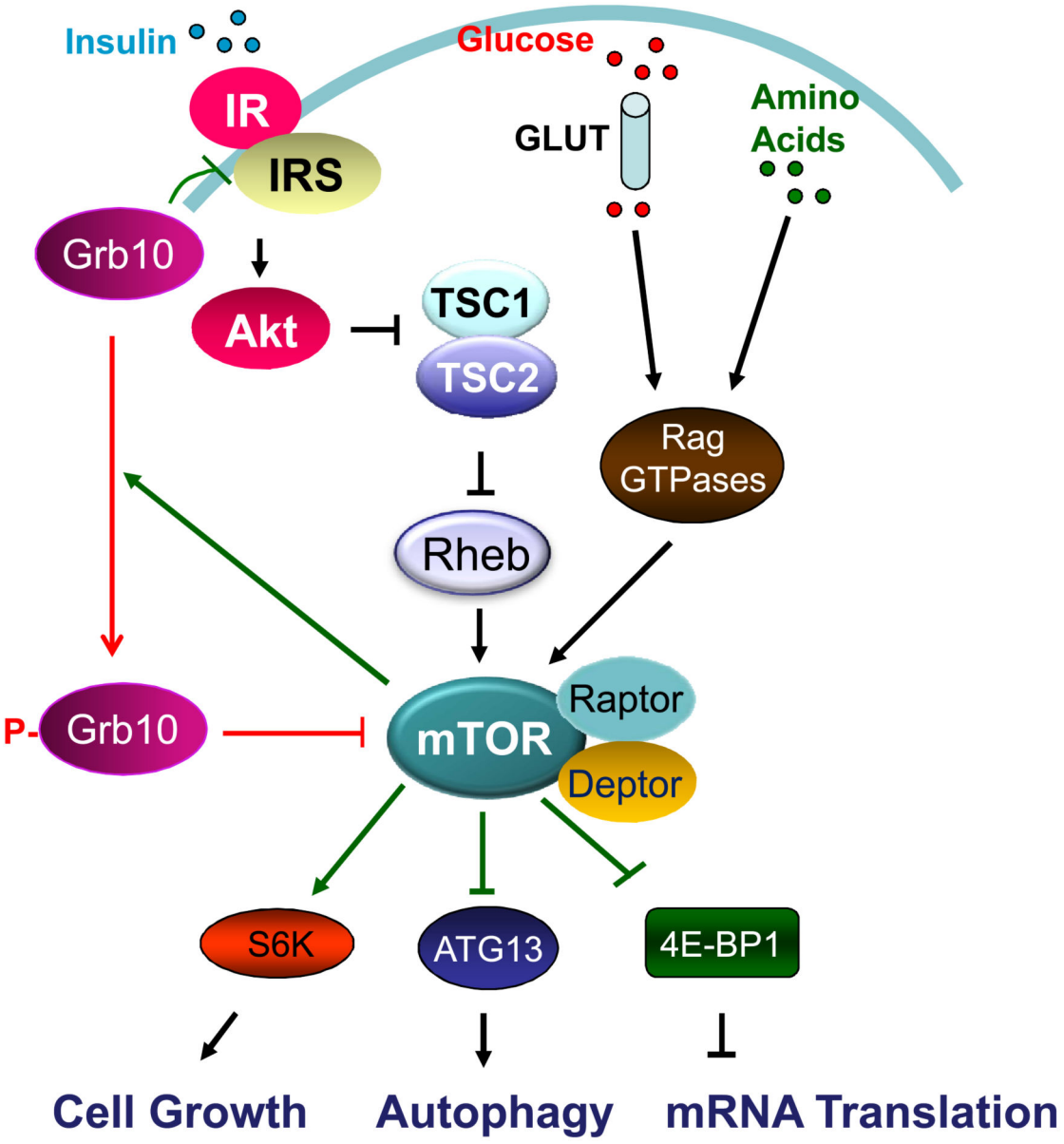

Figure 3. mTORC1 signaling and regulation

mTORC1, which regulates various cellular events such as cell growth, autophagy, and mRNA translation, is activated by insulin-stimulated and Akt-mediated phosphorylation and inhibition of TSC1/2, a negative regulator of mTOR. Inhibition of TSC1/2 leads to the activation of small G protein Rheb and thus enhanced mTORC1 signaling. mTORC1-mediated phosphorylation of Grb10 at $Ser^{501/503}$ switches the binding preference of Grb10 from the insulin receptor to Raptor, leading to the dissociation of Raptor from mTOR and thus down-regulation of mTORC1 signaling.