Aging and Disease
www.aginganddisease.org
Volume 1, Number 2; 147-157, October 2010

Review

Insulin, IGF-1 and longevity

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[Received July 23, 2010; Revised August 23, 2010; Accepted August 24, 2010]

ABSTRACT: It has been demonstrated in invertebrate species that the evolutionarily conserved insulin and insulin-like growth factor (IGF) signaling (IIS) pathway plays a major role in the control of longevity. In the roundworm *Caenorhabditis elegans*, single mutations that diminish insulin/IGF-1 signaling can increase lifespan more than twofold and cause the animal to remain active and youthful much longer than normal. Likewise, substantial increases in lifespan are associated with mutations that reduce insulin/IGF-1 signaling in the fruit fly *Drosophila melanogaster*. In invertebrates, multiple insulin-like ligands exist that bind to a common single insulin/IGF-1 like receptor. In contrast, in mammals, different receptors exist that bind insulin, IGF-1 and IGF-2 with different affinities. In several mouse models, mutations that are associated with decreased GH/IGF-1 signaling or decreased insulin signaling have been associated with enhanced lifespan. However, the increased complexity of the mammalian insulin/IGF-1 system has made it difficult to separate the roles of insulin, GH and IGF-1 in mammalian longevity. Likewise, the relevance of reduced insulin and IGF-1 signaling in human longevity remains controversial. However, studies on the genetic and metabolic characteristics that are associated with healthy longevity and old age survival suggest that the conserved ancient IIS pathway may also play a role in human longevity.

Key words: Insulin; IGF-1; longevity; signaling

Caenorhabditis elegans mutants with defective dauer formation

Much of the evidence that aging is hormonally regulated and that the evolutionarily conserved insulin/IGF-1 signaling (IIS) pathway plays a key role in the hormonal regulation of aging stems from studies on the roundworm *Caenorhabditis elegans* (*C.elegans*) [1]. After hatching, *C.elegans* develops through four successive juvenile (larval) stages into an adult hermaphrodite worm [2]. Under laboratory conditions, the life cycle is normally completed in about three weeks [3]. Natural populations of *C. elegans* have been found in soil and humus and in various sorts of decomposing organic matter (such as rotten fruits, compost and cadavers) [2]. In such natural environments, *C. elegans* will experience strong fluctuations in several key environmental cues, including food availability, temperature, and concentrations of oxygen and ethanol as well as the presence of competitors for food sources. Under unfavorable conditions, *C. elegans* larvae can temporarily exit the cycle of growth and development at the third larval stage, to postpone reproduction and form a so called dauer larva. Dauer larvae are morphologically and physiologically specialized, developmentally arrested, non-feeding and stress-resistant which allows for diapause and dispersal to new habitats once a food source or habitat has been exploited. When conditions become favorable again, the cycle of growth and development into reproductive maturation is resumed. The vast majority

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ISSN: 2152-5250

of isolated *C. elegans* larvae were found to be in the dauer stage, indicating that environmentally induced dauer formation is common in nature. Various forms of stress can trigger dauer formation, including food limitation, crowding and high temperature. Dauer larvae are resistant to a variety of stressors (e.g. starvation, desiccation, extreme temperatures, and toxins). Dauer larvae can survive up to eight times longer than normal under laboratory conditions [3]. Different mutants have been identified that show defects in dauer formation (daf mutants). Strong daf mutations can cause young larvae to permanently arrest as dauers. Subtle daf mutations can cause the larvae to develop into a reproducing adult, while some of the characteristics of the dauer state are maintained [4,5]. These later daf mutants are often long-lived due to preserved dauer-like features, such as enhanced resistance to stress and/or changes in (carbohydrate, lipid and amino acid) metabolism. In the nineties of the last century the genes mutated in these long-lived *C. elegans* daf mutants were cloned and sequenced and the identified genes were shown to exhibit strong homology to components of the mammalian insulin and insulin-like growth factor (IGF) signal transduction cascade (IIS) [6-8].

Insulin and IGF-1 signaling and longevity in invertebrates

The IIS system is an ancient system that is highly conserved and coordinates growth, differentiation and metabolism in response to changing environmental conditions and nutrient availability (see Figure 1) [1]. In invertebrates, such as *C.elegans*, insulin signaling starts with the secretion of multiple, insulin-like peptides in response to food or the sensory perception of food. Insulin-like ligands can bind to a common single insulin/IGF-1 like tyrosine kinase receptor (DAF-2). After ligand binding, the signal is transduced from the activated receptor, either directly or via the adaptor protein IST-1 [9] to the phosphatidylinositol 3-kinase AGE-1 [10]. AGE-1 converts the phospholipid PIP_2 into the second messenger PIP_3, whose elevated levels activate the 3-phosphoinositide dependent protein kinase-1 (PDK1) [11] and the protein kinases B (PKB1-2), thus leading to the phosphorylation of DAF-16, a homolog of the mammalian FoxO family of transcription factors [7,8]. Phosphorylation of DAF-16 causes its translocation from the nucleus to the cytosol. PIP_3 can be dephosphorylated to PIP_2 by the phospatase DAF-18, a homologue of the mammalian phosphatase and tensin homolog PTEN. Reduction-of-function mutations in *daf-2* and the kinase components of the IIS pathway can extend *C.elegans* life span (Table 1) [1]. Conversely, reduction of function mutations in *daf-18* abolishes the life-span extensions of *daf-2* and *age-1* mutants [12]. Downstream targets of DAF-16 include cellular stress response genes, genes encoding antimicrobial peptides and metabolic genes [13].

The organization of the IIS pathway in the fruit fly *Drosophila melanogaster* shows strong similarities to that in *C. elegans*, and consists of multiple extracellular ligands which bind to a common single transmembrane insulin/IGF-1 tyrosine kinase receptor to induce a cascade of intracellular phosphorylation events, culminating in the phosphorylation and nuclear exclusion of dFOXO [14]. In *Drosophila*, numerous subtle loss of function mutations have been associated with enhanced lifespan (Table 1), including those in the insulin receptor (*InR*) [15] and its substrate (CHICO) [16]. Interestingly, the observed effects on longevity were found to be more pronounced in the female sex. Interestingly, foxo null mutant flies were found incapable of adjusting their circadian rhythms under low doses of paraquat and showed an enhanced age-related decline in their ability to maintain circadian rhythms [17].

Insulin and IGF-1 signaling in mammals

Although the core of the insulin/IGF-1 signaling pathway is conserved between invertebrates and mammals (Figure 1), the mammalian IIS network has greatly increased in complexity [18]. In mammals, three different insulin/IGF-1 receptor ligands are present: insulin, IGF-1 and IGF-2. Three different mammalian insulin/IGF tyrosine kinase receptors have been identified: the insulin receptor (IR), IGF-1 receptor (IGF-1R), and the orphan IR related receptor (IRR). In addition, a structurally and functionally distinct mannose-6-phosphate IGF-2 receptor exists, which is thought to have evolved primarily as a scavenger receptor for IGF-2 [19]. After ligand binding, the activated IGF-1 or insulin receptor phosphorylates several intracellular substrates, including IR substrates (IRS) and the Src-homology-2-domain containing transforming protein (Shc). The phosphorylated substrates provide specific docking sites for intracellular effectors, including the p85 regulatory subunit of PI-3K and Growth-factor-receptor-bound protein-2 (Grb2), thus leading to the

activation of two major signaling pathways, the PI-3K-PKB/AKT pathway and the Ras-MAPK pathway (Figure 1). The PI-3K- PKB/AKT pathway has been shown to regulate most of the metabolic effects of insulin/IGF-1 signaling, whereas the Ras-MAPK pathway had been shown to regulate most of the mitogenic effects of insulin/IGF-1 signaling [18].

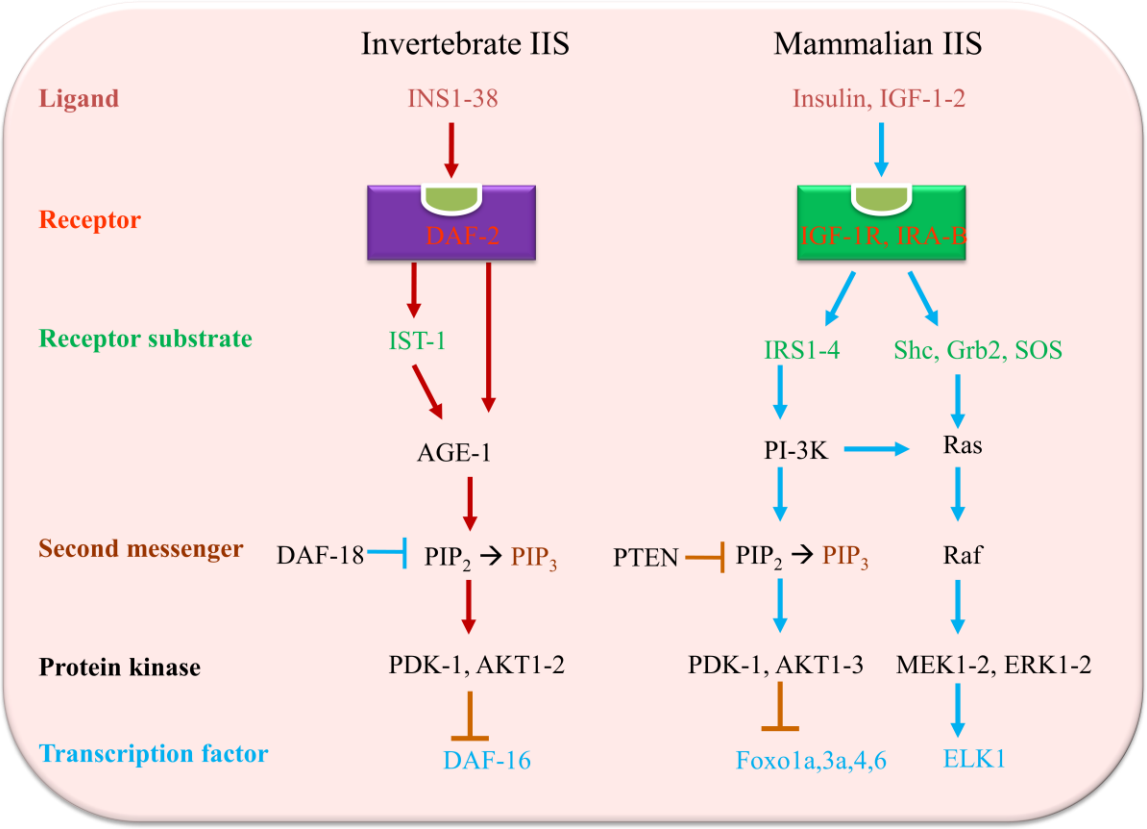

Figure 1. Simplified description of the insulin/IGF-1 signal transduction (IIS) pathway in invertebrates and mammals. In the invertebrate *C.elegans*, multiple insulin/IGF-1 like ligands (INS1-38) bind to a single common receptor (DAF-2). After ligand binding, the signal is transduced from the activated receptor, either directly or via the insulin receptor substrate homolog protein-1 (IST-1) to the phosphatidylinositol 3-kinase (PI-3K) AGE-1 (ageing alteration-1)/AAP-1 [9], which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-trisphosphate (PIP_3). Elevated levels of the second messenger PIP_3 activate the 3-phosphoinositide dependent protein kinase-1 (PDK1) and the protein kinases B (PKB1-2 also known as AKT1-2), thus leading to the phosphorylation of DAF-16, a homolog of mammalian FoxO family of transcription factors by PKB1-2/AKT1-2. In mammals, three different insulin/IGF-1 receptor ligands are present: insulin, IGF-1 and IGF-2, which can bind to the insulin receptor isoforms A or B (IRA-B) or the IGF-1 receptor (IGF-1R). Upon ligand binding, activated insulin or IGF-1 receptors phosphorylate several intracellular substrates, including IR substrates (IRS1-4) and the Src-homology-2-containing protein (Shc). The phosphorylated substrates provide specific docking sites for intracellular effectors, including the p85 regulatory subunit of PI-3K and Growth-factor-receptor-bound protein-2 (Grb2). PI-3K converts PIP_2 in the second messenger PIP_3. Elevated levels of the second messenger activate PDK1 and PKB1-3 (also known as AKT1-3), which culminates, amongst others, in the phosphorylation the mammalian FoxO family of transcription factor members Foxo1a, 3a, 4, 6. Grb2 recruits the GDP/GTP exchange factor Son-of-Sevenless (SOS), upon which the small G-protein Ras is converted in its active conformation, leading to the activation of successively the intracellular kinases Raf (part of the family of mitogen activated protein kinase (MAPK) kinase kinases), Mitogen activated protein kinase/Extracellular-signal-regulated-Kinase kinases MEK1-2 (part of the family of MAPK kinases) and Extracellular-signal-Regulated-Kinases ERK1-2 (part of the family of MAPKs), culminating in the activation of transcription, amongst others, via the transcription factor ELK1 (member of ETS oncogene family).

Further adding to the complexity, for most of the critical components of the mammalian insulin/IGF-1 signaling cascade different forms encoded by different genes and/or different isoforms encoded by a single gene have been identified [18]. Two isoforms exist of the insulin receptor (encoded by one gene), IR-A (lacking exon 11) and IR-B (including exon 11), that show pronounced functional differences [20]. IR-A was found to exhibit high affinity for IGF-2, and has been predominantly implicated in mitogenic IGF signaling, whereas IR-B has been predominantly implicated in metabolic insulin signaling. Moreover, hybrid IR-IGF-1R complexes might be formed that show distinct affinities for insulin, IGF-1 and IGF-2. Likewise, three different isoforms exist of PKB/AKT (PKB α, β and γ or AKT1-3) [18]. Four distinct IRS proteins have been identified in various mammals (IRS1-4), and two additional IRS proteins have been detected in humans (IRS5-6). IRS5 and IRS6 were shown to be involved in insulin signaling, but not in PI-3K activation [21]. For class 1A of PI-3K, eight different isoforms (derived from three genes [22]) have been identified of the regulatory subunit that can associate with three different forms of the PI-3K catalytic subunit (p110α, β and δ) [23]. Moreover, four different members of the mammalian FoxO family of transcription factors have been identified (FOXO1A, FOXO3A, FOXO4 and FOXO6) [24]. The existence of different forms and isoforms that differ in their tissue distribution, subcellular localization and interactions with downstream targets and components from other signaling pathways has greatly enhanced the possibilities for tissue specificity, diversification and fine-tuning of IIS transduction under various physiological states [18]. Concordantly, the complexity of the IIS network has increased enormously. In addition, the mammalian IIS network is tightly linked to growth hormone (GH), that appeared relatively late in evolution within vertebrates. GH produced by the anterior pituitary regulates the biosynthesis and release of IGF-1 by the liver and peripheral tissues. However, GH has other effects on IIS in addition to the effects mediated by IGF-1. Binding of GH to the GH receptor (GHR) results in activation of the associated Janus kinase (JAK2) [25]. In turn, JAK2 activates several intracellular mediators leading to different signaling pathways. The main GH signaling pathways comprise besides STAT (signal transducers and activators of transcription) signaling, the PI-3K- PKB/AKT and the MAPK pathways. Moreover, additional pathways, independent of JAK2, have been described [25].

Insulin and IGF-1 signaling and longevity in mammals

Various mouse mutants with reduced GH/IGF-1/insulin have been shown to be long-lived (Table 1), but the increased complexity of the mammalian IIS network has made it difficult to disentangle the roles of GH, IGF-1 and insulin. *FIRKO* mice, in which the insulin receptor was specifically deleted in fat tissue, provide evidence for a link between longevity and reduced insulin signaling [26]. In addition to being long-lived, *FIRKO* mice exhibit a reduction in fat mass and lessened age related loss of insulin sensitivity [27]. Available evidence indicates that enhanced mitochondrial capacity of white adipose tissue may contribute to the resistance of *FIRKO* mouse to diet induced obesity [28]. Data from other mutant mouse models support a link between reduced GH/IGF-1 signaling and longevity. In mammals, GH produced by the anterior pituitary regulates the biosynthesis and release of IGF-1 by the liver and peripheral tissues to control mammalian growth. Four dwarf mouse models with impeded IGF-1 production, namely $Prop1^{df/df}$ [29], $Pit1^{dw/dw}$ [30], $GHRHR^{lit/lit}$ [30] and $GHR^{-/-}$ [31] all show a long-lived phenotype. Common characteristics of these long-lived GH-deficient dwarfs and GH-resistant dwarfs include reduced circulating levels of insulin and glucose and enhanced insulin sensitivity [32]. Results obtained with mice mutated for the IGF-1 receptor hint at a direct role for reduced IGF-1 signaling in mammalian longevity: $Igf1r^{+/-}$ females, but not males, exhibit a long-lived phenotype as well as increased resistance to oxidative stress [33]. Overexpression of *Klotho* can inhibit IIS [34] and extend lifespan, whereas *Klotho* mutant mice age prematurely [35]. Thus far, the strongest effects on life span in mouse mutants with defective GH/IGF-1 and/or insulin signaling have been observed in the GH deficient hypopituitary dwarfs and the GH resistant $GHR^{-/-}$ dwarfs. Recent evidence strongly suggests that enhancement of insulin sensitivity, in conjunction with reduced insulin levels, is a key factor in the longevity phenotype of these mice as well as in wild type mice subjected to caloric restriction [36]. However, insulin resistance has been reported for other mouse models with extended longevity, including *Klotho* transgenic mice [34], $IRS1^{-/-}$ mice [37] and mice with a brain specific

deletion of IRS2, which are long-lived when fed a high fat diet [38]. It has been suggested that a key feature shared between these insulin resistant mice and the insulin sensitive dwarfs is a reduced strength of the insulin signal in specific key insulin target tissues or organs [36].

IIS and human longevity: studies on genetic polymorphisms

Based on the observed associations between reduced insulin/IGF-1 signaling and longevity in organisms as diverse as worms, flies and mice and given the evolutionarily conservation of the core IIS pathway components, it could be speculated that the genes involved in insulin/IGF-1 signaling might be important for human longevity as well. However, results from human studies have been conflicting and controversial. In humans, defects in insulin signaling have been associated with insulin resistance and diabetes [18]. Also, defects in GH/IGF-1 signaling have been associated with defects in growth and increased risk of cardiovascular disease [39]. However, despite their obesity, patients with Laron syndrome, a human dwarf disease that is associated with IGF-1 deficiency, do not exhibit premature death and seem protected against cancer [40]. Moreover, common polymorphisms in several of the IIS genes have been associated with longevity across diverse cohorts. Genotype combinations at *IGF-IR* and *PI3KCB* genes were found associated with lower free IGF-I plasma levels and were found to be enriched in Italian centenarians [41]. In the Leiden 85-plus Study, a composite score was calculated based on the expected effects (increased or reduced signaling) of genetic variants in the *GHRHR*, *GH1*, *IGF-1*, *INS* and *IRS1* loci [42]. In nonagenarian women of the Leiden 85-plus Study, a lower composite score was found to be associated with shorter stature and improved old age survival [42], as well as with reduced cognitive decline [43]. In studies on Ashkenazi Jewish centenarians and their offspring, higher serum levels of IGF-1 were associated with smaller stature in female offspring of centenarians [44]. Sequence analysis showed overrepresentation of heterozygous mutations in the *IGF-1R* gene among centenarians that were associated with high serum IGF-I levels and reduced activity of the IGF-IR as measured in transformed lymphocytes. Also in Italian centenarians, a higher plasma IGF-I/IGFBP3 molar ratio was found that was positively associated with whole body glucose disposal rate [45]. Another study showed enrichment of a haplotype in the *INSR* gene in Japanese semisupercentenarians [46]. Variants in *AKT* were found associated with longevity across three Caucasian cohorts [47]. Variants in *FOXO3A* have been associated with longevity in an ethnic Japanese population in Hawaii [48], as well as in four different Caucasian cohorts [47,49,50] and in a Chinese cohort [51]. Variation in *FOXO1A* was found associated with higher Hb1Ac levels and mortality in old age [52]. To date, although only few findings have been systematically replicated in different cohorts and confirmed in meta-analyses, these data seem to indicate that of the single genes of the IIS pathway that have been systematically analyzed across different cohorts, variation in *FOXO3A* is most consistently associated with human longevity [1].

IIS and human longevity: studies on insulin sensitivity

In mammals, carbohydrates are an essential fuel source for the central nervous system and the immune system. In response to high levels of circulating glucose, the pancreas secretes insulin, which stimulates the uptake of glucose and its subsequent metabolism (glycolysis and glucose oxidation in the muscle) and the storage of excess carbohydrates (glycogenesis in the liver and lipogenesis in adipose tissue). In response to low circulating glucose levels, metabolism is shifted towards the breakdown of fat reserves (lipolysis in adipose tissue): the liberated fatty acids are used for fatty acid oxidation in the muscle and glycerol is used for the synthesis of glucose in the liver (gluconeogenesis), thus proving carbohydrates for the central nervous system. Under conditions of low circulating glucose levels, glycolysis and lipogenesis are suppressed. With age, insulin sensitivity progressively declines, which significantly contributes to the increased incidence of type 2 diabetes mellitus in older people [53]. Remarkably, centenarians [54] and their offspring [55], as well as the offspring of nonagenarian siblings were found to have a reduced risk of diabetes [56]. In a sample of 52 healthy subjects representing three different age categories of the Italian population (adults, aged subjects and centenarians), centenarians were found to exhibit preserved glucose tolerance, as well as preserved insulin sensitivity as assessed by the hyperinsulinemic euglycemic clamp technique [57]. Whole body glucose disposal rate (per kg fat-free

mass) was significantly higher in centenarians (mean age: 102 years) than in aged subjects (mean age: 78 years) and was comparable to that of adults (mean age: 44.5 years) [57]. In a sample of 466 healthy Italian subjects, covering an age range from 28-110 years, insulin resistance (as determined by homeostasis model assessment) was shown to increase with age and reach a peak around the age of 80 years. However, beyond the ages of 85-90 years, insulin resistance declined again and a group of subjects with a lower degree of insulin resistance emerged [58]. It is unresolved to what extent the preserved insulin sensitivity in centenarians reflects selective survival of subjects with genetically determined favorable insulin sensitivity. Recently, offspring of long-lived nonagenarian siblings were also found to have lower levels of fasting glucose and insulin, a hallmark of enhanced insulin sensitivity, as well as better glucose tolerance compared to a control group of similar age and body composition [59]. Likewise, it is not clear which biological mechanisms contribute to the preservation of insulin sensitivity in centenarians. Interestingly, centenarians were shown to have higher serum levels of insulin sensitizing hormones, most notably adiponectin [60].

Taken together these data suggest that, as in the GH deficient hypopituitary dwarfs and the GH resistant *GHR*$^{-/-}$ dwarfs, low glucose, low insulin and preserved insulin sensitivity may represent a key metabolic feature of a human longevity phenotype. Although speculatively, these metabolic features might reflect a state of reduced flux through the IIS pathway and enhanced FoxO activation.

Strength of the insulin signal: insulin versus Foxo

Class O forkhead box transcription factors (FoxOs) may act as a "master-switch" to adapt cells and organisms to food shortage and ensure metabolic stability under conditions of food shortage and thus opposes many of insulin functions [24]. In addition to PI-3K/PKB-AKT, other kinases, including AMPK (5' adenosine monophosphate-activated protein kinase), JNK (c-Jun NH_2-terminal kinase), and IKKβ (inhibitor of nuclear factor kappa-B kinase subunit beta) can also phosphorylate FoxO, centralizing its role as a master-switch [61]. Moreover, in addition to phosphorylation, other posttranslational modifications, including acetylation and ubiquitination are important in the regulation of FoxO activity [24,61].

Low glucose (low insulin drive), low insulin (reduced strength of the insulin signal) and FoxO activation cause a similar metabolic shift. Cells rely on fatty acid breakdown and oxidative phosphorylation, with concomitant upregulation several anti-oxidant enzymes, including calatase and manganese superoxide dismutase (MnSOD), and of the fatty acetyl-CoA carriers sterol carrier protein x (SCP-x) and SCP2 [62] which protect unsaturated fatty acids from oxidative damage and ensure their proper processing. FoxOs increase insulin sensitivity by feedback control, inducing expression of the insulin receptor and of IRS2 [63]. In addition, FoxO activation enables cellular survival under conditions of food shortage by induction of cell cycle arrest and quiescence, reminiscent of the Dauer switch induced in *C. elegans* [24]. However, depending on the cell type, quality and strength of the stress, FoxO activity can also shift the cellular response from survival towards apoptosis. The mechanisms through which a low insulin drive with enhanced FoxO activation may contribute to longevity include a metabolic shift from glucose to lipid oxidation, with concomitant enhancement of cellular stress resistance and protection, suppression of inflammation and enhanced mitochondrial biogenesis (Figure 2).

As lipids are far more energy dense than glycogen, the ability to store and burn lipids would yield an essential survival advantage when faced with famine and injury/infection [64]. However, lipids are highly susceptible to oxidative damage, especially the unsaturated fatty acids, due to their readily oxidisable double bonds. Lipid peroxidation causes structural damage but will also provide a strong inflammatory signal activating NFκB, which may explain why the ability for lipid storage and metabolism has become tightly coupled to increased oxidative stress resistance and suppression of inflammation. Various intertwining signaling networks have evolved which integrate modulation of energy metabolism with suppression of inflammation and increased resistance to oxidative stress [64,65]. Impairment of signaling via PI-3K/AKT-PKB will induce FoxO activation, while enhanced PI-3K/AKT-PKB will stimulate NFκB (Nuclear transcription Factor kappa-B) signaling via activation of the IKK (Inhibitor of nuclear factor Kappa-B Kinase) complex [65].

Besides upregulation of anti-oxidative stress capacity, another mechanism by which FoxO activation or low insulin drive may reduce oxidative stress includes the activation of Peroxisome

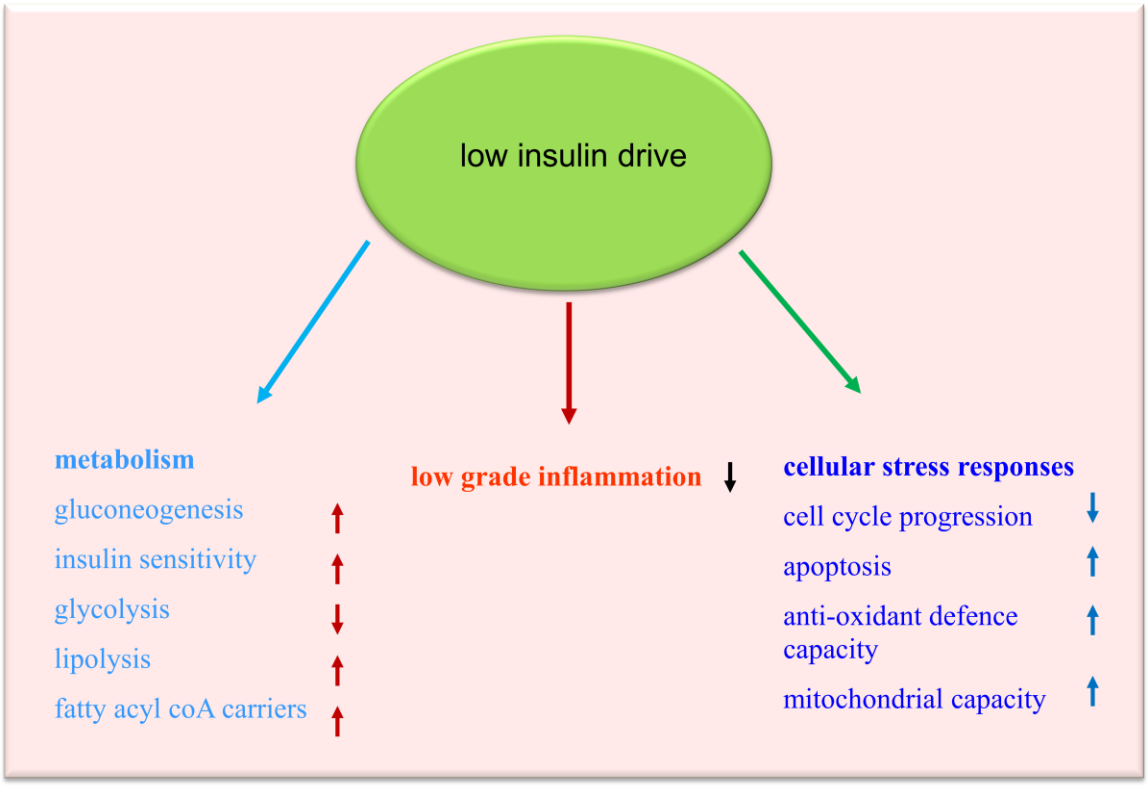

Figure 2. A low insulin drive may be associated with differences in metabolism, low grade inflammation and cellular stress responses.

Proliferator-activated receptor Gamma Coactivator 1α (PPGC-1α), a nutrient sensing system that shifts substrate utilization towards fat and away from carbohydrate and increases mitochondrial biogenesis [66]. Mitochondria are the major consumers of cellular oxygen and 2-5% of consumed oxygen is associated with the production of reactive oxygen species (ROS) as a by-product of oxidative phosphorylation [67]. The most important function of mitochondria is to produce ATP. This is done by transporting protons from the inside to the outside through the inner membrane, which is coupled with the movement of electrons on the electron transport system. Studies in mammals have shown that calorie restriction is associated with increased mitochondrial number [68,69], but decreased oxidative stress and mitochondrial oxygen consumption [70,71]. Also in humans, calorie restriction has been shown to decrease 24 hour energy expenditure and markers of oxidative stress and to improve muscle mitochondrial function and mass [72]. Taken together, these data suggest that calorie restriction improves whole body energy efficiency by inducing the biogenesis of "efficient" mitochondria that utilize less oxygen and produce less reactive oxygen species. Low mitochondrial content may be associated with an increased "workload", leading to higher membrane potential and increased ROS production [73].

Besides protection against oxidative stress, cellular defense mechanisms associated with reduced IIS activity include enhancement of autophagy [74]. In addition to modulation of IIS activity, modulation of the activity of other evolutionarily conserved nutrient sensing and stress sensing signaling pathways, most notably TOR (target of rapamycin) signaling, has been shown to confer life extending effects across different species, possibly through partly overlapping, partly distinct mechanisms [75]. It will be important to determine the precise mechanisms underlying the lifespan extension associated with modulation of IIS activity and the activity of other nutrient and stress sensing pathways.

Conclusion

Table 1. Life span extension in different species with alteration in insulin/IGF-1 signaling

C .elegans	*D. melanogaster*	*M. musculus*	*Affected IIS component*
		Prop1$^{df/df}$ ↑	Prophet of Pit1
		Pit1$^{dw/dw}$ ↑	Pituitary-specific positive transcription factor 1
		GHRHR$^{lit/lit}$ ↑	Growth hormone release hormone receptor
		GHR$^{-/-}$ ↑	Growth hormone receptor
ins-1 ↓, *ins-7* ↑			Insulin-like ligand
		Klotho$^{-/-}$ ↓	Inhibitor of intracellular insulin/IGF-1 signaling (amongst others)
daf-2 ↑	*dInsR* ↑	*FIRKO* ↑ *IGF-1R*$^{+/-}$ ↑♀	(fat) insulin receptor IGF-1 receptor
	CHICO ↑	*Irs1*$^{-/-}$ ↑♀ *Irs2*$^{+/-}$ ↑ or ↔ *bIRS2*$^{-/-}$ ↑ *bIRS2*$^{+/-}$ ↑	Insulin receptor substrate (brain) insulin receptor substrate 2
		p66$^{Shc-/-}$ ↑	P66 isoform of the Src-homology-2-domain containing transforming protein
age-1↑			phosphatidylinositol 3-kinase catalytic subunit
aap-1 ↑			phosphatidylinositol 3-kinase regulatory subunit
daf-18 ↓	*dPTEN* ↓		phosphatase and tensin homolog PTEN
pdk-1 ↑			3-phosphoinositide dependent protein kinase-1
pkb-1/pkb-2 ↑			protein kinases B 1-2
daf-16 ↓			FoxO family of transcription factors

Arrows indicate the effects of mutations or RNAi on lifespan: ↑: increased lifespan, ↓: decreased lifespan, ↔: no chance in life span. In some mutants, effects on life span are only observed in the female (♀) sex.

Studies in different model organisms have shown that the evolutionarily conserved insulin/IGF-1 signal transduction pathway plays a key role in the coordination of growth, differentiation and metabolism in response to changing environmental conditions and nutrient availability. Down-regulation of the IIS pathway in response the harsh environmental conditions leads to FoxO activation. FoxO activation causes a metabolic shift from glucose to lipid oxidation, with concomitant enhancement of cellular stress resistance and protection, suppression of low-grade inflammation and enhanced mitochondrial biogenesis. Preliminary data indicate that genetic variation in IIS pathway genes and metabolic features reminiscent of enhanced FoxO activation associate with human longevity as well. However, clearly more research is necessary to systematically analyze the effects of genetic variation in the IIS pathway and intertwining signaling cascades on human health and metabolic features. Preferably, such analyses should be performed across different human cohorts, and include analyses of different tissues and organ systems during the different phases of the human life span.

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