

Role of insulin/insulin-like growth factor 1 signaling pathway in longevity

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Abstract

The insulin/insulin-like growth factor 1 (IGF-1) signaling pathway is evolutionary conserved in diverse species including *C.elegans*, *saccharomyces cerevisiae*, *Drosophila melanogaster*, rodents and humans, which is involved in many interrelated functions that are necessary for metabolism, growth and reproduction. Interestingly, more and more research has revealed that insulin/IGF-1 signaling pathway plays a pivotal role in the regulation of longevity. Generally, disruption of the power of this pathway will extend longevity in species ranging from *C.elegans* to humans. The role of insulin/IGF-1 in longevity is probably related to stress resistance. Although the underlying mechanisms of longevity are not fully understood, the Insulin/IGF-1 signaling pathway has attracted substantial attention and it will be a novel target to prevent or postpone age-related diseases and extend life span. In this review, we mainly focus on the similar constitution and role of insulin/IGF-1 signaling pathway in *C.elegans*, *saccharomyces cerevisiae*, rodents and humans.

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INTRODUCTION

Why and how do we age? What regulates longevity?

Researchers have asked these questions for long, and some answers are finally emerging. However, the underlying mechanisms of longevity remain elusive.

Recently, some researchers have concluded that the incidence of insulin resistance increases with age and type 2 diabetes can accelerate aging syndromes. Concomitant reduction in plasma insulin and plasma glucose levels, which implies increased sensitivity to insulin, emerges as a hallmark of increased longevity^[1,2]. Interestingly, organisms including *C.elegans*, *saccharomyces cerevisiae*, *Drosophila melanogaster*, rodents and humans have similar insulin/insulin-like growth factor 1 (IGF-1) signaling pathway and mutations in pathway extend life span^[3]. Thus, the potential link between ageing and insulin/IGF-1 signaling has attracted substantial attention.

Here we will review the evidence for the role of insulin/IGF-1 signaling pathway in the control of longevity in species ranging from *C.elegans* to humans.

Caenorhabditis elegans

The insulin/IGF-1 signaling pathway remains in the *C.elegans*. The pathway is composed of proteins encoded by daf-2, age-1, akt-2, daf-16 and daf-18, which not only regulate the dauer diapause and reproduction but also influence the lifespan of the adult^[4]. The gene daf-2 encodes insulin/IGF-1 receptor-like protein, which is probably the ancestor of human insulin receptor, IGF-1 receptor and insulin related receptor, because the predicted DAF-2 protein is 35% identical to the human insulin receptor, 34% identical to the IGF-1 receptor, and 33% identical to the human insulin receptor-related receptor^[5].

Animals with weak daf-2 mutation age slowly and have an extended lifespan compared to wild-type^[5]. The downstream of the gene age-1, which encodes the protein similar to the mammalian p110 catalytic subunit of PI3K, leads to a 65% increase in mean life span^[6]. These effects need the integrity of daf-16, which can help the formation of the dauer. Dauer diapause is a nonfeeding stress-resistant larval state evolved for endurance and dispersal under adverse conditions. The protein daf-16 is similar to a family of mammalian forkhead transcriptional regulator (FOXO). Also, daf-16 encodes a member of the hepatocyte nuclear and plays a central role in the regulation of lifespan^[7].

Interestingly, daf-2 also regulates metabolism. The mutation of daf-2 makes the higher expression of antioxidant enzymes such as catalase and superoxide dismutase (SOD)^[3,8]. The downstream gene of daf-2, age-1 mutation can protect the decline of catalase with age. Also, DAF-16 is a key regulator of heat and oxidative stress resistance, fat storage,

development arrest, fertility, and metabolism. Elevated stress resistance combined with down-regulated central metabolism and reproduction may be coordinated physiological states associated with slow aging^[2]. Down-regulation of *daf-2* signaling upon adult life-span and stress resistance is independent of larval dauer and of adult reproduction.

There are 37 insulin-like ligands in the genome of *C. elegans* and they mainly express in neurons, but are also found in intestine, muscle, epidermis, and gonad^[3]. The same signals from different tissues have different influence on aging. The most important signal is located in the nervous system^[9]. However, recently Libina *et al.*, reported that DAF-16 activity in the intestine completely restores the longevity of *daf-16* (-) germline-deficient animals, and increases the life spans of *daf-16*(-) insulin/IGF-1-pathway mutants substantially. These results indicate that DAF-16 may control two types of downstream signals: DAF-16 activity in signaling cells upregulates DAF-16 in specific responding tissues, possibly via regulation of insulin-like peptides, and also evokes DAF-16-independent responses^[10].

Drosophila melanogaster

In the fly *D. melanogaster*, the insulin/IGF-1 pathway is constituted of insulin/IGF receptor INR, insulin receptor substrate (IRS) CHICO, the PI3K Dp110/p60, and the PI3K target protein PKB (Akt), which regulates growth, size and longevity^[11]. The gene of IGF receptor *inR* is similar to the insulin receptor gene and the IGF-1 receptor gene^[12]. The mutation of *inR* in fly *D. melanogaster* can significantly extend adult longevity. Interestingly, it has been reported that a heteroallelic genotype *InR*^{P5545}/*InR*^{E19} in females can lead to small, infertile and 85% longer life than wild-type^[3]. Also, the long-lived flies share some important characteristics with wild-type adults that are in reproductive diapause, including increased triglycerides and SOD and reduced synthesis of juvenile hormone.

Clancy has reported that the life span of female *D. melanogaster* is also extended by mutation of the IRS homolog *chico*^[13]. Null mutation of the *chico* gene that encodes IRS increases the life span of homozygous *chico1/chico1* female fruit flies by 48%. Interestingly, homozygous males are short-lived, whereas heterozygous animals of both sexes have increased longevity ($\leq 36\%$ in females and 13% in males).

In *D. melanogaster*, insulin-like receptor mediates phosphorylation of dFOXO, the equivalent of nematode *daf-16* and mammalian FOXO3a. Recently, Tatar *et al.*^[14], have reported that dFOXO regulates *D. melanogaster* aging when activated in the adult pericerebral fat body. Interestingly, this limited activation of dFOXO reduces the expression of the insulin-like peptide *dilp-2* synthesized in neurons, and represses endogenous insulin-dependent signaling in peripheral fat body. These results suggest that autonomous and non-autonomous roles of insulin signaling combine to control aging.

Rodents

The decrease of insulin/IGF-1 signaling pathway has also been shown to extend longevity in several rodent models, which include murine genetic models and caloric restricted (CR) rodents. The character of these models again explains

the role of insulin/IGF-1 pathway in longevity.

The gene *pit-1* and *prop-1* in mice can code transcription factor that regulates the development of pituitary. The Snell dwarf (*Pit*^{dw}/*Pit*^{dw}) mice and the Ames dwarf (*Prop1*^{df}/*Prop1*^{df}) mice come from the homozygous mutation of gene locus *pit-1* and *prop-1*, respectively. These mice are dwarfs but live 25-65% longer than wild type^[15,16]. Also, they are deficient in serum growth hormone (GH), thyroid stimulating hormone, and prolactin as well as for IGF-I, which is secreted by liver cells upon stimulation with GH. Furthermore, dwarf mice with high plasma GH but a 90% lower IGF-I [GH receptor/binding protein (GHR/BP) null mice] live longer than the wild-type mice^[17]. Taken together, these studies suggest that the reduction in plasma IGF-I is responsible for a major portion of the life-span increase in dwarf, GH-deficient, and GHR/BP null mice^[18].

Recently, very strong support for the role of insulin/IGF-1 signaling pathway in the control of mammalian aging and for the involvement of this pathway in longevity of IGF-1 deficient mice was provided by Hsieh *et al.*^[19,20]. It was shown that in the Snell dwarf mice, GH deficiency would lead to a decreased IRS-2 pool level, decrease in PI3K activity and its association with IRS-2 and decreased docking of p85 α to IRS-2. The authors conclude that the *Pit-1* mutation may result in physiological homeostasis that favors longevity, and that the Snell dwarf mutant conforms to the nematode longevity paradigm.

To investigate whether IGF-1R also controls longevity in mammals, Holzenberger *et al.*^[21], inactivated the IGF-1R gene in mice (*Igf1r*). Using heterozygous knockout mice because null mutants are not viable, they report that *Igf1r*^{+/-} mice live on average 26% longer than their wild-type littermates ($P < 0.02$). Female *Igf1r*^{+/-} mice live up to 33% longer than wild-type females ($P < 0.001$), whereas the equivalent male mice show an increase in life span by 16%, which is not statistically significant. Also, long-lived *Igf1r*^{+/-} mice do not develop dwarfism, their energy metabolism is normal, and their nutrient uptake, physical activity, fertility and reproduction are unaffected. The spontaneous tumor incidence in the aging cohort of *Igf1r*^{+/-} mice was similar to that in wild-type controls. It is very important that these *Igf1r*^{+/-} mice, and mouse embryonic fibroblasts derived from them, were more resistant to oxidative stress than controls, a known determinant of aging. At the molecular level, IRS and the p52 and p66 isoforms of Shc, both main substrates of IGF-1 receptor, showed decreased tyrosine phosphorylation. p66^{Shc} mediated cellular responses to oxidative stress^[22]. Two main pathways the extracellular-signal regulated kinase/mitogen-activated protein kinase pathway and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway were downregulated in *Igf1r*^{+/-} mice. These results indicate that the IGF-1 receptor may be a central regulator of mammalian life span.

Using the Cre-loxP system, Bluher *et al.*^[23], created mice with fat-specific disruption of the insulin receptor gene (FIRKO mice) and the extension of longevity was observed in this model. These mice have low fat mass, loss of the normal relationship between plasma leptin and body weight, and are protected against age-related and hypothalamic

lesion-induced obesity, and obesity-related glucose intolerance, although their food intake is normal. Both male and female FIRKO mice were found to have an increase in mean life span of approximately 134 d (18%), with parallel increases in median and maximum life spans. Extended longevity in FIRKO mice was associated with a shift in the age at which age-dependent increase in mortality risk becomes appreciable and a decreased rate of age-related mortality, especially after 36 mo of age. In FIRKO mice, the resistance to obesity, despite normal food intake, suggests that metabolic rate is increased, rather than decreased^[24]. The authors believe that decreased fat mass could lead to a decrease in oxidative stress in FIRKO mice. Another possibility is that the increased longevity in these mice is the direct result of altered insulin signaling^[25].

Shimokawa *et al.*^[26], designed another transgenic rat model that can be used to elucidate a role for insulin /IGF-1 or their overlapping signaling pathways in the modulation of longevity in mammals. These transgenic rats were produced from founders created by introducing a fusion gene into rat embryos. Transgenic offspring expressed the rat GH anti-sense RNA in the pituitary gland, spleen, and thymus but not in the lung, liver, heart, kidney, or testes. Male rats homozygous for the transgene (tg/tg) had a reduced number of pituitary GH cells, a lower plasma concentration of IGF-1, and a dwarf phenotype. Heterozygous rats (tg/-) had an intermediate phenotype in plasma IGF-1, food intake, and body weight between tg/tg and control (-/-) rats. The life span of tg/tg rats was 5-10% shorter than -/- rats. In contrast, the life span of tg/- rats was 7-10% longer than -/- rats. It was found that tumors caused earlier death in tg/tg rats; in contrast, tg/- rats had reduced non-neoplastic diseases and a prolonged life span. Immunological analysis revealed a smaller population and lower activity of splenic natural killer cells in homozygous tg/tg rats. The present data on a reduction in plasma glucose and insulin in the transgenic rats suggested a similarity between daf-2 and age-1 mutants in nematodes for the insulin signaling pathway and provided evidence that an optimal level of the GH-IGF-1 axis function needs for longevity in mammals.

CR extends longevity in organisms from yeast to mice and postpones or prevents a remarkable array of diseases and age-dependent deterioration, without causing irreversible developmental or reproductive defects^[27]. Interestingly, CR increases life span of rodents up to 35-40%. Furthermore, CR rodents encompass changes in both insulin and IGF-1 signaling and levels. These animals have lower insulin, glucose and IGF-1 levels, several-fold decrease fat stores, and boosted immune system and defenses against free radicals damage. Low insulin levels in CR animals suggest increased insulin sensitivity^[28]. Indeed, when stimulated with similar levels of insulin, CR rodents have improved ability to increase peripheral glucose uptake, to promote glycogen synthesis and to suppress hepatic glucose production compared to ad libitum fed animals. Thus, the reduced levels of plasma IGF-I in dwarf mice may contribute to disease prevention and life-span extension by simulating CR or more severe starvation conditions. Consistent with this notion is the role of IGF-I in reversing the protection of CR against carcinogen-induced bladder cancer. Apoptosis in the tumor

is decreased 10-fold in CR mice in which the levels of IGF-I are restored, indicating that the activation of antiapoptotic pathways contributes to tumor incidence^[29].

Interestingly, de Cabo *et al.*^[30], described a valuable *in vitro* model for the study of CR. They use sera obtained from either Fisher 344 rats or Rhesus monkeys that were fed ad libitum (AL) or CR diets to culture various cell types. They show that treatment of cultured cells with CR sera caused reduced cell proliferation, enhanced tolerance to oxidants and heat, and heightened expression of stress-response genes. These phenotypic features mirror the effects of CR in animals. Supplementation of CR serum with insulin and IGF-1 partially restored the proliferative and stress-response phenotype that was seen in cells cultured with AL serum, indicating that reduced levels of insulin and IGF-1 likely contribute to the CR-related effects.

Human

Although human ageing is more complicated than *C.elegans* and rodents, centenarian, sporadic mutations, and diseases studies have given significant insight on the role of the insulin/IGF-1 signaling in human longevity. In humans, insulin sensitivity normally declines during aging, and insulin resistance is an important risk factor associated with a variety of intermediate phenotypes (hypertension, atherosclerosis, obesity) strongly affecting morbidity, disability, and mortality among the elderly^[3,31].

Roth *et al.*^[32], reported that the most people with insulin levels live longer. More recently, data from 466 healthy subjects with a wide age range (range 28-110 years) demonstrated a significant reduction of insulin resistance in subjects 90-100 year old^[33]. These data suggest an intriguing peculiarity of this age category and indicate that an efficient insulin response has an impact on human longevity.

Interestingly, polymorphisms at *IGF-IR*, *PI3KCB*, *IRS-1*, *FOXO1A* have been investigated^[34]. IGF-I plasma levels, which decrease significantly with age, are affected by the polymorphisms at *IGF-IR* and *PI3KCB* genes, both alone and in combination. In particular, individuals bearing at least one allele A at the *IGF-IR* locus (*IGF-IR* A⁺) have lower plasma IGF-I levels than the rest of the population. Also, *IGF-IR* A⁺ subjects are found in increased proportion in long-lived individuals. Moreover, genotype combinations of an A allele at the *IGF-IR* locus and a T allele at the *PI3KCB* locus (A⁺/T⁺ subjects) affect IGF-I plasma levels (having A⁻/T⁻ individuals the highest free IGF-I plasma levels), as well as longevity, and the proportion of A⁺/T⁺ subjects significantly increased among long-lived individuals.

Recently, Anisimov *et al.*^[35], firstly reported that mutation located downstream to daf-16 in human insulin signal transduction system is associated with longevity. They invested a group of 137 elderly individuals and concluded that a greater frequency of the apolipoprotein C-III-455C allele was correlated with aging ($P < 0.005$). Also, it is worth noting that centenarians display lower degree of resistance to insulin and lower degree of oxidative stress as compared with elderly persons before 90 years^[36]. Moreover, Ruiz-Torres and Soares de Melo Kirzner compared ageing parameters of young (up to 39 years) and old (over 70 years) individuals

having similar IGF-1 blood levels, the result was that old males with IGF-1 levels similar to young ones do not show the age-dependent decrease in serum testosterone and lean body mass, nor the increase in fat body mass. This provides powerful evidence on the important life-potential role of this peptide^[37].

DISCUSSION

From the data presented, the insulin/IGF-1 pathway has the similar characters in *C.elegans*, *D.melanogaster*, rodents and humans, which include the constitution of the gene, the role in regulation of aging and longevity. All these can be concluded that the pathway exists long ago and the mechanism of aging is evolutionarily conserved.

Reviewing the available data on the benefits and adverse effects of caloric restriction and genetic modifications Longo and Finch suggested three categories of drugs which may have the potential to prevent or postpone age-related diseases and extend life span: drugs that (1) stimulate dwarf mutations and therefore decrease pituitary production of GH; (2) prevent IGF-1 release from the liver, or (3) decrease IGF-1 signaling by the action on either extracellular or intracellular targets^[18]. According to this, Anisimov concluded and reported that effects of antidiabetic biguanides seems to be more adequate in the prevention of age-related deteriorations in glucose metabolism and in insulin signaling pathway as well as in such important for longevity parameters as a fertility and a resistance to oxidative stress and tumorigenesis than those induced by caloric restriction and genetic manipulations^[25].

Although, the insulin/IGF-1 pathway can regulate the life span in different species, the molecular mechanism largely remains unknown. The most possible is that the pathway can enhance the stress resistance. Murakami *et al.*, reported that fibroblasts from Snell dwarf mice show resistance to a variety of forms of lethal injury, including ultraviolet light, heat, paraquat, H₂O₂, and the toxic metal cadmium. This cellular stress resistance may lead to resistance to late-life diseases and frailty, and thereby increase longevity^[38]. Using DNA microarray analysis, Murphy *et al.*^[39], found the insulin/IGF-I pathway not only do cells function non-autonomously to regulate life span but also exert their effect on life span by upregulating a wide variety of genes, including cellular stress-response, antimicrobial and metabolic genes, and by downregulating specific life-shortening genes. In human, Barbieri *et al.*^[36], suggest that centenarians may have been selected for appropriate insulin regulation as well as for the appropriate regulation of tyrosine hydroxylase gene, whose product is rate limiting in the synthesis of catecholamines, stress-response mediators. It was shown that catecholamine may increase free radical production through induction of the metabolic rate and auto-oxidation in diabetic animals^[25,40].

On the other hand, although the insulin/IGF-1 pathway is evolution conserved, the pathway in mammals is more complicated than other low animals. While disruption of the insulin/IGF-1 receptor in nematodes and flies increases lifespan significantly, mammals with genetic or acquired defects in insulin signaling pathway are at a risk for age-

related diseases and increased mortality. This contradiction can be explained by the acquisition of more complicated metabolic pathways in mammals over evolution. Mammals have insulin/IGF-1 receptors in many organs, but their functions are opposite if they are located in the central nervous system or in the periphery; whereas lower species have insulin/IGF-1 receptors signaling mainly through the nervous system. Furthermore, mammals have different and very specific receptors for insulin and IGF-1, with distinct pathways and diverse functions^[41].

Human longevity is mysterious. Though we have found the role of insulin/IGF-1 pathway in regulation of longevity, further investigation would shed light on the molecular mechanism of the pathway so that we will get more methods to decrease the age-related diseases and everyone will be centenarians.

REFERENCES

- 1 **Bartke A**, Chandrashekar V, Dominici F, Tutyn D, Kinney B, Steger R, Kopchick JJ. Insulin-like growth factor 1 (IGF-1) and aging: Controversies and new insights. *Biogerontology* 2003; **4**: 1-8
- 2 **Tatar M**, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; **299**: 1346-1351
- 3 **Barbieri M**, Bonafe M, Franceschi C, Paolisso G. Insulin/IGF-I-signaling pathway: An evolutionarily conserved mechanism of longevity from yeast to humans. *Am J Physiol Endocrinol Metab* 2003; **285**: 1064-1071
- 4 **Claeys I**, Simonet G, Poels J, Van Loy T, Vercammen L, De Loof A, Vanden Broeck J. Insulin-related peptides and their conserved signal transduction pathway. *Peptides* 2002; **23**: 807-816
- 5 **Kimura KD**, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 1997; **277**: 942-946
- 6 **Morris JZ**, Tissenbaum HA, Ruvkun G. A phosphatidylinositol-3-OH kinase family member regulates longevity and diapause in *Caenorhabditis elegans*. *Nature* 1996; **382**: 536-539
- 7 **Henderson ST**, Johnson TE. daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr Biol* 2001; **11**: 1975-1980
- 8 **Honda Y**, Honda S. Oxidative stress and life span determination in the nematode *Caenorhabditis elegans*. *Ann N Y Acad Sci* 2002; **959**: 466-474
- 9 **Wolkow CA**, Kimura KD, Lee MS, Ruvkun G. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 2000; **290**: 147-150
- 10 **Libina N**, Berman JR, Kenyon C. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell* 2003; **115**: 489-502
- 11 **Weinkove D**, Leevers SJ. The genetic control of organ growth: Insights from *Drosophila*. *Curr Opin Genet Dev* 2000; **10**: 75-80
- 12 **Tatar M**, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 2001; **292**: 107-110
- 13 **Clancy DJ**, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ, Partridge L. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 2001; **292**: 104-106
- 14 **Hwangbo DS**, Gersham B, Tu MP, Palmer M, Tatar M. *Drosophila* dFOXO controls lifespan and regulates insulin signaling in brain and fat body. *Nature* 2004; **429**: 562-566
- 15 **Brown-Borg HM**, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature* 1996; **384**: 33
- 16 **Flurkey K**, Papaconstantinou J, Harrison DE. The Snell dwarf mutation Pit1(dw) can increase life span in mice. *Mech Ageing Dev* 2002; **123**: 121-130

- 17 **Coschigano KT**, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* 2000; **141**: 2608-2613
- 18 **Longo VD**, Finch CE. Evolutionary medicine: From dwarf model systems to healthy centenarians? *Science* 2003; **299**: 1342-1346
- 19 **Hsieh CC**, DeFord JH, Flurkey K, Harrison DE, Papaconstantinou J. Implications for the insulin signaling pathway in Snell dwarf mouse longevity: A similarity with the *C. elegans* longevity paradigm. *Mech Ageing Dev* 2002; **123**: 1229-1244
- 20 **Hsieh CC**, DeFord JH, Flurkey K, Harrison DE, Papaconstantinou J. Effects of the Pit1 mutation on the insulin signaling pathway: implications on the longevity of the long-lived Snell dwarf mouse. *Mech Ageing Dev* 2002; **123**: 1245-1255
- 21 **Holzenberger M**, Dupont J, Ducos B, Leneuve P, Geloën A, Even PC, Cervera P, Le Bouc Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003; **421**: 182-187
- 22 **Holzenberger M**. The GH/IGF-I axis and longevity. *Eur J Endocrinol* 2004 ; **151**(Suppl 1): S23-S27
- 23 **Bluher M**, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 2003; **299**: 572-574
- 24 **Bluher M**, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR. Adipose tissue selective insulin receptor knock-out protects against obesity and obesity-related glucose intolerance. *Dev Cell* 2002; **3**: 25-38
- 25 **Anisimov VN**. Insulin/IGF-1 signaling pathway driving aging and cancer as a target for pharmacological intervention. *Exp Gerontol* 2003; **38**: 1041-1049
- 26 **Shimokawa I**, Higami Y, Utsuyama M, Tuchiya T, Komatsu T, Chiba T, Yamaza H. Life span extension by reduction in growth hormone-insulin-like growth factor-1 axis in a transgenic rat model. *Am J Pathol* 2002; **160**: 2259-2265
- 27 **Finch CE**, Ruvkun G. The genetics of aging. *Annu Rev Genomics Hum Genet* 2001; **2**: 435-462
- 28 **Gupta G**, She L, Ma XH, Yang XM, Hu M, Cases JA, Vuguin P, Rossetti L, Barzilai N. Aging does not contribute to the decline in insulin action on storage of muscle glycogen in rats. *Am J Physiol Regul Integr Comp Physiol* 2000; **278**: R111-117
- 29 **Dunn SE**, Kari FW, French J, Leininger JR, Travlos G, Wilson R, Barrett JC. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res* 1997; **57**: 4667-4672
- 30 **de Cabo R**, Furer-Galban S, Anson RM, Gilman C, Gorospe M, Lane MA. An *in vitro* model of caloric restriction. *Exp Gerontol* 2003; **38**: 631-639
- 31 **Facchini FS**, Hua N, Abbasi F, Reaven GM. Insulin resistance as a predictor of age-related diseases. *J Clin Endocrinol Metab* 2001; **86**: 3574-3578
- 32 **Roth GS**, Lane MA, Ingram DK, Mattison JA, Elahi D, Tobin JD, Muller D, Metter EJ. Biomarkers of caloric restriction may predict longevity in humans. *Science* 2002; **297**: 811
- 33 **Paolisso G**, Barbieri M, Rizzo MR, Carella C, Rotondi M, Bonafe M, Franceschi C, Rose G, De Benedictis G. Low insulin resistance and preserved beta-cell function contribute to human longevity but are not associated with TH-INS genes. *Exp Gerontol* 2001; **37**: 149-156
- 34 **Bonafe M**, Barbieri M, Marchegiani F, Olivieri F, Ragno E, Giampieri C, Mugianesi E, Centurelli M, Franceschi C, Paolisso G. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: Cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocrinol Metab* 2003; **88**: 3299-3304
- 35 **Anisimov SV**, Volkova MV, Lenskaya LV, Khavinson VK, Solovieva DV, Schwartz EI. Age-associated accumulation of the apolipoprotein C-III gene T-455C polymorphism C allele in a Russian population. *J Gerontol A Biol Sci Med Sci* 2001; **56**: B27-32
- 36 **Barbieri M**, Rizzo MR, Manzella D, Grella R, Ragno E, Carbonella M, Abbatecola AM, Paolisso G. Glucose regulation and oxidative stress in healthy centenarians. *Exp Gerontol* 2003; **38**: 137-143
- 37 **Ruiz-Torres A**, Soares de Melo Kirzner M. Ageing and longevity are related to growth hormone/insulin-like growth factor-1 secretion. *Gerontology* 2002; **48**: 401-407
- 38 **Murakami S**, Salmon A, Miller RA. Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J* 2003; **17**: 1565-1566
- 39 **Murphy CT**, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 2003; **424**: 277-283
- 40 **Singal PK**, Beamish RE, Dhalla NS. Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. *Adv Exp Med Biol* 1983; **161**: 391-401
- 41 **Rincon M**, Muzumdar R, Atzmon G, Barzilai N. The paradox of the insulin/IGF-1 signaling pathway in longevity. *Mech Ageing Dev* 2004; **125**: 397-403