PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

*Phil. Trans. R. Soc. B* (2011) **366**, 28–34
doi:10.1098/rstb.2010.0281

*Review*

# Single-gene mutations and healthy ageing in mammals

**Andrzej Bartke**[1,2,*]

[1]*Department of Physiology, and* [2]*Department of Internal Medicine, Southern Illinois University School of Medicine, 801 North Rutledge Street, Room 4389, Springfield, IL 62794-9628, USA*

Studies of the effects of single-gene mutations on longevity in *Caenorhabditis elegans, Drosophila melanogaster* and *Mus musculus* identified homologous, highly conserved signalling pathways that influence ageing. In each of these very distantly related species, single mutations which lead—directly or indirectly—to reduced insulin, insulin-like growth factor (IGF) or insulin/IGF-like signalling (IIS) can produce significant increases in both average and maximal lifespan. In mice, most of the life-extending mutations described to date reduce somatotropic (growth hormone (GH) and IGF-1) signalling. The reported extensions of longevity are most robust in GH-deficient and GH-resistant mice, while suppression of somatotropic signalling 'downstream' of the GH receptor produces effects that are generally smaller and often limited to female animals. This could be due to GH influencing ageing by both IGF-1-mediated and IGF-1-independent mechanisms. In mutants that have been examined in some detail, increased longevity is associated with various indices of delayed ageing and extended 'healthspan'. The mechanisms that probably underlie the extension of both lifespan and healthspan of these animals include increased stress resistance, improved antioxidant defences, alterations in insulin signalling (e.g. hypoinsulinaemia combined with improved insulin sensitivity in some mutants and insulin resistance in others), a shift from pro- to anti-inflammatory profile of circulating adipokines, reduced mammalian target of rapamycin-mediated translation and altered mitochondrial function including greater utilization of lipids when compared with carbohydrates.

**Keywords:** IGF-1; growth hormone; lifespan; longevity; mutation

## 1. INTRODUCTION

The process of biological ageing is surprisingly difficult to define, but there is little doubt that it is associated with and/or due to a multitude of changes in the function of all organ systems and in the resistance to stress and disease. Like most phenotypic characteristics, ageing and longevity appear to be determined by a complex interplay of the genetic endowment with environmental influences. Anecdotal evidence that surviving to exceptionally old age 'runs in the families' existed for millennia and is amply supported by recent studies of centenarians and their progeny.

Greatly increased interest in the study of the genetic control of ageing during the past 20 years stems from the exciting observations that mutation of a single gene can markedly increase the lifespan in a microscopic worm *Caenorhabditis elegans* [1,2], and that 'longevity genes' discovered in this organism have homologues in the mammalian genome [3–7]. Existence of single-gene mutations that extend life creates unique opportunities for elucidating the mechanisms of ageing and identifying potential targets for interventions designed to slow down or delay this process. What this article hopes to accomplish is to present some key findings from the studies of mutations that extend longevity in mice and to discuss conclusions and hypotheses concerning mechanisms of mammalian ageing that can be derived from these studies.

*abartke@siumed.edu

One contribution of 15 to a Discussion Meeting Issue 'The new science of ageing'.

## 2. MUTATIONS AFFECTING GROWTH HORMONE SIGNALLING

Substantial and reproducible increases in the average, median and maximal longevity in both males and females were documented in mice with mutations that block growth hormone (GH) signalling. These include spontaneous recessive loss-of-function mutations of the pituitary factor 1 (Pit1) and Prophet of Pit 1 (Prop-1) genes, which block differentiation of GH-producing (as well as prolactin and thyrotropin-producing) cells in the anterior pituitary in Snell dwarfs and Ames dwarfs, respectively, and targeted disruption 'knockout' of the gene encoding GH receptor and GH-binding protein in Ghr−/− (GHRKO) mice [8–10]. 'Little' mice with a mutation of the receptor for GH-releasing hormone (GHRH), a hypothalamic peptide that stimulates GH release, also live longer than their normal siblings but the increase in their longevity apparently depends on the interaction of their genetic background with fat content in the diet [9].

In addition to the extended lifespan, Ames dwarf, Snell dwarf and GHRKO ('Laron dwarf') mice exhibit longer healthspan and various features of delayed ageing. These characteristics include delayed onset and reduced incidence of fatal neoplastic disease [11–13]; delayed immune system and collagen ageing in Snell dwarfs [9], and maintenance of cognitive functions into advanced age in Ames dwarf and GHRKO mice [14,15]. Although the potential roles of prolactin deficiency, thyroid-stimulating hormone deficiency and the resulting hypothyroidism in the remarkable longevity of Snell and Ames dwarf mice remains to be thoroughly evaluated ([9,13]; J. Panici, M. Masternak & A. Bartke 2008, unpublished data), overlap of the phenotypes of these mutants with the characteristics of GHRKO and the Little mice suggests that deficiency of GH is the leading cause of their delayed ageing and extended survival.

Ames dwarf, Snell dwarf, GHRKO and Little mice share a number of phenotypic characteristics that can be postulated to account for, or more likely, contribute to their extended longevity. Prominent among these characteristics are:

— Reduced hepatic IGF-1 expression and circulating IGF-1 levels. Proliferative and anti-apoptotic actions of IGF-1 are believed to enhance the progression (and perhaps also initiation) of neoplastic disease, and tumours are the most common cause of death in laboratory mice.
— Increased resistance of dermal fibroblasts to a variety of oxidative, cytotoxic and metabolic stresses *in vitro* [16,17], which in Snell dwarfs is combined with somewhat greater capacity for DNA base excision repair [18]. In Ames dwarfs, this is associated with increased activity of antioxidant enzymes [19] and increased *in vivo* resistance to paraquat, an agent producing oxidative stress, primarily in the lungs [20]. Association of increased stress resistance with longevity is well documented in worms and insects.
— Reduced insulin levels and enhanced insulin sensitivity. These characteristics are opposite to the key features of the metabolic syndrome and are believed to delay and/or slow ageing and to protect from a number of age-related diseases [21–24].
— Increased adiponectin levels. Adiponectin is a major product of white adipose tissue, which promotes insulin sensitivity and exerts anti-inflammatory and anti-atherogenic effects. In contrast to the findings in genetically normal (wild-type) animals, increased adiponectin levels in long-lived mutants are associated with normal or increased rather than reduced adiposity [25–27]. Interestingly, high levels of adiponectin were detected in centenarians from different populations (reviewed in [28]).
— Reduced body size. Within species, smaller body size is generally associated with longer lifespan and this relationship is particularly striking in laboratory mice and domestic dogs ([29,30]; reviewed in [31]). It is very unlikely that body size *per se* influences ageing, but it represents a biomarker of several processes that are very likely to be involved, including somatotropic and mammalian target of rapamycin (mTOR) signalling. GH and IGF-1 promote proliferation and survival of cells and mTOR regulates translation, protein synthesis and cell size. Expression and/or activation of mTOR are reduced in Ames dwarf and GHRKO mice [23,32]. Pharmacological suppression of mTOR or genetic deletion of one of its targets extends longevity in mice [33,34].

Other physiological characteristics of GH-related mutants that may contribute to their longevity are not discussed here because of limitations of space or because they have been examined in only one long-lived mutant.

## 3. MUTATIONS AFFECTING INSULIN-LIKE GROWTH FACTOR SIGNALLING

Association of reduced plasma IGF-1 levels with extended longevity was consistently observed in GH-deficient and GH-resistant mice [8,10,35,36]. However, in view of the well-documented role of hormones homologous to IGF-1 and insulin in the control of ageing in worms and insects, it was of great interest to determine whether a more direct targeting of IGF-1 signalling can affect longevity in mammals.

While homozygous deletion of genes encoding IGF-1 receptor or IGF-1 is lethal or produces severe developmental defects, extended longevity was reported in mice heterozygous for the deletion of IGF-1 receptor [37], expressing a hypomorphic IGF-1 allele that leads to a reduction in circulating IGF-1 levels [38] as well as in mice homozygous for the deletion of insulin receptor substrate (IRS)-1 [39] or heterozygous for the deletion of IRS2 [40]. Activation of IRS1 and IRS2 mediates cellular transmission of IGF-1 as well as insulin signals. Life extension in these mutants was relatively modest and, in contrast to the results obtained in hypopituitary dwarfs and GHRKO mice, was significant only in females. Moreover, data concerning longevity of Igflr+/− and IRS2+/− mice were not readily reproducible in other laboratories, presumably due to differences in the genetic background of the animals and composition (in particular, fat content) of the diet [28,41,42]. However, heterozygous deletion of IGF-1 receptor gene consistently improved resistance to oxidative stress [37,41], and IRS1−/− mice had phenotypic characteristics of delayed ageing [39]. It was also recently shown that Alzheimer's model mice can be protected from developing various transgenic pathological symptoms and cognitive deficits by deficiency of IGF-1 receptor or IRS2 [43–45].

Since IGF-1 mimics some of the key insulin actions and GH is anti-insulinaemic, it is not surprising that alterations in insulin signalling detected in GH-deficient and GH-resistant mice were not seen in animals with disruption of genes encoding IGF-1, IGF-1 receptors or IRSs. For example, IRS1−/− mice are insulin and IGF-1 resistant [39], resembling the situation described earlier in long-lived transgenic mice overexpressing Klotho [46].

It is of particular interest that deleting IRS2 only in the brain was sufficient to increase lifespan [40]. It was also reported that selective reduction of IGF-1 signalling in the brain by heterozygous deletion of IGF-1

receptor gene in this organ reduced the size of the pituitary and suppressed GH signalling, and that these changes were associated with marked reduction of early mortality and increase in the average (although not the maximal) lifespan in both sexes [47]. It has previously been documented that in the invertebrates altering expression of longevity genes selectively in a particular organ can increase lifespan and that the central nervous system is particularly important in this regard.

The physiological role of IGF-1 in the control of mammalian ageing is strongly supported by the recent demonstration of a significant extension of longevity in mice by deletion of pregnancy-associated plasma protein A (PAPP-A) [48]. PAPP-A is a protease that degrades IGF-1 binding proteins, in particular IGFBP4 (which is widely expressed), and as a result increases local levels of bioavailable (free, i.e. not complexed with IGF-BPs) IGF-1.

## 4. MUTATIONS SELECTIVELY AFFECTING INSULIN SIGNALLING

Ageing in *C. elegans* and *Drosophila* is regulated by signalling molecules homologous to both IGF-1 and insulin, and acting via a single common receptor. In mammals, biosynthesis of insulin and IGF-1 is regulated by different mechanisms and these two hormones signal mostly via distinct, separate receptors. However, their intracellular signalling pathways overlap and interact, and the results of deleting IRS1 or IRS2 discussed earlier in this paper may have reflected reduced action of insulin, rather than of IGF-1.

The amount of information on the impact of specific alterations in insulin signalling on mammalian ageing is very limited. Similar to the effects of deleting IGF-1 or its receptor, complete elimination of insulin signalling is incompatible with survival. However, mice with selective deletion of insulin receptor (IR) in specific organs survive and have been used to obtain a wealth of information on the local and systemic insulin actions [49]. In 2003, Blüher and his colleagues reported that adipose tissue-specific deletion of IR in Fat-specific insulin receptor knockout (FIRKO) mice leads to significantly extended longevity [50]. These very interesting animals appear to develop normally but exhibit progressive depletion of both brown and white adipose tissue, increased adiponectin, increased oxidative metabolism, mild hypoglycaemia, very pronounced hyperinsulinaemia and insulin resistance [50–52]. The unique phenotype of FIRKO mice contrasts with the characteristics of animals subjected to calorie restriction (CR), which are also lean and long-lived but are hypo- rather than hyperinsulinaemic, and insulin-sensitive rather than -resistant. The FIRKO mouse phenotype is also very different from the GHRKO and hypopituitary mouse in which adiposity is increased or normal rather than reduced, circulating insulin is very low and insulin sensitivity is enhanced. Consequently, it is difficult to know, or even hypothesize which of the characteristics of FIRKO mice are causally related to their extended longevity. It is interesting that plasma adiponectin levels are increased in FIRKO, CR, GHRKO, Ames dwarf and Snell dwarf mice.

## 5. OTHER LONG-LIVED MOUSE MUTANTS

Ablation of the p66Shc adaptor protein increases resistance to oxidative stress and extends longevity in mice, without significant alterations in growth or fertility [53]. Subsequent studies have shown that p66Shc is the only proapoptotic member of this protein family and that it acts as a downstream target of an important tumour suppressor p53, mediating its effects on the levels of intracellular oxidants, cytochrome *c* release and apoptosis [54]. p66Shc associates with mitochondrial heat shock protein 70 and regulates mitochondrial transmembrane potential, leading to its collapse after oxidative stress [55]. Macrophages of long-lived p66Shcc−/− mice exhibit reduced superoxide production [56]. Thus, putative mechanisms linking p66Shc with the control of ageing overlap those related to the somatotropic and insulin signalling: oxidative stress resistance, mitochondrial function, reactive oxygen species production and apoptosis.

Involvement of defences against oxidative stress in the control of mammalian ageing is further supported by the studies in transgenic animals overexpressing catalase in mitochondria, the mitochondrial-targeted catalase (MCAT) mice [57]. These animals exhibit delays in the development of age-related cardiac and ocular pathology, produce less hydrogen peroxide and have fewer mitochondrial deletions [57]. At the end of life, MCAT mice have reduced disease burden including a decrease in non-haematopoietic malignant tumours [58]. However, the exact role of oxidative stress and defences in the control of mammalian ageing is currently a subject of extensive debate.

Increase in lifespan was also produced by deletion or overexpression of genes not directly related to the insulin/IGF-like signalling (IIS) pathway. Thus, deletion of the RIIβ regulatory isoform of protein kinase A in mice leads to a significant extension of longevity in males, along with various indices of healthy ageing, including maintenance of a lean phenotype and insulin sensitivity, and a decrease in tumour incidence [59]. Deletion of a homologous gene in yeast was previously shown to extend lifespan [60].

Mice with a genetic disruption of type 5 adenylyl cyclase live longer than control mice and are protected from different types of cardiac stress as well as from age-related cardiac myopathy [61]. This was associated with activation of the Raf/MEK/ERK signalling pathway and increased fibroblast resistance to oxidative stress *in vitro* [61]. This latter characteristic is shared with other long-lived mutants and provides yet another example of enhanced stress resistance of animals with genetic predisposition for extended longevity.

Increased lifespan and protection from neurotoxic effects of kainic acid were described in mice with knockout of *Surf1*, a gene related to cytochrome *c* oxidase [62].

Various indices of delayed ageing along with an increase in lifespan were described in mice overexpressing a combination of telomerase reverse transcriptase and tumour suppressors p53, p16 and p19ARF [63]. Anti-ageing effects of enhanced but normally regulated expression of Arf and p53 were demonstrated earlier by the same group [64].

Conti and his colleagues reported increased longevity in both sexes of transgenic mice in which core body temperature was reduced by 0.3–0.5°C following overexpression of uncoupling protein 2 in hypothalamic hypocretin neurons [65]. In these animals, reduction in core body temperature was induced by a local increase in hypothalamic temperature and was associated with increased energy efficiency without alterations in food intake. These results relate in very interesting ways to the reciprocal relationships between environmental temperature and lifespan in invertebrates and in fish and also to the findings concerning somatotropic control of ageing in mammals. GH-resistant long-lived hypopituitary mice are hypoinsulinaemic and their core body temperature measured hourly for 24 h was significantly or numerically reduced at each time point [66]. Both GH and insulin are thermogenic.

Increased longevity of transgenic mice overexpressing Klotho [46] was mentioned in §3 in the context of relating insulin signalling to the control of ageing. In these very interesting animals, extended longevity is associated with resistance to both IGF-1 and insulin, perhaps offering protection from normal exposure to these signals. Klotho is a single-pass transmembrane protein that is expressed in the kidney and interacts with various fibroblast growth factor (FGF) receptors. It is an obligatory co-receptor of FGF23 and thus plays a major role in phosphate balance. Deletion of the Klotho gene causes phosphate retention and multiple symptoms of premature ageing that can be ameliorated by correcting hyperphosphataemia [67]. Extracellular domain shedding produces a circulating factor (secreted Klotho) which acts on surfaces of cells to modulate function of channels and growth factors including IGF-1, insulin and Wnt [68]. Klotho transgenics are resistant to oxidative stress, likely owing to reduced insulin/IGF-1 signalling, activation of Forkhead box, class O (FOXO) and increased expression of manganese superoxide dismutase [69].

## 6. DISCUSSION AND RELEVANCE TO OTHER MAMMALIAN SPECIES

It is now almost 40 years since Silberberg reported that Snell dwarf mice, animals homozygous for a spontaneous recessive mutation affecting pituitary development and somatic growth, were long-lived [70]. Studies conducted during the past 15 years have provided undeniable evidence that loss-of-function mutations (spontaneous or produced by targeted gene disruption or 'gene knockout') of many genes can lead to significant increases of healthspan and longevity in laboratory mice. The key and most striking conclusion that follows from these observations is that normal levels of expression of numerous genes are not optimal for maintenance of youthful phenotype or for long-term survival. Apparently normal physiological actions of these genes, or rather their protein products, incur significant 'costs' in terms of ageing and survival.

Studies in mutant mice demonstrated that somatotropic (GHRH, GH, IGF-1) signalling can have major impact on ageing, healthspan and lifespan. This echoes findings in *C. elegans* and *D. melanogaster* in which partial suppression of the actions of hormones homologous to IGF-1 and insulin can produce spectacular increases in longevity. As was mentioned earlier in this article, extension of longevity by mutations affecting GH signalling are generally greater and more consistent (between females and males and between different studies) than the effects of mutations acting directly on IGF-1 levels or actions. This could be ascribed to GH effects that are not mediated by increases in IGF-1 expression and/or circulating IGF-1 levels, or to actions of GH that are distinct from and in some cases opposite to the effect of IGF-1. Examples of such GH actions would include lipolysis and inducing insulin resistance by phosphorylating IRS1 at specific serine residues.

Moreover, mutations of genes encoding IGF-1, IGF-1 receptor, IRS1 and IRS2 alter IGF-1 signalling in all tissues of the affected individual, while reduced GH action selectively suppresses IGF-1 expression in some organs (e.g. liver and kidney) while preserving it elsewhere, including the brain and the heart [71,72]. Differences between the phenotypes of 'GH-related' and 'IGF-1-related' mutants may also stem from the fact that they are based on comparisons of the effects of complete blockade of GH signals and partial suppression of IGF-1 levels and actions. Unlike IGF-1, GH is not essential for foetal or early postnatal development and therefore animals completely lacking GH or GH receptors are viable and available for the study.

It is of obvious interest to ask to what extent findings obtained from studies of laboratory stocks of mice may apply to other mammals. Most of the laboratory mice used for contemporary research are derived from animals that have been domesticated as much as 100 years ago or even earlier and thus have been subjected for hundreds of generations to deliberate or inadvertent selection for genotypes (and epigenetic mechanisms) that favour thriving and fecundity under very artificial conditions of constant availability of food and little need or opportunity for physical activity. In some of the long-lived mouse mutants, puberty is delayed and fecundity reduced (for a review of data on Ghr−/− mice, please see [73]). Interestingly, age of puberty and litter size in these mutants resemble the characteristics of normal mice derived from animals recently caught in the wild [74]. However, negative association of adult body size (a biomarker of somatotropic signalling) and average lifespan applies not only to various mutants and transgenics and to genetically normal laboratory mice, but also to other species likely including humans [30,75–77]. While relationships between different ontogenic phases of growth, adult stature and lifespan in different human populations are complex and controversial, there is evidence that both GH deficiency and GH resistance can protect the affected individuals from age-related disease, including cancer and atherosclerosis [78–80]. There is also considerable evidence for a reciprocal relationship between adult height and risk of various types of cancer.

Finally, studies in cohorts of elderly subjects and in exceptionally long-lived people suggest that reduced somatotropic signalling as well as mild (subclinical) hypothyroidism are associated with lower rates of mortality in women [81–84]. Most relevant to the subject of this article are reports of association of human longevity with polymorphism of genes related to somatotropic and insulin signalling [82,83]. Interestingly, these associations include polymorphism of genes coding for adiponectin and its receptor [85] and genes for FOXO ([86]; review in [87]), an important transcription factor controlled by insulin via alterations in its phosphorylation state and intracellular location (cytoplasmic versus nuclear).

Studies of ageing in our laboratory received support from NIA, the Ellison Medical Foundation, the Glenn Foundation for Medical Research and SIU Geriatric Research Initiative. Sincere apologies are extended to those whose work pertinent to the subject was not cited because of inadvertent omission or space limitations. Assistance of Carol Thomas and Steve Sandstrom in preparing the manuscript is gratefully acknowledged.

## REFERENCES

1 Johnson, T. E., Conley, W. L. & Keller, M. L. 1988 Long-lived lines of *Caenorhabditis elegans* can be used to establish predictive biomarkers of aging. *Exp. Gerontol.* **23**, 281–295. (doi:10.1016/0531-5565(88)90031-9)
2 Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. 1993 A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464. (doi:10.1038/366461a0)
3 Kimura, K. D., Tissenbaum, H. A., Liu, Y. & Ruvkun, G. 1997 *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**, 942–946. (doi:10.1126/science.277.5328.942)
4 Morris, J. Z., Tissenbaum, H. A. & Ruvkun, G. 1996 A phosphatidylinositol-1-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**, 536–539. (doi:10.1038/382536a0)
5 Tatar, M., Bartke, A. & Antebi, A. 2003 The endocrine regulation of aging by insulin-like signals. *Science* **299**, 1346–1351. (doi:10.1126/science.1081447)
6 Guarente, L. & Kenyon, C. 2000 Genetic pathways that regulate ageing in model organisms. *Nature* **408**, 255–262. (doi:10.1038/35041700)
7 McElwee, J. J. *et al.* 2007 Evolutionary conservation of regulated longevity assurance mechanisms. *Genome Biol.* **8**, R132. (doi:10.1186/gb-2007-8-7-r132)
8 Brown-Borg, H. M., Borg, K. E., Meliska, C. J. & Bartke, A. 1996 Dwarf mice and the ageing process. *Nature* **384**, 33. (doi:10.1038/384033a0)
9 Flurkey, K., Papaconstantinou, J., Miller, R. A. & Harrison, D. E. 2001 Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc. Natl Acad. Sci. USA* **98**, 6736–6741. (doi:10.1073/pnas.111158898)
10 Coschigano, K. T., Holland, A. N., Riders, M. E., List, E. O., Flyvbjerg, A. & Kopchick, J. J. 2003 Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. *Endocrinology* **144**, 3799–3810. (doi:10.1210/en.2003-0374)
11 Ikeno, Y., Bronson, R. T., Hubbard, G. B., Lee, S. & Bartke, A. 2003 Delayed occurrence of fatal neoplastic diseases in Ames dwarf mice: correlation to extended longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **58**, 291–296.
12 Ikeno, Y. *et al.* 2009 Reduced incidence and delayed occurrence of fatal neoplastic diseases in growth hormone receptor/binding protein knockout mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **4**, 522–529.
13 Vergara, M., Smith-Wheelock, M., Harper, J. M., Sigler, R. & Miller, R. A. 2004 Hormone-treated Snell dwarf mice regain fertility but remain long-lived and disease resistant. *J. Gerontol. A Biol. Sci. Med. Sci.* **59**, 1244–1250.
14 Kinney, B. A., Coschigano, K. T., Kopchick, J. J., Steger, R. W. & Bartke, A. 2001 Evidence that age-induced decline in memory retention is delayed in growth hormone resistant GH-R-KO (Laron) mice. *Physiol. Behav.* **72**, 653–660. (doi:10.1016/S0031-9384(01)00423-1)
15 Kinney, B. A., Meliska, C. J., Steger, R. W. & Bartke, A. 2001 Evidence that Ames dwarf mice age differently from their normal siblings in behavioral and learning and memory parameters. *Horm. Behav.* **39**, 277–284. (doi:10.1006/hbeh.2001.1654)
16 Murakami, S., Salmon, A. & Miller, R. 2003 Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J.* **17**, 1565–1566. (doi:10.1096/fj.02-1092fje)
17 Salmon, A. B., Murakami, S. & Bartke, A. 2005 Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress. *Am. J. Physiol. Endocrinol. Metab.* **289**, E23–E29. (doi:10.1152/ajpendo.00575.2004)
18 Page, M. M., Salmon, A. B., Leiser, S. F., Robb, E. L., Brown, M. F., Miller, R. A. & Stuart, J. A. 2009 Mechanisms of stress resistance in Snell dwarf mouse fibroblasts: enhanced antioxidant and DNA base excision repair capacity, but no differences in mitochondrial metabolism. *Free Radic. Biol. Med.* **46**, 1109–1118. (doi:10.1016/j.freeradbiomed.2009.01.014)
19 Brown-Borg, H. M. 2006 Longevity in mice: is stress resistance a common factor? *Age* (*Dordr*) **28**, 145–162. (doi:10.1007/s11357-006-9003-y)
20 Bokov, A. F., Lindsey, M. L., Khodr, C., Sabia, M. R. & Richardson, A. 2009 Long-lived Ames dwarf mice are resistant to chemical stressors. *J. Gerontol. A Biol. Sci. Med. Sci.* **64**, 819–827.
21 Bartke, A. 2008 Insulin and aging. *Cell Cycle* **7**, 3338–3343.
22 Bonkowski, M. S., Rocha, J. S., Masternak, M. M., Al Regaiey, K. A. & Bartke, A. 2006 Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. *Proc. Natl Acad. Sci. USA* **103**, 7901–7905. (doi:10.1073/pnas.0600161103)
23 Bonkowski, M. S. *et al.* 2009 Disruption of growth hormone receptor prevents calorie restriction from improving insulin action and longevity. *PLoS ONE* **4**, e4567. (doi:10.1371/journal.pone.0004567)
24 Masternak, M. M., Panici, J. A., Bonkowski, M. S., Hughes, L. F. & Bartke, A. 2009 Insulin sensitivity as a key mediator of growth hormone actions on longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **64**, 516–521.
25 Berryman, D. E., List, E. O., Coschigano, K. T., Behar, K., Kim, J. K. & Kopchick, J. J. 2004 Comparing adiposity profiles in three mouse models with altered GH signaling. *Growth Horm. IGF Res.* **14**, 309–318. (doi:10.1016/j.ghir.2004.02.005)
26 Al-Regaiey, K. A., Masternak, M. M., Bonkowski, M., Sun, L. & Bartke, A. 2005 Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor 1/insulin signaling and caloric restriction. *Endocrinology* **146**, 851–860. (doi:10.1210/en.2004-1120)
27 Wang, Z., Al-Regaiey, K., Masternak, M. M. & Bartke, A. 2006 Adipocytokines and lipid levels in Ames dwarf and

calorie-restricted mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**, 323–331.
28 Bartke, A. 2008 Impact of reduced insulin-like growth factor-1/insulin signaling on aging in mammals: novel findings. *Aging Cell* **7**, 285–290. (doi:10.1111/j.1474-9726.2008.00387.x)
29 Miller, R. A., Harper, J. M., Galecki, A. & Burke, D. T. 2002 Big mice die young: early life body weight predicts longevity in genetically heterogeneous mice. *Aging Cell* **1**, 22–29. (doi:10.1046/j.1474-9728.2002.00006.x)
30 Patronek, G. J., Waters, D. J. & Glickman, L. T. 1997 Comparative longevity of pet dogs and humans: implications for gerontology research. *J. Gerontol. A Biol. Sci. Med. Sci.* **52A**, B171–B178.
31 Bartke, A. 2009 The somatotropic axis and aging: mechanisms and persistent questions about practical implications. *Exp. Gerontol.* **44**, 372–374. (doi:10.1016/j.exger.2009.04.001)
32 Sharp, Z. D. & Bartke, A. 2005 Evidence for down-regulation of phosphoinositide 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR)-dependent translation regulatory signaling pathways in Ames dwarf mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **60**, 293–300.
33 Harrison, D. E. *et al.* 2009 Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* **460**, 392–395. (doi:10.1038/nature08221)
34 Selman, C. *et al.* 2009 Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science* **326**, 140–144. (doi:10.1126/science.1177221)
35 Chandrashekar, V. & Bartke, A. 1993 Induction of endogenous insulin-like growth factor-I secretion alters the hypothalamic-pituitary-testicular function in growth hormone-deficient adult dwarf mice. *Biol. Reprod.* **48**, 544–551. (doi:10.1095/biolreprod48.3.544)
36 Zhou, Y. *et al.* 1997 A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc. Natl Acad. Sci. USA* **94**, 13 215–13 220. (doi:10.1073/pnas.94.24.13215)
37 Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Geloen, A., Evens, P., Cervera, P. & LeBouc, Y. 2003 IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**, 182–187. (doi:10.1038/nature01298)
38 Sell, C. & Lorenzini, S. 2007 Aging in IGF-1 hypomorphic mice. Program presentation, *American Aging Association 36th Annual Meeting, San Antonio, TX, 1–4 June 2007.*
39 Selman, C. *et al.* 2008 Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J.* **22**, 807–818. (doi:10.1096/fj.07-9261com)
40 Taguchi, A., Wartschow, L. M. & White, M. F. 2007 Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* **317**, 369–372. (doi:10.1126/science.1142179)
41 Ladiges, W., Van Remmen, H., Strong, R., Ikeno, Y., Treuting, P., Rabinovitch, P. & Richardson, A. 2009 Lifespan extension in genetically modified mice. *Aging Cell* **4**, 346–352.
42 Selman, C., Lingard, S., Gems, D., Partridge, L. & Withers, D. J. 2008 Comment on 'Brain IRS2 signaling coordinates life span and nutrient homeostasis'. *Science* **320**, 1012. (doi:10.1126/science.1152366)
43 Cohen, E. *et al.* 2009 Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* **139**, 1157–1169. (doi:10.1016/j.cell.2009.11.014)
44 Freude, S. *et al.* 2009 Neuronal IGF-1 resistance reduces Abeta accumulation and protects against premature death in a model of Alzheimer's disease. *FASEB J.* **23**, 3315–3324. (doi:10.1096/fj.09-132043)
45 Killick, R. *et al.* 2009 Deletion of IRS2 reduces amyloid deposition and rescues behavioural deficits in APP transgenic mice. *Biochem. Biophys. Res. Commun.* **386**, 257–262. (doi:10.1016/j.bbrc.2009.06.032)
46 Kurosu, H. *et al.* 2005 Suppression of aging in mice by the hormone Klotho. *Science* **309**, 1829–1833. (doi:10.1126/science.1112766)
47 Kappeler, L. *et al.* 2008 Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism. *PLoS Biol.* **6**, e254. (doi:10.1371/journal.pbio.0060254)
48 Conover, C. A. & Bale, L. K. 2007 Loss of pregnancy-associated plasma protein A extends lifespan in mice. *Aging Cell* **6**, 727–729. (doi:10.1111/j.1474-9726.2007.00328.x)
49 Kahn, C. R. 1995 Causes of insulin resistance. *Nature* **373**, 384–385. (doi:10.1038/373384a0)
50 Blüher, M., Kahn, B. & Kahn, C. R. 2003 Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **299**, 572–574. (doi:10.1126/science.1078223)
51 Katic, M. *et al.* 2007 Mitochondrial gene expression and increased oxidative metabolism: role in increased lifespan of fat-specific insulin receptor knock-out mice. *Aging Cell* **6**, 827–839. (doi:10.1111/j.1474-9726.2007.00346.x)
52 Russell, S. J. & Kahn, C. R. 2007 Endocrine regulation of ageing. *Nat. Rev. Mol. Cell Biol.* **8**, 681–691. (doi:10.1038/nrm2234)
53 Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P. P., Lanfranconce, L. & Pelicci, P. G. 1999 The p66Shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* **402**, 309–313. (doi:10.1038/46311)
54 Trinei, M. *et al.* 2002 A p53-p66Shc signaling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncongene* **21**, 3872–3878. (doi:10.1038/sj.onc.1205513)
55 Orsini, F. *et al.* 2004 The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential. *J. Biol. Chem.* **279**, 25 689–25 695. (doi:10.1074/jbc.M401844200)
56 Tomilov, A. A., Bicocca, V., Schoenfeld, R. A., Giorgio, M., Migliaccio, E., Ramsey, J. J., Hagopian, K., Pelicci, P. G. & Cortopassi, G. A. 2010 Decreased superoxide production in macrophages of long-lived p66Shc knock-out mice. *J. Biol. Chem.* **285**, 1153–1165. (doi:10.1074/jbc.M109.017491)
57 Schriner, S. E. *et al.* 2005 Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* **308**, 1909–1911. (doi:10.1126/science.1106653)
58 Treuting, P. M., Linford, N. J., Knoblaugh, S. E., Emond, M. J., Morton, J. F., Martin, G. M., Rabinovitch, P. S. & Ladiges, W. C. 2008 Reduction of age-associated pathology in old mice by overexpression of catalase in mitochondria. *J. Gerontol. A Biol. Sci. Med. Sci.* **63**, 22–813.
59 Enns, L. C., Morton, J. F., Treuting, P. R., Emond, M. J., Wolf, N. S., McKnight, G. S., Rabinovitch, P. S. & Ladiges, W. C. 2009 Disruption of protein kinase A in mice enhances healthy aging. *PLoS ONE* **4**, e5963. (doi:10.1371/journal.pone.0005963)
60 Lin, S. J., Defossez, P. A. & Guarente, L. 2000 Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* **289**, 2126–2128. (doi:10.1126/science.289.5487.2126)
61 Yan, L., Vatner, D. E. & O'Connor, J. P. 2007 Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* **130**, 247–258. (doi:10.1016/j.cell.2007.05.038)

62 Dell'Agnello, C. *et al.* 2007 Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. *Hum. Mol. Genet.* **16**, 431–444.
63 Tomas-Loba, A. *et al.* 2008 Telomerase reverse transcriptase delays aging in cancer-resistant mice. *Cell* **135**, 609–622. (doi:10.1016/j.cell.2008.09.034)
64 Matheu, A. *et al.* 2007 Delayed ageing through damage protection by the Arf/p53 pathway. *Nature* **9**, 375–448. (doi:10.1038/nature05949)
65 Conti, B. *et al.* 2006 Transgenic mice with a reduced core body temperature have an increased life span. *Science* **314**, 825–828. (doi:10.1126/science.1132191)
66 Hauck, S. J., Hunter, W. S. & Danilovich, N. 2001 Reduced levels of thyroid hormones, insulin, and glucose, and lower body core temperature in the growth hormone receptor/binding protein knockout mouse. *Exp. Biol. Med.* **226**, 552–558.
67 Kuro-o, M. 2010 A potential link between phosphate and aging—lessons from Klotho-deficient mice. *Mech. Ageing Dev.* **131**, 270–275. (doi:10.1016/j.mad.2010.02.008)
68 Kuro-o, M. 2009 Klotho and aging. *Biochim. Biophys. Acta* **1790**, 1049–1058.
69 Yamamoto, M. *et al.* 2005 Regulation of oxidative stress by the anti-aging hormone Klotho. *J. Biol. Chem.* **280**, 34–38.
70 Silberberg, R. 1972 Articular aging and osteoarthrosis in dwarf mice. *Pathol. Microbiol. (Basel)* **38**, 417–430.
71 Lupu, F., Terwilliger, J. D., Lee, K., Segre, G. V. & Efstratiadis, A. 2001 Roles of growth hormone and insulin-like growth factor I in mouse postnatal growth. *Dev. Biol.* **229**, 141–162. (doi:10.1006/dbio.2000.9975)
72 Sun, L. Y., Al-Regaiey, K., Masternak, M. M., Wang, J. & Bartke, A. 2005 Local expression of GH and IGF-1 in the hippocampus of GH-deficient long-lived mice. *Neurobiol. Aging* **26**, 929–937. (doi:10.1016/j.neurobiolaging.2004.07.010)
73 Chandrashekar, V., Zaczek, D. & Bartke, A. 2004 The consequences of altered somatotropic system on reproduction. *Biol. Reprod.* **71**, 17–27. (doi:10.1095/biolreprod.103.027060)
74 Miller, R. A., Harper, J. M., Dysko, R. C., Durkee, S. J. & Austad, S. N. 2002 Longer life spans and delayed maturation in wild-derived mice. *Exp. Biol. Med. (Maywood)* **227**, 500–508.
75 Rollo, C. D. 2002 Growth negatively impacts the life span of mammals. *Evol. Dev.* **4**, 55–61. (doi:10.1046/j.1525-142x.2002.01053.x)
76 Samaras, T., Elrick, H. & Storms, L. 2003 Is height related to longevity? *Life Sci.* **72**, 1781–1802. (doi:10.1016/S0024-3205(02)02503-1)
77 Brosnahan, M. M. & Paradis, M. R. 2003 Demographic and clinical characteristics of geriatric horses: 467 cases (1989–1999). *J. Am. Vet. Med. Assoc.* **223**, 93–98. (doi:10.2460/javma.2003.223.93)
78 Laron, Z. 2005 Do deficiencies in growth hormone and insulin-like growth factor-1 (IGF-1) shorten or prolong longevity? *Mech. Ageing Develop.* **126**, 305–307. (doi:10.1016/j.mad.2004.08.022)
79 Shevah, O. & Laron, Z. 2007 Patients with congenital deficiency of IGF-I seem protected from the development of malignancies: a preliminary report. *Growth Horm. IGF Res.* **17**, 54–57. (doi:10.1016/j.ghir.2006.10.007)
80 Oliveira, J. L. *et al.* 2007 Congenital growth hormone (GH) deficiency and atherosclerosis: effects of GH replacement in GH-naive adults. *J. Clin. Endocrinol. Metab.* **92**, 4664–4670. (doi:10.1210/jc.2007-1636)
81 Gussekloo, J., van Exel, E., de Craen, A. J., Meinders, A. E., Frolich, M. & Westendorp, R. G. 2005 Thyroid status, performance, and survival in old age. *JAMA* **292**, 2591–2599. (doi:10.1001/jama.292.21.2591)
82 Suh, Y., Atzmon, G., Cho, M.-O., Hwang, D., Liu, B., Leahy, D. J., Barzilai, N. & Cohen, P. 2008 Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl Acad. Sci. USA* **105**, 3438–3442. (doi:10.1073/pnas.0705467105)
83 van Heemst, D., Beekman, M., Mooijaart, S. P., Heijmans, B. T., Brandt, B. W., Zwaan, B. J., Slagboom, P. E. & Westendorp, G. J. 2005 Reduced insulin/IGF-1 signaling and human longevity. *Aging Cell* **4**, 79–85. (doi:10.1111/j.1474-9728.2005.00148.x)
84 Atzmon, G., Barzilai, N., Surks, M. I. & Gabriely, I. 2009 Genetic predisposition to elevated serum thyrotropin is associated with exceptional longevity. *J. Clin. Endocrinol. Metab.* **94**, 4768–4775. (doi:10.1210/jc.2009-0808)
85 Atzmon, G. *et al.* 2008 Adiponectin levels and genotype: a potential regulator of life span in humans. *J. Gerontol. A Biol. Sci. Med. Sci.* **63**, 447–453.
86 Li, Y. *et al.* 2009 Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. *Hum. Mol. Genet.* **18**, 4897–4904. (doi:10.1093/hmg/ddp459)
87 Greer, E. L. & Brunet, A. 2008 FOXO transcription factors in ageing and cancer. *Acta Physiol.* **192**, 19–28. (doi:10.1111/j.1748-1716.2007.01780.x)