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Links between growth hormone and aging

Hormon wzrostu a proces starzenia się

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Abstract

Studies in mutant, gene knock-out and transgenic mice have demonstrated that growth hormone (GH) signalling has a major impact on ageing and longevity. Growth hormone-resistant and GH-deficient animals live much longer than their normal siblings, while transgenic mice overexpressing GH are short lived. Actions of GH in juvenile animals appear to be particularly important for life extension and responsible for various phenotypic characteristics of long-lived hypopituitary mutants.

Available evidence indicates that reduced GH signalling is linked to extended longevity by multiple interacting mechanisms including increased stress resistance, reduced growth, altered profiles of cytokines produced by the adipose tissue, and various metabolic adjustments such as enhanced insulin sensitivity, increased oxygen consumption (VO_2/g) and reduced respiratory quotient. The effects of removing visceral fat indicate that increased levels of adiponectin and reduced levels of pro-inflammatory cytokines in GH-resistant mice are responsible for their increased insulin sensitivity. Increased VO_2 apparently represents increased energy expenditure for thermogenesis, because VO_2 of mutant and normal mice does not differ at thermoneutral temperature. Recent studies identified GH- and IGF-1-dependent maintenance of bone marrow populations of very small embryonic-like stem cells (VSELs) as another likely mechanism of delayed ageing and increased longevity of GH-deficient and GH-resistant animals.

Many of the physiological characteristics of long-lived, GH-related mouse mutants are shared by exceptionally long-lived people and by individuals genetically predisposed to longevity. **(Endokrynol Pol 2013; 64 (1): 46–52)**

Key words: *growth hormone (GH), calorie restriction (CR), insulin-like growth factor (IGF-1), Ames dwarf mice, growth hormone receptor knockout (GHRKO) mice*

Streszczenie

W badaniach na zmutowanych i transgenicznych myszach wykazano, że przekazywanie sygnałów przez hormon wzrostu (GH) wywiera istotny wpływ na procesy starzenia się organizmu i długowieczność. Zwierzęta, u których stwierdza się oporność na hormon wzrostu lub jego niedobór, żyją dłużej niż ich normalne rodzeństwo, podczas gdy myszy transgeniczne wykazujące nadekspresję GH żyją krótko. Działania, jakie wywiera GH u młodocianych zwierząt wydają się odgrywać szczególnie ważną rolę w wydłużaniu życia i są odpowiedzialne za różnorodne cechy fenotypowe długowiecznych mutantów z niedoczynnością przysadki.

Z dostępnych dowodów naukowych wynika, że osłabione przekazywanie sygnałów przez GH wiąże się ze zwiększeniem długowieczności, u podstawy czego leży wiele współzależnych mechanizmów obejmujących zwiększoną odporność na stres, osłabione wzrastanie, zmieniony profil cytokin wytwarzanych przez tkankę tłuszczową, a także różne adaptacje metaboliczne, np. zwiększona insulinowrażliwość, zwiększone zużycie tlenu (VO_2/g) i zmniejszony współczynnik oddechowy. Konsekwencje usunięcia trzewnej tkanki tłuszczowej wskazują, i że zwiększoną insulinowrażliwość u myszy opornych na GH odpowiedzialne jest zwiększone stężenie adyponektyny i zmniejszone stężenie cytokin prozapalnych. Zwiększone VO_2 wydaje się wynikać ze zwiększenia wydatkowania energii na termogenezę, bowiem w temperaturze termoneutralnej VO_2 u myszy zmutowanych i myszy niezmutowanych nie różni się. W najnowszych badaniach — jako kolejny prawdopodobny mechanizm opóźnionego starzenia się organizmu i zwiększonej długowieczności u zwierząt z niedoborem GH i opornością na GH — zidentyfikowano zależne od GH i IGF1 podtrzymywanie szpikowych populacji bardzo małych komórek macierzystych podobnych do zarodkowych (VSEL, *very small embryonic-like stem cells*).

Wiele z cech fizjologicznych długowiecznych myszy z mutacjami związanymi z GH występuje też u bardzo długo żyjących ludzi i osób genetycznie predysponowanych do długowieczności. **(Endokrynol Pol 2013; 64 (1): 46–52)**

Słowa kluczowe: *hormon wzrostu (GH), ograniczenie kaloryczne, insulinopodobny czynnik wzrostu 1 (IGF1), myszy karłowate szczepu Ames, myszy z wyłączonym genem kodującym receptor hormonu wzrostu (GHRKO)*

Introduction

In laboratory populations of house mice (*Mus musculus*), the strength of growth hormone (GH) signals is a major determinant of ageing and longevity. In transgenic mice expressing various GH genes under the control of metallothionein or phosphoenolpyruvate carboxykinase promoters, massive overproduction of GH leads to drastically reduced lifespan and many symptoms of accelerated ageing [1]. In contrast, GH deficiency in

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hypopituitary mutants and GH resistance in mice with targeted deletion of GH receptors are associated with approximately 30%–60% lifespan extension, depending on the mutation involved, genetic background, sex and diet composition [2–5]. Importantly, GH-deficient and GH-resistant mice exhibit many symptoms of delayed ageing and have an extended 'health span' i.e. a period of life free from major disease or functional impairments [3, 5, 6].

The incidence of fatal neoplastic disease is reduced, and its onset delayed, in these long-lived GH-related mutants [7–9]. Studies of the phenotypes of these mutant animals have revealed partial overlap with the characteristics of genetically normal mice subjected to long-term calorie restriction (CR; a life-extending intervention) and identified a number of very likely (although at this time not conclusively proven) candidate mechanisms of their delayed ageing and extended longevity. These mechanisms include enhanced activity of antioxidant enzymes and reduced oxidative damage [10, 11]; increased cellular resistance to a variety of toxic substances and stressful conditions [12, 13]; reduced activity of a nutrient-sensing mammalian target of rapamycin (mTOR) pathway that regulates translation and growth [14, 15]; and improved sensitivity to insulin coexisting with reduced insulin levels [15–17]. Importantly, negative association of GH action with longevity discovered in mutant, gene knock-out and transgenic mice also applies to genetically normal ('wild-type') animals. Comparisons of mice from different strains, as well as individual animals from a genetically heterogeneous normal population, has revealed that smaller animals live longer [18, 19], and circulating levels of insulin-like growth factor 1 (IGF-1, the key mediator of GH stimulatory action on somatic growth) are negatively correlated with longevity [19, 20].

The ground-breaking discovery of significant homology of longevity genes of a tiny, free-living worm, *Caenorhabditis elegans*, with mammalian genes involved in insulin and IGF-1 signalling [21, 22] led to identification of a conserved pathway of endocrine signalling that controls ageing and longevity in organisms ranging from yeast to mammals [23, 24]. This pathway comprises multiple insulin-like and IGF-like hormones in worms and insects, insulin and IGF in vertebrates, the corresponding receptors (one in worms and insects but several in invertebrates) as well as their signalling intermediates including insulin receptor substrates, phospatidylinositol kinase, protein kinase B (Akt) and an important FOXO family of transcription factors that indirectly control cellular defences from oxidative stress along with other functions. In mammals, GH is intimately involved in the functioning of this pathway by serving as the key determinant of hepatic IGF-1 expression and circulating IGF-1 levels. It also acts as a counter-regulatory hormone in carbohydrate homeostasis by promoting insulin resistance and exerting various anti-insulinaemic effects.

While pathological excess of GH secretion reduces life expectancy in people as it does in mice [1, 25], the impact of hypopituitarism, GH deficiency and GH resistance on human longevity is poorly understood, controversial and probably relatively minor [26–28]. However, individuals with genetic GH deficiency or GH resistance are remarkably, and in some cases apparently completely, protected from major age-related diseases including atherosclerosis, diabetes and, most strikingly, cancer [26, 27, 29–31].

In this article, we will briefly review and discuss some recent findings in GH-deficient and GH-resistant mice that identify novel candidate mechanisms of their extended longevity and raise new questions about relationships between somatotropic (GH;IGF) signalling and ageing.

Energy metabolism and role of ambient (environmental) temperature

Indirect calorimetry studies have revealed that in both hypopituitary Ames dwarf (Prop1df) and insulin-resistant GH receptor-deleted (GHRKO) mice, oxygen consumption (VO_2) per gram of body weight is increased during both active (dark) and resting (light) portions of the 24 h period [32]. These differences were evident regardless of the presence or absence of food during recording and were accompanied by a reduction in respiratory quotient (RQ) [32]. Respiratory quotient represents ratio of carbon dioxide produced to oxygen consumed and provides an estimate of the relative amounts of carbohydrate vs. lipids (primarily fatty acids) utilised as energy substrates.

The finding that long-lived dwarf mice have increased metabolic rate (as assessed by VO_2) was counter-intuitive and contrary to our expectations because these animals have reduced levels of anabolic and thermogenic hormones, GH, IGF-1 and insulin. Ames dwarf mice are also severely hypothyroid. However, the observed differences were relatively large and reproducible, and apparently did not represent an artifact of the fact that this data was expressed per unit of body mass rather than per unit of calculated lean or 'metabolic' body mass. Estimates of lean body mass are based on the assumption that smaller animals are proportionally leaner, while the amount of adipose tissue in these mutants is either increased or nearly normal (depending on the mutation, sex and age) [33–35]. When we re-calculated VO_2 data in GHRKO and normal mice in terms of the actual percent of lean body mass as determined by

dual energy X-ray absorptiometry (DEXA), the increase of VO_2 in the mutants not only persisted but became exaggerated [Westbrook and Bartke, unpublished].

In small animals maintained in a standard animal room temperature (approx. 23°C), a considerable proportion of the energy budget is devoted to thermogenesis. Consequently, we suspected that the increase in VO_2 in dwarf mice may reflect increased energy demand for compensation for the increased heat loss by radiation in these diminutive animals. This compensation would allow them to maintain stable body temperature. To test the validity of this hypothesis, we repeated indirect calorimetry studies in GHRKO and normal mice at a thermoneutral temperature, which in mice is approximately 30°C. In the thermoneutral environment, the metabolic rate decreased as expected, but this decrease was incomparably greater in GHRKO than in normal mice. As a result, the difference in VO_2 between the normal and mutant animals disappeared [Westbrook and Bartke, unpublished]. It is tempting to speculate that, at a standard animal room temperature, increased thermogenesis in dwarf mice leads to greater utilisation of lipids as an energy source ('fat burning') and more efficient function of mitochondria with reduced production of reactive oxygen species, and that these metabolic adaptations contribute to the extended longevity of these animals. In support of these speculations, similar metabolic adaptations develop in response to calorie restriction, and some of the beneficial effects of calorie restriction were reported to be attenuated if the mice were housed at a thermoneutral temperature [36].

Inflammation; secretory activity of adipose tissue

There is considerable, although largely only correlative, evidence that inflammatory processes and circulating inflammation markers are involved in the control of human ageing, age-related disease and longevity [37, 38]. Growth hormone has been reported to exert both anti- and pro-inflammatory effects [39-41], while inflammation can influence GH release and actions [42, 43]. Available information indicates that the expression as well as tissue and blood levels of pro-inflammatory cytokines, interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) are reduced in long-lived GH-deficient and GH-resistant mice [44, 45]. Moreover, circulating levels of an anti-inflammatory adipokine, adiponectin, are consistently elevated in these animals [15, 45]. This latter observation was unexpected because the absence of GH signals in these mutants leads to increased adiposity, and because, in both laboratory animals and humans, plasma levels of adiponectin have been repeatedly shown to be inversely rather than directly related to adiposity. Thus, circulating adiponectin levels are normally reduced in obese individuals, while leanness and calorie restriction are associated with increased plasma adiponectin. Apparently, in Ames dwarf and in GHRKO mice, absence of GH action overrides the influence of increased adiposity on adiponectin secretion. In support of this interpretation, over-expressing GH adiposity in transgenic mice is reduced, and yet adiponectin levels are reduced rather than elevated [46].

Because adiponectin promotes insulin sensitivity, increased adiponectin levels in GHRKO and Ames dwarf mice are consistent with improved insulin signalling in these animals, and indeed provide a very plausible explanation as to why these generally obese animals are insulin sensitive rather than insulin resistant. It is also likely that the anti-inflammatory and anti-atherogenic activity of adiponectin contribute to the extension of health span and lifespan in these mutants.

Recent studies involving surgical removal of visceral adipose tissue have provided strong support for the suspected role of increased adiponectin levels in the enhancement of insulin sensitivity in GHRKO mice. Visceral fat is an important source of cytokines, and in obese individuals it is believed to be involved in producing a state of chronic, low-grade inflammation leading to insulin resistance and increased risk of age-related disease. Consistent with this role of visceral fat in glucose homeostasis and ageing, surgical removal of most of the abdominal fat from male rats resulted in improved insulin sensitivity and a significant extension of lifespan [46]. To address the paradox of the coexistence of obesity and enhanced insulin sensitivity in GHRKO mice, we compared the effects of removing visceral (epididymal and perinephric) fat in GHRKO and normal mice. In normal animals, visceral fat removal improved insulin sensitivity (measured by insulin tolerance tests, ITT) and clearance of injected glucose (measured in glucose tolerance tests, GTT), as anticipated. In contrast, in GHRKO mice, the same surgical procedure led to a reduction of plasma adiponectin levels and deterioration of glucose homeostasis as measured by ITT and GTT [47].

Studies of epididymal fat pads confirmed increased expression of adiponectin and reduced expression of interleukin 6 (IL-6) in GHRKO compared to normal mice [47]. It can be concluded that, in the absence of GH signals, the secretory profile of adipose tissue (or perhaps, specifically, intraabdominal adipose tissue) is shifted from pro-inflammatory to anti-inflammatory adipokines. This shift importantly contributes to paradoxically improved insulin signalling in obese GHRKO males. Together with the documented anti-atherogenic actions of adiponectin, these changes probably contribute to the extension of longevity in GHRKO mice. Studies of gene expression in peripheral blood leukocytes

in Ames dwarf mice have indicated that activation of anti-inflammatory pathways in long-lived GH-related mutants extends beyond the adipose tissue [48].

Intriguingly, some of the interactions between inflammatory processes and metabolism demonstrated in mutant mice appear to also apply to humans. In humans, as in mice, the absence of GH signals leads to obesity, which is strongly associated with insulin resistance and increased risk of chronic disease. However, hereditary GH resistance in an extensively studied population of individuals with Laron dwarfism in Ecuador was associated with striking, nearly complete, protection from cancer and diabetes [27]. Moreover, a cohort of GH-deficient dwarfs studied in Brazil was shown to be unexpectedly protected from atherosclerosis in spite of obesity and unfavourable serum lipid profiles [26].

Maintenance of bone marrow populations of pluripotent stem cells

Although the role of stem cells in the control of longevity and protection from age-related disease remains to be fully elucidated, there is considerable evidence for their involvement in the maintenance and repair of adult tissues [49–52]. A population of pluripotent very small embryonic-like stem cells (VSELs) in the bone marrow has been identified on the basis of a unique combination of markers and ability to differentiate into a wide variety of cell types *in vitro* [53–57]. It has been postulated that these developmentally primitive cells are dormant precursors of more restricted tissue-committed stem cells, and thus play a role in steady state conditions in tissue organ rejuvenation and regeneration after injury [57]. In mice, the number of VSELs in the bone marrow declines with age [58], and their maintenance has been linked to the control of methylation state of a number of imprinted genes related to IGF signalling [59]. It was therefore interesting to assess the abundance of VSELs in GH-related mutants in which circulating levels of IGF-1 are dramatically suppressed. Results of these studies revealed that the numbers of VSELs are greater in Ames dwarf and GHRKO mice than in normal animals from the same strains [60, 61 and unpublished observations]. These differences were large, with little or no overlap between the values measured in mutant and normal animals, and were present in both young adult and middle-aged mice [60, 61 and unpublished observations]. In interesting contrast, the number of VSELs was reduced in GH transgenic mice in which circulating IGF-1 levels are chronically elevated [62]. Hormonal replacement therapy with GH in Ames dwarf mice and with IGF-1 in GHRKO animals, as well as treatment of normal mice with large doses of GH, reduced the number of VSELs in their bone marrow [61]. These results clearly establish that in animals with GH deficiency or GH resistance and the consequent suppression of plasma IGF-1 levels, the abundance of VSELs in the bone marrow is increased, probably reflecting reduced differentiation and/or other mechanisms of age-dependent depletion.

In the same studies, the effects of altered GH levels or actions on the number of haematopoietic stem cells were generally parallel to the above-described impact on the number of VSELs [60–62]. It is interesting to speculate that the observed relationships between the activity of the somatotropic axis and the populations of VSELs may apply more broadly, and include various categories of stem and progenitor cells. This in turn could prove important for the repair and maintenance of different tissues and organs and thus may represent yet another mechanism that links reduced GH/IGF-1 signalling with the extension of health span and lifespan.

Impact of prepubertal GH signalling on longevity

Failure of somatotroph differentiation and the resulting GH deficiency in Ames (Prop1-df) and Snell (Pit1-dw) mice led to dramatic reductions in the rate of postnatal growth and adult body size. These mutants are also thyroid-stimulating hormone (TSH) and prolactin (PRL) deficient [51]. The effects of treating dwarf mice with thyroxine (T4) or PRL indicate that hypothyroidism resulting from TSH deficiency contributes to the reduced growth and adult size of these animals. The role of PRL deficiency in reduced growth of these mutants is less clear and almost certainly minor. The unexpected remarkable increase of longevity of both Ames and Snell dwarf mice, as well as in other GH-related mutants, raised a question of the possible significance of the rate of postnatal growth in the determination of lifespan. The fact that the negative correlation of longevity with adult body size detected in mutant, gene knock-out and transgenic mice also applies to genetically normal mice [18, 19], domestic dogs [55] and other species [56, 57] provided additional rationale for probing the role of post-natal growth in the determination of longevity.

Studies conducted in the Miller laboratory in Snell dwarf mice [58], as well as our initial studies in Ames dwarfs, demonstrated that treatment of juvenile dwarf mice with GH, T4 or a combination of GH and T4 produced the expected increase in body weight and length. Longevity, however, was not affected. In these studies, Snell dwarfs were given hormonal treatments between four and 15 weeks of age, while Ames dwarf mice were injected with GH (once daily), T4 (three times per week) or both starting at two weeks of age and continuing for six weeks. In a follow-up study, we have increased

the dose of GH as well as the frequency of injections (twice a day on weekdays and once a day on weekends) and observed a major reduction in the longevity of GH-treated Ames dwarf mice [59]. During the period of GH treatment, these animals grew at a rate roughly comparable to the growth of normal mice; but when the treatment was stopped, their growth rate levelled off and their adult body size was intermediate between the size of normal mice and control (vehicle-injected) dwarfs. Thus, it would appear that GH signalling during the normally rapid early (prepubertal) growth has an important role in the determination of ageing and lifespan. Preliminary data derived from recent and ongoing studies in our laboratory suggests that this 'critical period' of the action of GH on lifespan begins fairly early (during the period of suckling), and there are some intriguing indications that it may be advanced and/or shortened by treatment with T4 [Spong and Bartke, unpublished].

The importance of early 'developmental' effects of GH in the determination of lifespan was not expected because GH is believed to have a very limited role, if any, in the control of pre-weaning growth in rodents [60]. However, the involvement of early postnatal events in the determination of longevity received strong support from the recent demonstration in the Miller laboratory that reducing pre-weaning growth in genetically normal mice by increasing the number of pups suckled by one female significantly extends their average and maximal lifespan [61].

Working with dwarf rats, which have reduced somatotropic signalling but normal lifespan, Sonntag et al. reported that twice-daily injections of GH between the ages of four and 14 weeks led to a significant increase in longevity [62].

Although more work is obviously needed to fully reconcile findings in different species and in long-lived mutants vs. normal animals, it appears to be justified to conclude that ageing and lifespan are significantly related to early postnatal growth and to the actions of GH during this period. This might appear counterintuitive, but is not surprising when viewed in the light of the well-documented early ('organisational') effects of steroid hormones on reproductive behaviour, fertility and responses to stress. Moreover, there is rapidly accumulating evidence for the important impact of early, nutrition-related 'developmental programming' on adult metabolism and susceptibility to chronic disease [63, 64].

We are currently searching for mechanisms that may link GH actions in juvenile animals with ageing and longevity. As the initial step, we are interested in identifying longevity-related traits that are influenced by early GH therapy and remain altered after GH treatment is stopped. The most recent data suggests that GH-induced insulin resistance is not a likely mechanism of shortened lifespan in GH-injected dwarfs. However, increased oxygen consumption per unit body mass (VO_2/g) and reduced respiratory quotient (RQ), which we have previously identified as metabolic characteristics of Ames dwarf and GHRKO mice [32], were completely (VO_2) or partially (RQ) normalised ('rescued') in GH-injected dwarfs five months after completion of GH therapy [Westbrook and Bartke, unpublished]. Adult hepatic expression of genes coding for several detoxification enzymes was also normalised by treatment of Ames dwarf mice with GH between two and eight weeks of age [Sun and Bartke, unpublished]. In as much as improved mitochondrial function, increased utilisation of fatty acids for energy metabolism (as indicated by reduced RQ), and improved breakdown (detoxification) of xenobiotics are probable contributors to the extended longevity of Ames dwarf versus normal mice, the elimination or suppression of these characteristics in GH-injected dwarfs may represent candidate mechanisms of reduced life expectancy.

Conclusion

Studies in mutant, gene knock-out and transgenic animals with extreme, well-defined, hereditary alterations in somatotropic signalling have revealed a prominent role of GH in the control of mammalian ageing and identified multiple interactive mechanisms that appear to be responsible for these relationships. It is hoped that further studies in these animals will help clarify the mechanisms by which GH influences ageing and elucidate the role of variations in the somatotropic axis activity within the normal (physiological) range in the control of ageing in genetically normal ('wild-type') animals and in humans.

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