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Consequences of growth hormone (GH) overexpression and GH resistance

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Summary Development of transgenic mice overexpressing GH and GHR-KO mice with GH resistance provided novel animal models for study of the somatotropic axis and for identifying GH actions that may be relevant to its current and contemplated use in medicine and agriculture. Studies of phenotypic characteristics of these animals revealed previously unsuspected actions of GH and IGF-I on neuroendocrine functions related to reproduction and to the release of ''stress hormones'' (glucocorticoids and prolactin). These studies also provided novel and still-disputed evidence for involvement of somatotropic axis in the control of aging and life span and in mediating the actions of longevity genes.

Nearly twenty years have elapsed since Palmiter and Brinster reported their success in producing giant transgenic mice overexpressing a rat growth hormone (GH) gene in multiple organs (Palmiter et al., 1982). The striking phenotype of these animals and transmission of the transgene to their progeny produced enormous interest in the use of biotechnology to produce animals with economically desirable traits. In the context of the potential utility of GH transgenics and GH-treated animals, the consequences of overexpression of various GH genes in the transgenic mice were studied in considerable detail. Although many of the observed responses to GH overexpression have been predictable, these studies produced much new information on the consequences of life-long exposure to supraphysiological levels of GH and on the mechanisms involved.

Analysis of phenotypes of GH transgenic mice is important for identifying the full range of GH effects and for assessment of the potential of very high doses of GH to produce adverse consequences. However, effects of overexpression of GH in transgenic animals may be difficult to relate to normal physiology. A unique opportunity to study the physiological role of GH signaling and the normal functions of the somatotropic axis arose when Kopchick's laboratory succeeded in targeted disruption ''knock out'' of the GH receptor/GH binding protein gene (Zhou et al., 1997). Growth hormone receptor knock out (GHR-KO) mice exhibit complete GH resistance and mimic many features of the analogous condition (Laron dwarfism) in the human (Kopchick and Laron, 1999).

In this article, we will review the effects of GH overexpression in transgenic mice and effects of disruption of the somatotropic axis in GHR-KO mice.

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GH TRANSGENIC MICE

The effects of overexpression of heterologous GH depend on the level of expression and the resulting plasma levels of GH and insulin-like growth factor I (IGF-I), the main mediator of its actions (Naar et al., 1991; Sotelo et al., 1993; review in Bartke et al., 1994). Information on the binding of different GHs to murine GH receptors suggests that overexpression of rat, bovine, or ovine GH in the mouse has essentially the same consequences (Posner et al., 1974). The actions of these hormones presumably mimic the actions of endogenous (mouse) GH, and this conclusion is supported by the studies of transgenic mice which overexpress hypothalamic GH-releasing hormone and consequently produce abnormally high levels of endogenous (mouse) GH (Mayo et al., 1988). In contrast, expression of human GH in transgenic mice cannot be considered as equivalent to elevation of endogenous GH

(as in gigantism or acromegaly in the human) because hGH binds to both GH and PRL receptors in the mouse (Posner et al., 1974). Thus, the often unique phenotypic consequences of hGH expression in the mouse (Naar et al., 1991; Bartke et al., 1994; Chandrashekar et al., 1988) must be viewed as responses to a physiological equivalent of combined hypersomatotropism and hyperprolactinemia. Some of the effects of hGH expression in transgenic mice are absent in animals expressing purely somatogenic GH (e.g. bGH) and thus can be assumed to represent the actions of PRL rather than GH (Cecim et al., 1994; Prins et al., 1992).

Key phenotypic characteristics of GH transgenics

Overexpression of GH leads to an increase in IGF-I levels, stimulation of growth, an increase in adult body size ranging from approximately 30% to nearly 100% in different lines, organomegaly, and reduced percent of body fat (Palmiter et al., 1982; Bartke et al., 1994; Searle et al., 1992). These characteristics of GH transgenic mice are consistent with the well-documented actions of GH and with biological activity of heterologous GHs in the mouse. Increased size of GH transgenic mice is associated with differential enlargement of internal organs including hepatomegaly (Shea et al., 1987; Orian et al., 1989; Ulshen et al., 1993) and alterations in body proportions which produce a readily recognizable "acromegalic" appearance.

Bovine GH transgenic mice were recently shown to have impaired cardiac function and an increase in mean arterial blood pressure which was related to greater peripheral vascular resistance (Bollano et al., 2000; Bohlooly-y et al., 2001a). In addition to these abnormalities, overexpression of GH is associated with major deficits in immune function (Gonzalo et al., 1996; Hall, Bartke and Martinko, unpublished), and arthritic disorder apparently due to production of self-antibodies (Ogueta et al., 2000).

In a line of transgenic mice overexpressing rat GH, sleep time is greatly increased and locomotor activity during wakefulness is reduced (Rollo et al., 1997). However, increased locomotor activity was reported in transgenic mice overexpressing bovine GH under control of the same promoter (Bohlooly-y et al., 2001b). Overexpression of GH in transgenic mice is also associated with indices of altered function of the central nervous system. These include a shortened free-running period in constant light (Ferraro et al., 1994), altered performance in behavioral tests designed to measure learning and memory (Meliska et al., 1997; Rollo et al., 1999), and gender specific changes in ethanol and nicotine preference (Meliska et al., 1995). It remains to be determined whether behavioral changes in GH transgenic mice are causally related to altered levels and metabolism of neurotransmitters in different brain regions of these animals (Cecim et al., 1995a; Steger et al., 1994; Steger et al., 1993; Soderpalm et al., 1999).

Human GH transgenic mice exhibit some unique characteristics, including increased incidence and early onset of mammary tumors (Cecim et al., 1994), hypertrophy of male accessory reproductive glands accompanied by loss of androgen receptors (Prins et al., 1992), increased luteinizing hormone (LH) ß subunit gene expression (Tang et al., 1993) and plasma LH levels, increased number of tuberoinfundibular dopaminergic neurons (Phelps and Bartke, 1997) and suppression of plasma PRL levels (Bartke et al., 1994; Chandrashekar et al., 1988). These characteristics are not shared by mice overexpressing bovine GH, and therefore are assumed to be due to stimulation of PRL receptors or, perhaps, to concomitant signaling via GH and PRL receptors.

Fertility of GH transgenic mice

Reproductive function of GH transgenic mice was studied in some detail in the context of potential utility of GH treatment and GH overexpression in the animal industry. Male mice overexpressing different GH hybrid genes are usually fertile, although they may exhibit alterations in the levels of adenohypophyseal hormones related to reproduction and in sexual behavior (Bartke et al., 1994; Chandrashekar et al., 1988; Meliska and Bartke, 1997). Moreover, their reproductive life span is reduced (Bartke et al., 1994; Milton, Johnson & Bartke, unpublished). Reproductive competence of most GH transgenic mice may well be related to the practice of developing transgenic lines from fertile male founders and maintaining them by breeding hemizygous transgenic males to normal females.

In GH transgenic female mice, puberty is advanced and ovulation increased, but fertility is reduced, with reproductive deficits ranging from an increased interval between litters to sterility of all females in some of the lines (Naar et al., 1991; Bartke et al., 1994; Cecim et al., 1995b). In general, suppression of female fertility is proportional to plasma GH levels with consequences of overexpression of hGH being more severe than the effects of purely somatotropic GHs (Naar et al., 1991; Bartke et al., 1994).

In a line overexpressing bovine GH under control of the phosphoenolpyruvate carboxykinase (PEPCK) promoter, female sterility was shown to be due to luteal failure resulting from suppression of mating-induced surges of PRL release (Cecim et al., 1995c; Cecim et al., 1995a). Interestingly, infertility due to luteal failure was detected also in mice overexpressing ovine GH under control of the modified ovine metallothionein promoter that drives GH overexpression only in response to increased intake of heavy metals and thus allows tight temporal control of hypersomatotropism (Thomas et al., 2001). Authors of

the latter study ascribed luteal failure to elevation of plasma corticosterone rather than to disruption of the post-mating pattern of PRL release (Thomas et al., 2001).

In contrast to the suppression of PRL release during early pregnancy, plasma PRL levels in cycling and in ovariectomized bGH transgenic mice are generally elevated (Steger et al., 1994; Chandrashekar and Bartke, 1996). Mild hyperprolactinemia in these animals is associated with and possibly due to reduced expression of dopaminergic D2 receptors and increased expression of estrogen receptors in their pituitaries (Vidal et al., 1999). Alterations in the control of PRL release in GH transgenic mice represent previously unsuspected actions of GH, and may be related to the stimulatory effects of IGF-I on the lactotrophs (Proger et al., 1998).

In adult murine metallothionein I-bovine GH (MT-bGH) transgenic mice, the ovariectomy-induced increases in plasma FSH levels were decreased, and the FSH response to GnRH treatment was attenuated (Chandrashekar and Bartke, 1996), suggesting an impairment of FSH secretion as the possible cause for reduced fertility in these animals.

Reduced life expectancy of GH transgenic mice

Mice overexpressing GH do not live as long as their normal siblings, and their life expectancy appears to be inversely related to plasma GH levels (Wolf et al., 1993; Rollo et al., 1996; Bartke et al., 1998). This is illustrated by the results of an ongoing study of longevity in PEPCK-hGH transgenic mice (Fig. 1). Accelerated onset and increased severity of glomerulosclerosis in the kidneys of GH transgenic mice along with histopathological changes in their livers and other organs (Shea et al., 1987; Wolf et al., 1993), undoubtedly contribute to their early mortality. In addition, GH transgenic mice exhibit various symptoms of early aging including reduced replicative potential

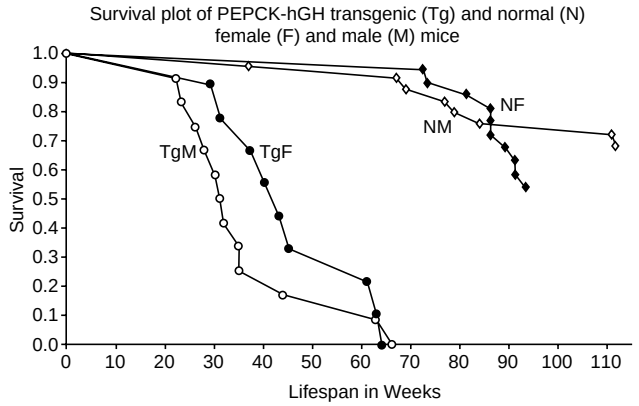

Fig. 1 Survival plot of PEPCK-hGH transgenic (Tg) and normal (N) female (F) and male (M) mice. The animals were produced by mating hemizygous Tg males to normal C57BL/6 × C3H F_1 hybrid females in every generation.

of their cells in vitro (Pendergrass et al., 1993), reduced turnover of hypothalamic neurotransmitters (Steger et al., 1993), increased expression of glial fibrillary acidic protein in the brain (Miller et al., 1995), exacerbated age-related decline in learning and memory (Meliska et al., 1997), and early loss of reproductive competence (Cecim et al., 1995b).

Numerous studies were aimed at identification of the possible causes of reduced life expectancy in GH-Tg mice. Rollo and his collaborators analyzed energy budgets of GH-Tg and normal mice and proposed a very interesting hypothesis that overexpression of GH produces a state of energy deficit. Disproportional resource allocation for growth driven by high GH levels would lead to insufficient availability of energy for other functions, including repair mechanisms (Rollo et al., 1997). Turyn and his colleagues documented severe hyperinsulinemia and insulin resistance in PEPCK-bGH transgenic mice with reduced responses to insulin in both liver and muscle of these animals (Dominici et al., 1999a; Dominici et al., 1999b). Insulin resistance is a risk factor for numerous age-related diseases in the human (Facchini et al., 2001). Elevated insulin levels and poor glucose control have been proposed as important mechanisms of aging (Parr, 1997; Baynes and Monnier, 1989). There is evidence for increased oxidative processes and reduced activity of some of the anti-oxidant enzymes in GH-transgenic mice (Rollo et al., 1996; Hauck and Bartke, 2001), but the relationship of these findings to reduced life span of GH transgenic mice remains to be clarified (Carlson et al., 1999). Oxidative damage is believed to be the key mechanism of cellular aging (Harman, 1988). Marked increase in plasma corticosterone levels is a very consistent finding in GH transgenic mice (Miller et al., 1995; Cecim et al., 1996), and it becomes particularly striking as the animals age (Miller et al., 1995). The relationships of increased glucocorticoid secretion to aging and life expectancy is unclear. Cumulative exposure to stress and the resulting increases in glucocorticoids were proposed as important mechanisms of aging of the central nervous system (Sapolsky et al., 1986). However, elevation of plasma corticosterone is associated with caloric restriction, an intervention which delays aging and prolongs life (Masoro, 2001). Alterations in lipid levels in the liver and serum of GH transgenic mice (Quaife et al., 1989; Peluso & Bartke, unpublished) could also play a role in reduced life expectancy of these animals.

There is very strong evidence that in mice, similar to several other species, adult body size is negatively correlated to average life span (reviews in Bartke et al., 1998; Bartke et al., 2000). The mechanisms underlying this somewhat counterintuitive relationship are unknown, but it can be suspected that some of the characteristics of GH-transgenic mice listed above may link their giant phenotype with reduced longevity.

GROWTH HORMONE RECEPTOR KNOCK OUT MICE. KEY PHENOTYPIC CHARACTERISTICS

Developing a line of mice with targeted disruption of the GH receptor (GHR)/GH binding protein (GHBP) in Kopchick's laboratory (Zhou et al., 1997) provided an exciting opportunity to re-examine the physiological role of GH. Homozygous knock out (GHR-KO; -/-) animals are viable and of nearly normal size at birth, but lag behind their normal siblings in postnatal growth and reach approximately 50% of normal adult body weight. GHR mRNA, GHR protein and GHBP are absent from the liver of these animals (Zhou et al., 1997). Interestingly, hepatic GH binding, although very low, is not entirely eliminated (Zhou et al., 1997), hinting at the possible presence of a second type of GHR. As expected from the absence of GHR, the levels of IGF-I in peripheral circulation are either extremely low or undetectable (Zhou et al., 1997; Chandrashekar et al., 1999), and the levels of GH-dependent IGFBP3 are severely suppressed (Coschigano et al., 2000). Consistent with the actions of GH and IGF-I on body composition and bone metabolism, GHR-KO mice have reduced percent of lean body mass, increased percent of fat mass (Heiman, Kopchick and Bartke, unpublished data), and reduced bone turnover and mineral density (Sims et al., 2000; Sjorgen et al., 2000).

Animals heterozygous (+/−) for the null mutation in the GHR/GHBP gene appear to be normal, but body weight of juvenile and young adult females is significantly below the values measured in wild type (+/+) animals from the same line (Zhou et al., 1997; Coschigano et al., 2000). Plasma IGF-I levels and other physiological parameters that have been measured in these animals are not significantly different between +/+ and +/− animals, although small numerical differences were occasionally noted (Zhou et al., 1997, Chandrashekar, unpublished).

Plasma PRL levels in adult male GHR-KO mice are significantly elevated (Chandrashekar et al., 1999). This was not expected from the ability of GH to stimulate lactotroph function in mice (Steger et al., 1994; Wolf et al., 1993; details earlier in this article) and may represent a compensatory mechanism. Growth hormone and PRL are structurally related and their biological functions overlap in many instances. In the mouse, PRL can stimulate somatic growth (Romero and Phelps, 1993). Most of the GHR-KO animals are fertile (details in the next section).

Reproductive development and function in GHR-KO mice

In female mice, vaginal opening is established several days before first ovulation and serves as a convenient marker of sexual maturation. In comparison to normal animals, vaginal opening in GHR-KO mice is delayed by approximately seven days and can be advanced by treatment with IGF-I (Danilovich et al., 1999). Sexual maturation of GHR-KO males is also delayed, as indicated by the age of balano-preputial separation (a marker of penile development), by the time course of growth of accessory reproductive glands and appearance of elongated spermatids in the testis (Keene abstract), and by attainment of fertility (Zaczek & Bartke, unpublished).

Most of GHR-KO females are fertile but the estrous cycle is often irregular and/or prolonged, the number of preovulatory follicles and corpora lutea, ovulation rate, implantation rate, fetal weight, and litter size are significantly reduced (Zhou et al., 1997; Danilovich et al., 1999; Zaczek, Hamond & Bartke, unpublished). Interestingly, placental weight is increased (Danilovich et al., 1999), resembling the findings in mice produced by cloning (Tanaka et al., 2001). Ductal development of mammary glands in virgin GHR-KO mice is impaired (Gallego et al., 2001), but those females that are fertile produce sufficient amounts of milk to allow survival of most of their young till weaning (Zhou et al., 1997; Danilovich et al., 1999; and unpublished observations).

Reproductive deficits of GHR-KO females are consistent with the known influence of the somatotropic axis (GH and IGF-I) on the hypothalamic-pituitary-gonadal (H-P-G) axis, and with the well-documented actions of IGF-I on the development and function of ovarian follicles (Adashi et al., 1997). The expression and levels of IGF-I in the ovaries of IGF-I females are significantly reduced (Zaczek abstract). The plasma LH response to GnRH treatment was attenuated in adult female GHR-KO mice (Chandrashekar et al., unpublished), suggesting an impairment in pituitary function in these mice.

The testes of adult GHR-KO males are reduced in size approximately in proportion to the difference in body weight between normal and KO animals but are normal in terms of gross histological appearance, and almost all males can sire litters (Zhou et al., 1997; Chandrashekar et al., 1999). Morphometric studies revealed reductions in the length and diameter of seminiferous tubules and in the volume density (percent) of Leydig cells and seminiferous tubule lumen (Chandrashekar et al., 2001).

The numbers of testicular LH and PRL receptors are significantly reduced in GHR-KO mice in terms of total content, concentration, and number per μg of Leydig cells (Chandrashekar et al., 2001). Plasma FSH levels are reduced in adult GHR-KO males (Chandrashekar et al., 2001). Plasma testosterone levels are normal in these animals, but acute testosterone responses to stimulation with LH in vivo or in vitro are significantly compromised (Chandrashekar et al., 1999; Chandrashekar et al., 2001). Similarly, LH response to acute LHRH stimulation in vivo is reduced in GHR-KO as compared to normal mice (Chandrashekar et al., 1999).

The fact that most GHR-KO males are fertile and that their reproductive deficits are relatively mild fits well with the findings in GH deficient mutant ("little") mice (Chubb, 1987) and in transgenic animals that are GH resistant as a consequence of overexpression of an antagonistic GH analog (Chen et al., 1991). Alterations in the function of the H-P-G axis in GHR-KO male mice are consistent with the effects of GH on hypophysectomized rats and with the documented role of IGF-I in the maintenance of LH receptors in the testis (review in Bartke, 2000).

Extended longevity of GHR-KO mice; possible relationship to IGF-I and insulin signaling

In 2000, Coschigano et al. reported that GHR-KO (−/−) mice live much longer than their phenotypically normal siblings (+/+ or +/−) (Coschigano et al., 2000). Results of an ongoing study of GHR-KO animals maintained in a different laboratory and on slightly different genetic background (Fig. 2) fully support this observation.

Assessment of long-term memory in old GHR-KO mice using the inhibitory avoidance learning task provided evidence that age-related decline in memory retention in these animals is significantly delayed (Kinney et al., 2001). These findings, together with the increase in maximal life span (Fig 2), thoroughly imply that increased longevity of GHR-KO mice is associated with and most likely due to delayed aging.

The mechanisms by which GH resistance of GHR-KO mice confers longevity advantage are not known, but recent studies point to several possibilities. GHR-KO mice have profoundly suppressed plasma insulin levels, increased levels of hepatic insulin receptors, and a small reduction in plasma glucose levels which was significant in some, but not all, studies (Zhou et al., 1997; Coschigano et al., 1999; Hauck et al., 2001). Increased insulin sensitivity implied by these findings was confirmed by measuring phosphorylation of hepatic insulin receptors and suppression of plasma glucose after administration of a single large dose of insulin (Coschigano et al., 1999; Dominici et al., 2000). Surprisingly, glucose tolerance was reduced rather than enhanced (Coschigano et al., 1999). Other characteristics of GHR-KO mice that may be pertinent to their longevity include mild hypothyroidism (presumably caused by absence of IGF-I action on the thyroid), reduction in body core temperature (Tco) during several periods of the diurnal Tco cycle (Hauck et al., 2001), and diminutive body size. Negative correlation of adult body size and life expectancy was mentioned earlier in this article.

Prolonged longevity of IGF-I-deficient and hypoinsulinemic GHR-KO mice fits very well with rapidly accumulating evidence that the IGF/insulin signaling pathway(s) is importantly involved in the genetic control of longevity in animals ranging from worms to insects and mammals (Kimura et al., 1997; Clancy et al., 2001; Tatar et al., 2001). Discussion of the signaling and metabolic pathways that mediate the action of longevity genes in *Caenorhabditis elegans, Drosophila melanogaster*, and mice is outside the scope of this article, and the interested reader is referred to recent articles and reviews (Guarente and Kenyon, 2000; Bartke, 2001).

It is unclear why the effect of targeted disruption of GHR and the resulting GH resistance in GHR-KO mice on longevity is substantially greater than the effect of isolated GH deficiency in "little" ($GHRHR^{lit}$) mice. GHR-KO mice live approximately 40–50% longer than their normal siblings (Coschigano et al., 2000), while extension of life span in "little" mice is approximately 25% and appears to depend on feeding the animals a low fat diet (Flurkey et al., 2001). Incomplete suppression of GH release in "little" mice and their tendency to become extremely obese as they age may contribute to, or perhaps account for, this difference.

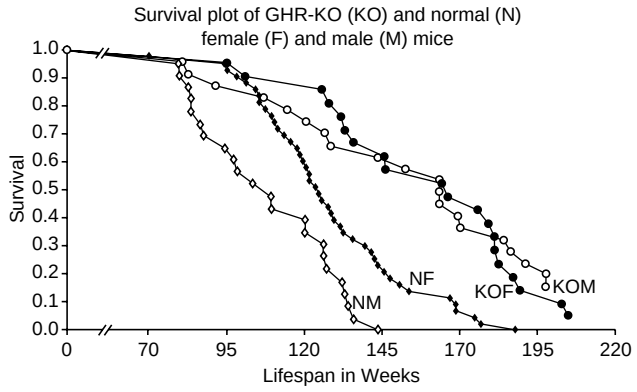

Fig. 2 Survival plot of GHR-KO (KO) and normal (N) female (F) and male (M) mice. The animals were derived from a random bred closed colony with heterogeneous genetic background.

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