
Reproductive Function in Long Lived Mice: Effects of Mutations and Caloric Restriction

Contents

Abstract

Introduction

 Hypopituitary Dwarf Mice

 GH Deficient Little Mice

 GH resistant Laron Dwarf Mice

 Partially IGF-1 Resistant Heterozygous IGF-1 Receptor KO Mice

 Other Long-lived Gene Knock-out Mice

Discussion

References

Abstract

In laboratory stocks of mice and rats, caloric restriction (CR) prolongs life but inhibits reproduction. It is believed that in response to food shortage, the animals can activate a “survival program” that favors stress resistance and repair processes at the expense of growth, reproductive development, and fertility. In two hypopituitary mouse mutants, Ames dwarf (Prop^{1^{df}}) and Snell dwarf (Pit^{1^{dw}}), life span is prolonged by as much as 65%, postnatal growth and adult body size are severely reduced, and puberty is greatly delayed. Fertility of male dwarf mice is reduced and females are sterile due to prolactin (PRL) deficiency and the consequent luteal failure. In “Laron dwarf mice” with targeted disruption of growth hormone (GH) receptor gene, GH resistance leads to dramatic depression of peripheral levels of insulin-like growth factor 1 (IGF-1), reduction of adult body size by approximately 50%, a slight but significant delay of female and male puberty,

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and extension of life span by 30 – 50%. Both genders are fertile but various measures of reproductive competence including time to conception and litter size in females and testosterone responses to LH in males are reduced. Plasma PRL levels are elevated while FSH levels are reduced. Holzenberger and his colleagues (2003) recently reported that female mice heterozygous for IGF-1 receptor knock-out live approximately 30% longer than normal mice, have nearly normal adult body weight, and are fully fertile. Males have similar phenotype but the apparent increase in longevity was not statistically significant. These results, as well as recent findings in worms and insects indicate that the well documented reciprocal relationship between reproductive effort and longevity does not apply to all animals with increased life expectancy. Reduced GH and IGF-1 signaling is compatible with fertility and a modest level of IGF-1 resistance can prolong life without a negative impact on reproduction. We are currently examining the interactive effects of CR and mouse longevity genes on reproductive functions.

Key words: reproduction, dwarf mice, growth hormone receptor knock-outs.

Invited Mini-review

Introduction

There are many examples of reciprocal relationship between reproduction and longevity. Most long lived species of mammals have late puberty, long gestations, small litter size, and large intervals between litters often combined with a long period of parental care. Large ungulates and primates provide many examples of this type of life history, with humans clearly fitting this pattern. In contrast, animals which mature early, have short gestation periods, and produce large and frequent litters are generally short lived. Most small rodents fit in this category. Comparison of mice derived from wild populations with mice from standard laboratory stocks and strains indicates that domestication in this species was associated with advancement of puberty, increase in litter size and reduction of life expectancy (Miller *et al.*, 2000).

In laboratory populations of mice and rats, aging can be delayed and lifespan extended by reducing the amount of food the animals are allowed to consume, the so-called dietary or caloric restriction (CR) (reviewed in Weindruch & Walford, 1988; Masoro, 2001; Hursting *et al.*, 2003). CR is the only intervention that consistently produces significant increase of longevity in mammals. It was also shown to be effective in domestic dogs (Kealy *et al.*, 2002) with evidence of similar responses in beef cattle (Pinney *et al.*, 1972) and in monkeys (Lane *et al.*, 2001), and there are reasons to believe that it could be applied to delay human aging. In rodents subjected to CR, puberty is delayed, and fertility is suppressed (Weindruch & Walford, 1988; Masoro, 2001; Hursting *et al.*, 2003). Female mice subjected to CR typically do not cycle and thus presumably are sterile while female

rats have reduced litter size (Weindruch & Walford, 1988; Masoro, 2001; Hursting *et al.*, 2003; Holehan & Merry, 1985). In the latter species, the effect of reduced litter size on life time fertility may be partially offset by a delay in reproductive aging and thus longer period of reproductive competence (Holehan & Merry, 1985). Separating the beneficial effect of CR on longevity from its detrimental effect on reproduction may be difficult. It is believed that CR affects aging by activating a genetic program which shifts the animals from a growth and reproduction mode to the survival/stress resistance mode, thus allowing the animal to respond to scarcity of food by postponing reproduction until environmental conditions improve.

Recent interest in the genetic control of aging resulted in identification of many mutations that prolong life in organisms ranging from yeast to mammals (reviewed in Guarente & Kenyon, 2000; Jazwinski, 2001; Tissenbaum & Ruvkun, 1998; Tatar *et al.*, 2003). Although exceptions exist, many of the long lived mutants in *Caenorhabditis elegans*, *Drosophila melanogaster* and mice have reduced fertility and in many cases one of the sexes is sterile. In *C. elegans* many of the known longevity genes promote entry into the so-called dauer, a larval stage that does not feed or reproduce. This presumably represents an adaptation to lack of food and increased population density, and provides an important mechanism for survival and dispersal.

There is great interest in utilizing the knowledge gained from the studies of CR and longevity genes to design anti-aging interventions that could be applied to the human. These could include dietary regimens acceptable to the general population, CR mimetics and pharmaceutical interventions based on the mechanism(s) of action of longevity genes. The generally reciprocal relationship between longevity and reproductive competence is of obvious concern in this regard. Compromising sexual and reproductive functions would represent unacceptable change in the quality of life, and reduced levels of gonadal steroids could lead to serious health problems including reduction in bone mineral density and increasing the risk of osteoporosis and fractures.

In this context, we will summarize available information on reproductive function in long lived mutant mice. Both natural mutations and targeted disruption of specific genes (the so-called knock-out, KO) will be discussed.

Hypopituitary Dwarf Mice

Mice homozygous for recessive loss-of-function mutations at the prophet of pituitary factor 1 (Prop 1^{df}) or pituitary factor 1 (Pit 1^{dw}) locus live 35% to 65% longer than normal animals from the same stock (Brown-Borg *et al.*, 1996; Flurkey *et al.*, 2001). Both Ames dwarf (Prop 1^{df}) and Snell dwarf (Pit 1^{dw}) mice have primary deficiency of growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone (TSH) because the corresponding cell types in the anterior pituitary fail to differentiate during fetal development (Li *et al.*, 1990; Sornson *et al.*, 1996). Consequently, postnatal growth and adult body size are greatly reduced, the animals are hypothyroid and exhibit a characteristic

dwarf phenotype (Bartke, 1979^a; Bartke, 1979^b; Bartke, 2000^a). Sexual maturation is delayed and gonadal function reduced with the extent of these impairments depending strongly on genetic background. Snell dwarf mice from the inbred Dwarf/J strain are severely hypogonadal with females failing to undergo puberty and males exhibiting significant reductions in the number of advanced germ cells in the testes and in the weight of accessory reproductive glands (Bartke & Lloyd, 1970). In contrast, in stocks maintained on heterogeneous genetic background, females undergo apparently normal, although delayed sexual development with establishment of estrous cycles and period of sexual receptivity, and males have complete and quantitatively nearly normal spermatogenesis, are fertile, and can be used as breeders (Bartke, 1979^a; Bartke, 1979^b; Bartke, 2000^a; Bartke & Lloyd, 1970). Plasma gonadotropin levels are reduced, presumably as a secondary consequence of GH, PRL, and TSH deficiency. Replacement therapy with GH, PRL or thyroxine can increase the levels of luteinizing hormone (LH) and/or follicle stimulating hormone (FSH) (Bartke *et al.*, 1977; Doherty *et al.*, 1980; Chandrashekar & Bartke, 1993). In Ames dwarf mice, treatment with bovine GH increased LH responses to exogenous LH releasing hormone (LHRH), partially normalized LH responses to gonadectomy and to testosterone replacement, and increased testosterone and androstenedione responses to human chorionic gonadotropin *in vitro* (Chandrashekar & Bartke, 1993).

Female dwarf mice with spontaneous or hormonally-induced sexual maturation can produce fertilizable ova and mate, but do not become pregnant because they lack PRL which, in a mouse, is absolutely required for establishment of normal luteal function and support of pregnancy. Providing PRL replacement by means of injections or transplants of normal pituitaries under the kidney capsule allows both Snell and Ames dwarf mice to become pregnant, produce live pups and raise them to weaning (Bartke, 1965; Bartke, 1973). During pregnancy, exogenous PRL administration is required only during the first 8 days after mating (Bartke, 1973), because afterwards mouse corpus luteum depends on placental lactogen rather than pituitary PRL, and production of placental lactogen in dwarf mice is normal or perhaps slightly increased (Soares *et al.*, 1984).

GH Deficient Little Mice

GH releasing hormone receptor deficient little (GHRHR^{lit}) mice have isolated GH deficiency and are smaller than normal (Eicher & Beamer, 1976). Life span of little mice on C57 BL/6 background is prolonged by approximately 25% if their obesity is prevented by maintaining them on a low fat diet (Flurkey *et al.*, 2001). Little mice are fertile, but male reproductive functions are quantitatively reduced (Chubb, 1987).

GH Resistant Laron Dwarf Mice

Targeted disruption of GH receptor/GH binding protein gene was used to produce mice with GH resistance due to deficiency of GH receptor, a syndrome analogous to Laron dwarfism in the human (Zhou *et al.*, 1997). Animals homozygous for the disrupted

gene (GHR-KO $-/-$) produce increased levels of GH, presumably due to absence of negative feedback, but fail to respond to GH and have dramatically reduced levels of insulin-like growth factor 1 (IGF-1) in peripheral circulation leading to reduced post weaning growth and diminutive adult body size (Zhou *et al.*, 1997). Lifespan of GHR-KO animals is increased by 46% (Coschigano *et al.*, 2000). Both females and males are fertile but their gonadal function and fertility are reduced in comparison to normal ($+/+$ and $+/-$) animals of the same strain. Females exhibit delayed puberty which can be advanced by a brief period of treatment with IGF-1 (Danilovich *et al.*, 1999). Adult GHR-KO females have reduced number of ovarian follicles and corpora lutea and reduced litter size (Zhou *et al.*, 1997; Bachelot *et al.*, 2002; Zaczek *et al.*, 2002). These deficits may be combined with early reproductive aging, although this conclusion is controversial (Bachelot *et al.*, 2002). In the ovaries of estrous GHR-KO mice produced in our colony, expression of IGF-1 was reduced but expression of key steroidogenic enzymes was not affected (Zaczek *et al.*, 2002). Bachelot *et al.* (2002), reported no attenuation in ovarian expression of IGF-1 or any of the examined IGF-1 binding proteins in GHR-KO mice.

In GHR-KO $-/-$ (compared to $+/+$ and $-/+$) males, puberty is delayed (Keene *et al.*, 2002), LH responses to LHRH as well as testicular responses to LH are reduced (Chandrashekar *et al.*, 1999) with the latter deficit likely being due to reduced number of LH receptors in the testis (Chandrashekar *et al.*, 2001). Moreover, the number of PRL receptors in the testis is reduced and the ratio of testosterone to androstenedione is diminished (Chandrashekar *et al.*, 2001), possibly reflecting reduced PRL signaling in spite of elevated PRL levels in the circulation (Chandrashekar *et al.*, 1999). Although most of GHR-KO males are fertile, several measures of fertility are reduced in comparison to normal ($+/+$ and $+/-$) animals (Chandrashekar *et al.*, 1999).

Partially IGF-1 Resistant Heterozygous IGF-1 Receptor KO Mice

In contrast to severe developmental deficits and early mortality of mice homozygous for disruption of the IGF-1 receptor, the animals heterozygous for this condition are only slightly smaller than normal and live significantly longer (Holzenberger *et al.*, 2003). Average life span was increased by 33% in females and appeared to be longer also in males (Holzenberger *et al.*, 2003). Both females and males are fertile and several indices of female reproductive function and reproductive aging were indistinguishable from normal ($+/+$) controls (Holzenberger *et al.*, 2003).

Other Long-lived Gene Knock-out Mice

Modest, but statistically significant extension of life span and normal fertility were reported in mice with targeted gene disruption of the $p66^{shc}$ gene which is involved in stress responses (Migliaccio *et al.*, 1999), and in mice with adipose tissue-specific disruption of the insulin receptor and the resulting decrease in body fat content (Bluhner *et*

al., 2003). We are not aware of any information on the age of sexual maturation, reproductive hormone levels, or quantitative measures of reproductive performance in these animals.

Discussion

Earlier in this chapter, we have listed seven spontaneous or experimentally produced mutations which extend life span in laboratory stocks of house mice (*Mus musculus*). Five of these seven mutations reduce function of the somatotrophic axis by causing GH deficiency, GH resistance or partial IGF-1 resistance. Existence of reproductive deficits in four of these five mutants is consistent with the well documented roles of GH and IGF-1 in the control of sexual maturation (Ramaley & Phares, 1980; Hiney *et al.*, 1996) and adult reproductive function (reviewed in Adashi, 1998; Bartke, 2000; Hull & Harvey, 2000^b; Hull & Harvey, 2002). These findings are also consistent with the generally reciprocal relationship between longevity and functions regulated by the somatotrophic axis including growth, maturation, and adult body size. However, the intriguing recent findings in IGF-1 receptor KO (+/-) mice suggest that a modest reduction in somatotrophic signaling can prolong life and be compatible with normal reproductive development and function (Holzenberger *et al.*, 2003). Apparent disassociations of the effects on life span from the effects on reproduction was achieved also by knockout of genes that increase resistance to oxidative stress or produce insulin resistance of adipocytes (Migliaccio *et al.*, 1999; Bluher *et al.*, 2003). Detailed information on reproductive development and function of p66^{shc} and fat insulin receptor KO (FIRKO) mice would be of considerable interest.

Interpretation of finding in GHR-KO mice is complicated by the possibility that effects of GH resistance caused by targeted disruption of the GHR gene may be partially offset by compensatory adjustments. Significant elevation of circulating prolactin (PRL) levels in male and in ovariectomized female GHR-KO mice (Chandrashekar *et al.*, 1999) represents such potential adjustment. Some of the biological actions of PRL overlap those of GH and it is conceivable that the preserved, although quantitatively reduced reproductive competence of GHR-KO animals is related to enhanced PRL secretion. Physiological compensatory adjustments can reduce and, in some cases, completely mask the phenotypic consequences of gene knockout. However, in GHR-KO mice, this appears unlikely, because most of their phenotypic characteristics correspond to the expected effects of GH resistance.

It is unclear whether reduced gonadal activity in GHR-KO, or other long lived mutant mice contributes in any way to extension of life span. Ablation of germ cells extends longevity in *C. elegans* (Hsin & Kenyon, 1999) and sterility can increase life span in *Drosophila* (Sgro & Partridge, 1999). Gonadectomy is reported to increase life span in males (Drori & Folman, 1976). Negative association of reproductive functions and longevity in mammals is more likely due to alterations in growth, metabolic rate, regulation of carbohydrate metabolism, and energy partitioning than to differences in gonadal status.

Ames and Snell dwarf mice are hypothyroid (Bartke, 1979; Li *et al.*, 1990; Sornson *et al.*, 1996; Bartke, 2000^a) and levels of thyroid hormones are reduced also in GHR-KO mice (Hauck *et al.*, 2001). Alterations in function of the somatotrophic axis together with the associated hypothyroidism and hypoinsulinemia are likely to influence energy metabolism, partitioning of food-derived energy, and generation of reactive oxygen species. Hypoglycemia likely reduces non-enzymatic glycation of proteins and generation of advanced glycation end products (AGE).

It should be emphasized that IGF-1 signaling, insulin signaling, and oxidative stress are believed to represent major evolutionary conserved mechanisms of aging. Therefore reproductive consequences of genetic manipulation of these pathways are likely to be relevant to most of the interventions that could slow aging and prolong life. Study of mutants with alterations in pituitary functions or GH signaling provide interesting opportunity to further clarify the roles of GH and IGF-1 in the control of female and male reproductive functions, to separate the roles of GH dependent vs. GH independent biosynthesis of IGF-1 and to gather new information in the intricate relationships between growth, reproduction, and aging.

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