MINIREVIEW

Effects of Growth Hormone Overexpression and Growth Hormone Resistance on Neuroendocrine and Reproductive Functions in Transgenic and Knock-Out Mice[2] (44434)

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Abstract. **Transgenic mice overexpressing growth hormone (GH) exhibit alterations in the function of the hypothalamic-pituitary-gonadal (HPG) axis and the H-P-adrenal axis. Alterations in the turnover of hypothalamic neurotransmitters, in plasma hormone levels, and in regulation of their release are associated with reproductive deficits, particularly in females. Results reported after publication of our minireview on this subject provided evidence that GH-transgenic mice have increased binding of GH to GH binding proteins in plasma, are hyperinsulinemic and insulin resistant, and have major alterations in energy budgets with increased allocation to growth. Reduced life span and fertility of these animals may be related to insufficient allocation of energy to reproduction and maintenance. Growth hormone resistance induced by transgenic expression of an antagonistic bGH analog or by targeted disruption (knock-out, KO) of the GH receptor (GH-R) gene leads to dramatic suppression of plasma levels of insulin-like growth factor-1 (IGF-1), and dwarf phenotype due to reduced growth and increased adiposity. In both models of GH resistance, there are marked reproductive deficits in females, decline of breeding performance of males, and alterations in the function of the HPG axis. In GH-R-KO females, puberty is delayed, and litter size is reduced. Fetal weights are reduced whereas placental weights are increased, and the weight of newborn pups is reduced despite an increase in the length of gestation. In GH-R-KO males, copulatory behavior and fertility are reduced, plasma PRL is elevated, and responses to lutenizing hormone releasing hormone (LHRH) *in vivo* and to LH *in vitro* are suppressed. However, reproductive deficits in GH-R-KO mice are**

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very mild when compared to those described previously in IGF-KO animals. Apparently, the amounts of IGF-1 that may be produced locally in the absence of GH stimulation are sufficient for sexual maturation and fertility in both sexes, whereas quantitative deficits in reproductive function reflect absence of GH-dependent IGF-1 production and other consequences of eliminating GH signaling. The reproduction phenotype of the GH-R-KO mice is also mild when compared to dwarf mice that lack GH, prolactin (PRL), and thyroid stimulating hormone (TSH). This is presumably related to the presence of redundant mechanisms in the stimulatory control of the gonads by the pituitary and the ability of animals capable of producing PRL and TSH to compensate partially for the absence of GH signaling. [P.S.E.B.M. 1999, Vol 222]

In a minireview entitled "Neuroendocrine and reproductive consequences of overexpression of growth hormone in transgenic mice," which appeared in this journal in 1994 (1), we summarized the effects of overexpression of bovine (b) growth hormone (GH) on the function of the hypothalamic-pituitary system and peripheral endocrine glands in transgenic mice. In animals used for these studies, bGH was expressed in several ectopic sites including the liver, the kidney, and the adipose tissue under control of phosphoenopyruvate carboxykinase (PEPCK) promoter. In each of the lines (pedigrees) used for these studies, plasma bGH levels were extremely high, ranging from several hundred to over 2000 ng/ml. Thus, these animals provide a model of exposure to pathologically elevated levels of GH throughout the postnatal life, a condition comparable to that in gigantism in the human and bearing some resemblance to acromegaly. This extreme situation allows identification of a wide range of functions that can be affected by GH, and provides an opportunity for study of the mechanisms involved. However, results obtained in these animals tell us very little about the physiological role of GH during early development, maturation, and adult life of normal individuals. In this "update" of our 1994 minireview, we will briefly reiterate the key findings in bGH transgenic mice, summarize some of the more recent observations in these and other GH transgenics, and concentrate on observations in animals with GH resistance or GH deficiency. We believe that data derived from animals that do not produce GH or cannot respond to it provide information on the physiological role of this hormone. Finally, we will briefly address the issue of difficulties that arise in interpretation of experimental results obtained from animals with targeted disruption (Knock-Out; KO) of specific genes.

Consequences of Overexpression of GH in Transgenic Mice

High levels of ectopically produced heterologous GH in the circulation of PEPCK-bGH transgenic mice are associated with increased expression of somatostatin (2) and reduced expression of GH releasing hormone (GHRH) in the hypothalamus (3) with the consequent drastic suppression of the production of endogenous GH in the pituitary (4). Transgenic PEPCK-bGH mice have normal or only marginally elevated plasma glucose levels, but are grossly hyperinsulinemic and insulin resistant (5, 6). Plasma corticosterone levels are elevated (7) apparently as a consequence of increased adrenocorticotropic hormone (ACTH) secretion (8). In males, plasma follicle-stimulating hormone (FSH) levels and expression or β subunits of FSH and lutenizing hormone (LH) in the pituitary are reduced (9, 10) whereas prolactin (PRL) levels are elevated or normal (10). Interestingly, mechanisms responsible for inducing twice daily surges of PRL in muted females appear to be disrupted leading to high incidence of infertility in these animals (11–13), details below). Alterations in the turnover of hypothalamic neurotransmitters, dopamine and norepinephrine (NE), suggest that alterations in the release of adenohypophyseal hormones are due, at least in part, to abnormalities of hypothalamic function induced by GH, directly or *via* the GH-induced increase in insulin-like growth factor-1 (IGF-1). In support of this interpretation, receptors for both GH and IGF-1 have been localized in the hypothalamus (14–16). In further support of the hypothalamic site of action of GH and/or IGF-1 being responsible for alterations in neuroendocrine function of these animals, we have recently detected reduced release of LH releasing hormone (LHRH) from hypothalami of PEPCK-bGH transgenic, as compared to normal mice *in vitro* in the presence of N-methyl-D, L-aspartic acid, a glutaminergic agonist (17). Glutamate is believed to be importantly involved in the control of LHRH-secreting neurons *in vitro* (18, 19).

Alterations in neuroendocrine function in GH-transgenic mice include changes in the release of pituitary hormones that control the gonads. Not surprisingly, these hormonal changes are associated with significant deficits in reproductive function. Thus most of PEPCK-bGH transgenic females are infertile (11). We were able to trace their infertility to deficiency of luteal function (12) which, in turn, could be explained by failure of stimuli associated with mating to induce the normal pattern of twice daily PRL surges (13). These PRL surges are necessary for maintenance of luteal function during the first half of gestation in the mouse (20, 21). In infertile PEPCK-bGH females, pregnancy could be rescued by administration of progesterone, PRL, or a dopaminergic antagonist that stimulates endogenous PRL release (12). In MT-hGH-transgenic female

mice we have found that the LH surge during the afternoon of proestrus was significantly lower than in their normal littermates (Debeljuk L, unpublished data).

Importantly, not all of the actions of chronically elevated GH on female reproduction are inhibitory. In the PEPCK-bGH females that did become pregnant, litter size was significantly increased, apparently reflecting enhanced ovulation rate (11). This is consistent with the stimulatory effects of GH on ovulation in this and other species (11, 22). We have recently obtained evidence that increased ovulation rate in transgenic (Tg) mice overexpressing bGH may be related to reduced apoptosis of follicular cells in proestrous ovaries and reduced atresia (23), combined with the increased expression of cytochrome P450 cholesterol side chain cleavage enzyme, 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase in the granulosa cells (24).

Transgenic males overexpressing bGH have plasma testosterone (T) levels in the normal range and usually are fertile, although their behavioral responses to females are suppressed and the interval from mating with normal females to conception is increased (25). Our recent study of the time course of plasma testosterone (T) levels in castrated PEPCK-bGH and normal mice injected with a single large dose of T suggests that T clearance is significantly slower in transgenic animals (Danilovich A, Bartke A, unpublished data). This would suggest that normal plasma T levels in these animals may signify reduced rather than normal rates of T secretion by their testes. The validity of this interpretation and its functional significance are difficult to determine without further studies. However, they raise an important issue of difficulty in interpreting the meaning of plasma hormone levels in animals with major alterations in body size and composition and plasma volume (26), as well as energy metabolism (details later in this review), and probably many aspects of hepatic function. Liver is the principal site of metabolism of steroid hormones, and overexpression of GH in transgenic mice is associated with hepatomegaly and pathological changes in hepatocytes (27).

In addition to the studies in PEPCK-bGH transgenic females in which plasma bGH levels are extremely high and fertility is significantly compromised, we have examined regulation of gonadotropin and PRL release in transgenic metallothionein-I (MT)-bGH mice from a line in which circulating levels of bGH are very modest, approximately 9% of the levels measured in PEPCK-bGH animals. Most of the females from this line of MT-bGH mice are fertile, and their reproductive characteristics are nearly normal except for the absence of pregnancies from postpartum estrus and the consequent significant increase in the average interval between litters (28). In ovariectomized MT-bGH females, plasma LH and FSH levels were lower, and plasma PRL levels were higher than the corresponding values measured in normal ovariectomized animals (29). Administration of estradiol benzoate (EB) caused the expected suppression of gonadotropins and stimulation of PRL release in both normal and transgenic females. However, plasma FSH levels in ovariectomized, EB-injected transgenics were significantly lower than in identically treated normal mice (29). Moreover, the acute FSH response to a single dose of LHRH was significantly smaller in ovariectomized EB-primed MT-bGH females than in the corresponding normal controls (29). These findings add to previously reported evidence that overexpression of human (h) GH alters the regulation of gonadotropin release by negative feedback of gonadal steroids in male mice (30) and extend these findings to animals overexpressing bGH and to females.

Vidal *et al.* (31) undertook studies aimed at identification of mechanisms responsible for elevation of plasma PRL levels in GH transgenic mice. They have shown that in MT-bGH females from a line in which plasma PRL levels are significantly elevated (1, 29), the expression of dopamine (DA) subtype 2 receptors in the anterior pituitary is significantly reduced, whereas expression of the estrogen receptors (ER) is significantly enhanced (31). Importantly, opposite changes in the expression of DA and estrogen receptors were detected in the pituitaries of transgenic MT-hGH females (31) in which plasma PRL levels are suppressed due to lactogenic activity of hGH in rodents (32). Further studies will be necessary to determine to what extent these changes in dopamine receptors (DA-R) and ER may represent consequences of alterations in the number, volume, surface area, and metabolic activity of the lactotrophs in response to altered regulatory inputs and to what extent they may represent primary effect of GH and/or IGF-1 on the lactotrophs. If chronic exposure to elevated peripheral levels of GH reduces the number of DA-R and increases the number of ER in the lactotrophs, these changes could lead to diminished response to the inhibitory influence of DA, and increased response to the stimulatory actions of estrogens, thus explaining the observed morphological indices of increased lactotroph activity (33) and increase in plasma PRL levels in MT-bGH females (1, 29).

Transgenic MT-hGHRH mice developed by Mayo *et al.* (34) provide an opportunity to examine the effects of overexpression of homologous (mouse) GH. In these animals, human GHRH driven by the MT promoter is produced in excessive amounts leading to overstimulation of somatotrophs, progressive enlargement of the pituitary, and extremely high levels of GH in the circulation (34). Thus, in contrast to the GH "transgenics" discussed above, these animals overproduce mouse rather than heterologous GH, and high levels of plasma GH originate from pituitary somatotrophs rather than from various ectopic sites. Preliminary data indicate that in comparison to normal mice, hGHRH transgenics have reduced turnover of DA in the median eminence of males and in the medial basal hypothalamus of females (Steger RW, unpublished data), no alterations in the turnover of NE in these regions of the hypothalamus, and normal levels of LH in spite of elevated levels of testosterone in males (35). However, LH responses to gonadectomy were severely attenuated in both sexes (Debeljuk L, unpublished data). As was the case in other lines

of transgenic mice overexpressing bGH, the hGH-RH transgenics also had significantly elevated plasma corticosterone levels (Debeljuk L, unpublished data).

Effects of Overexpression of GH on GH Transport in Peripheral Circulation, Insulin Signaling, and Energy Budgets

Overexpression of GH in transgenic mice is associated with an increase in hepatic GH and PRL receptors (36, 37). More recent studies provided evidence that serum levels of different fractions of GH binding proteins (GHBP) are also elevated in GH transgenic mice. Results of comparative experiments in MT-bGH and in two lines of PEPCK-bGH mice indicate that overexpression of GH is associated with significant increases in the GHBP binding capacity, in the amount of GH bound to GHBP in the circulation, and consequently, in the levels of GH-GHBP complexes (38). In spite of the increases in GHBP levels, and greater relative occupancy of GHBP by GH in transgenic mice (as evidenced by significant reduction in the levels of free GHBP in serum), the percentage of GH that was present in the serum in the bound form was significantly reduced in transgenic as compared to normal mice. These alterations in parameters related to GH transport in the circulation were numerically large. For example, serum levels of GH-GHBP complexes were approximately 10-fold higher in the serum of PEPCK-bGH transgenics than in the serum of normal control mice (38).

Insulin resistance of GH transgenic mice is manifested by marked hyperinsulinemia and normoglycemia (39). This is associated with significant reduction of the content of insulin receptors (IR) in both the particulate fraction and the solubilized membranes of the liver, corresponding to the expressed (functional) and nonexpressed (cryptic) receptors (40). There were no significant changes in IR affinity, but the activity of insulin-dependent tyrosine kinase in partially purified IR preparations was markedly increased (40). Subsequent studies provided evidence that both the decrease in the number of IR and the increase in their autophosphorylation activity are directly related to increased insulin levels in transgenic animals rather than to their abnormally elevated GH. Treatment with streptozotocin or fasting for 48 hr was used to suppress plasma insulin levels in transgenic mice without altering the levels of GH in their circulation. Both treatments were indeed effective in reducing insulin levels and produced an increase in IR and a decrease in their insulin-stimulated autophosphorylation (41). Thus, these treatments tended to normalize both the levels and the autophosphorylation of IR in spite of the persistence of grossly elevated GH.

In a very interesting series of studies, Rollo *et al.* (26, 42–46) addressed the issue of the impact of chronic overexpression to GH on energy budgets of transgenic mice. These investigators provided evidence that transgenic MT-rat (r)GH mice gain weight much faster than their normal siblings even though they consume the same (or slightly reduced) amount of food per gram body weight (26). These GH-transgenic mice exhibit enhanced efficiency of food utilization (in terms of the percentage of energy intake used for growth) (26) and appear to reduce their energy expenditures effectively by increased sleep time and reduced locomotor activity during wakefulness (45). Calculations of the energy budgets of these animals indicate that a disproportional amount of metabolizable energy is used for growth and thus the amount of energy available for other functions, including reproduction and maintenance, is reduced below the levels measured in normal controls. On the basis of these calculations, Rollo *et al.* (46) suggested that both reproductive deficits and reduction in life span of transgenic animals may be due to energy deficits. To test the validity of this interpretation, they increased caloric intake of MT-rGH transgenic mice by supplementing their diet with sucrose. This improved their reproductive performance and longevity (44, 46). The latter result is tantalizing since it is caloric restriction rather than increased caloric intake that is well documented to delay aging and prolong life in laboratory rodents. In fact, supplemental carbohydrate feeding of normal animals appeared to increase some indices of aging and reduce life span in the same experiment (44; Rollo CD, personal communication).

The results obtained by Rollo and the "energy stress paradigm" that he proposed (46) raise new and important issues in the search for mechanisms linking excessive GH levels with abnormalities in neuroendocrine control of reproduction and with accelerated aging. More specifically, his results suggest that the effects of GH excess on fertility and on aging may be due to the effects of GH on food intake, growth rate, and allocation of energy resources rather than to primary effects of altered release of hypothalamic, pituitary, or adrenal hormones.

In interpreting the consequences of GH overexpression on neuroendocrine function and reproduction in transgenic mice, it is also important to consider that these animals have a greatly reduced life span and multiple indices of premature aging including reduced replicative potential of their cells *in vitro* (47), early decline in the turnover of hypothalamic neurotransmitters (48), and increased age-related astrogliosis in many regions of the brain (49). Interestingly, life span of dwarf mice that are GH-, PRI-, and thyrotropin-stimulating hormone (TSH)-deficient and diminutive in size is significantly prolonged (50). The possible role of GH in determination of the life span and the relationship of findings in transgenic and dwarf mice to the reported "anti-aging" effects of GH in the human are outside the scope of this review. The reader is referred to our recent discussion of these issues in other publications (51, 52).

Effects of GH Resistance on Neuroendocrine Function in Transgenic and KO Animals

Resistance to the actions of GH due to mutations of the GH receptor (GH-R) was described in the human and

is known as Laron dwarfism (53). The affected individuals exhibit major abnormalities of growth and adult stature but are typically fertile (53). Recent availability of animals with GH resistance allows the study of the mechanisms responsible for the phenotype of individuals lacking functional GH-R and provides excellent model systems for identification and study of the physiological actions of GH.

A novel model for the study of GH resistance became available from serendipidous discovery that the GH molecule with substitution of arginine or lysine for glycine at position 119 in the third α-helix has GH antagonistic properties (54, 55). Transgenic animals overexpressing this antagonistic GH analog under control of MT promoter have reduced plasma IGF-1 levels, are small, and have a tendency to develop obesity (i.e., exhibit phenotypic characteristics consistent with GH resistance) (54, 55). In these "transgenic dwarfs" the levels of GH-R in the liver and GHBP in the serum are increased (55). However, *in vitro* binding of labeled hGH to serum of these animals was reduced to levels not distinguishable from nonspecific binding, and *in vivo* hepatic uptake of injected labeled bGH was reduced to approximately 1/5 of the values measured in normal animals (56). These observations, together with the results of chromatographic analysis of serum and liver extracts, suggest that almost all of GHBP present in serum of MT-bGH-Antagonist (Ant) mice is complexed with the antagonistic bGH analog, and thus no free GHBP is available for binding wild-type hormone. Moreover, high levels of this analog inhibit hepatic uptake of wild type GH (56). Additional mechanisms of bGH-Ant action is suggested by the evidence that this bGH antagonist apparently fails to induce the normal dimerization of GH-R (54, 56), and occupancy of most of the GH-R by the antagonist interferes with formation of the complexes of one molecule of wild-type GH with two GH-Rs (56). This further reduces the ability of endogenous or exogenous wild type GH to exert their normal effects in MT-bGH-Ant animals because dimerization of GH-Rs is required for normal GH signaling (57).

Although both female and male MT-bGH-Ant mice can reproduce, their fertility is suppressed with major deficits in litter size and postnatal survival of the pups, and increased intervals between mating and conception. Reduced postnatal survival of the pups was related to underdevelooped mammary glands of MT-bGH-Ant females (58). However, some of the females can raise their litters to weaning. Although turnover of dopamine in the median eminence region of the hypothalamus, a region importantly involved in the control of PRL release, is significantly reduced, we did not find alterations in plasma PRL levels or in PRL responses to pharmacological blockade of catecholamine synthesis (Steger RW, Bartke A, unpublished data). Plasma LH levels also appeared to be normal (Chandrashekar V, Bartke A, unpublished data). Further studies will be necessary to identify mechanisms responsible for reproductive deficits in these animals.

Interpretation of the findings in MT-bGH-Ant transgenic mice is somewhat complicated by the fact that these animals may not be completely GH-resistant (56) and by the possibility that antagonistic bGH analogs may be capable of exerting biological effects in some target organs of GH. For example, G119K-bGH appears to be capable of stimulating the synthesis of both GH-R and GHBP, although its potency in this regard is lower than that of the wild type hormone (54, 56, 59).

A model of complete GH resistance was developed recently by targeted disruption (knock-out, "KO") of the *GH-R-GHBP* gene in the mouse (60). Animals homozygous for this "null mutation" of the *GH-R-GHBP* gene (hereafter referred to as GH-R-KO mice) exhibit profound suppression of GH binding, nondetectable or extremely low plasma IGF-1 levels (depending on the assay system; 60, 61), reduced postnatal (and particularly postweaning) growth, and dwarf phenotype in spite of significantly elevated plasma GH levels (60). These phenotypic characteristics are fully consistent with complete GH resistance in this "Laron mouse." Plasma insulin levels in GH-R-KO animals are extremely low, whereas plasma glucose levels are significantly suppressed (60). Plasma corticosterone levels appeared to be elevated by approximately 50% in both sexes (Danilovich NA, Bartke A, unpublished data), but additional studies including measurements in samples collected under "basal" conditions will be necessary to verify this observation.

In GH-R-KO males, plasma LH levels are normal whereas PRL levels are increased (61). Acute increases in plasma LH and T levels after LHRH administration are attenuated. Moreover, testicular T secretion *in vitro* is reduced in GH-R-KO versus normal males both basally and in the presence of LH in the incubation media (61).

Although most males and females can reproduce, their reproductive potential is significantly suppressed. Males exhibit reduced copulatory behavior, increased interval between mating and conception, and increased incidence of infertility and sire smaller litters than their normal counterparts (60, 62). Moreover, the temporal distribution of successful copulation (as evidenced by the presence of vaginal plugs) differs between GH-R-KO and normal males mated to previously group-housed normal females. In normal males, the maximal number of plugs is found after 3 days of cohabitation with females, consistent with the pheromonally mediated ability of male mice to induce estrus synchrony, the so called Whitten effect (63). In contrast, the peak of mating with GH-R-KO males occurred on Day 4, suggesting delayed or reduced secretion of pheromones responsible for synchronizing the cycle and inducing ovulation in the female (64).

In female GH-R-KO mice, puberty is delayed, estrous cycle is prolonged and often irregular, litter size is reduced

apparently reflecting reduced ovulation rate, and pregnancy is prolonged (60, 62, 65), Danilovich and Bartke, unpublished data). Moreover, fetal size on Day 17 of gestation and birth weight of pups are significantly reduced in GH-R-KO as compared to normal females (62, 65). Comparison of the data obtained in GH-R-KO females mated to normal males with those obtained in GH-R-KO females mated to GH-R-KO males suggests that male genotype significantly influences litter size, but fetal size is not affected by the genotype of the fetus (62). Puberty in GH-R-KO females can be significantly advanced by injections of IGF-1 (65; Fig. 1).

Some of the reproductive abnormalities in GH-R-KO mice were not unexpected. Thus significant delay of vaginal opening in these mice as well as the ability of exogenous IGF-1 to accelerate their maturation (65) are consistent with considerable evidence that the GH/IGF-1 axis is involved in the control of the timing of puberty (66, 67). Indeed, increased production of IGF-1 has been proposed to act as a signal for the maturational activation of the hypothalamic LHRH pulse generator (67). Reduced litter size in GH-R-KO females is in excellent agreement with the well-documented ability of IGF-1 to potentiate the actions of the gonadotropins on the ovary (68) and with the observation of increased ovulation rate in transgenic mice overexpressing GH (11) and in GH-injected normal animals (11). However, some of the findings in GH-R-KO mice would have been difficult or impossible to predict from what is already known about the influence of the somatotropic axis on reproduction and thus may represent novel actions of GH or IGF-1. For example, we are not aware of any previous evidence that GH may normally act to limit placental size, control the length of gestation, influence production of pheromonal signals that control timing of ovulation in the presence of adult males. The elevation of plasma PRL levels in the GH-R-KO males was unexpected and indeed opposite to what may have been predicted from previous findings. As was discussed earlier in this review, there is considerable evidence for stimulation of the lactotrophs and increase in peripheral PRL levels in several lines of MT-bGH and PEPCK-bGH transgenic mice, in which GH signaling and IGF-1 levels are augmented.

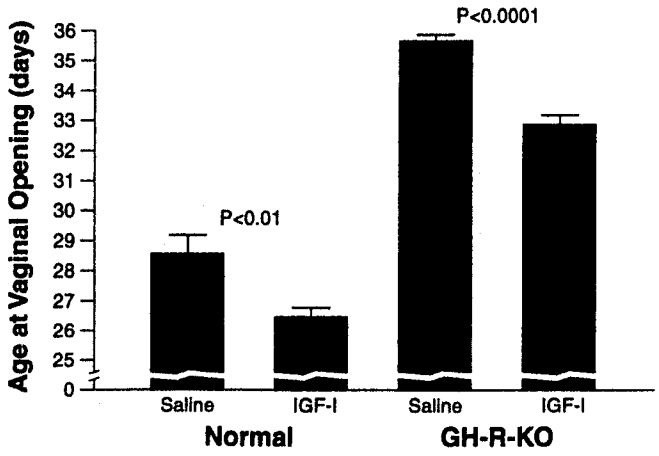

Figure 1. Age of vaginal opening in GH-R-KO and normal female mice injected twice daily with recombinant human IGF-1 (2 μg/g body weight; s.c.) or with saline vehicle starting one week before the expected age of sexual maturation.

The impact of GH resistance on reproductive functions in GH-R-KO mice is generally similar to the consequences of isolated GH deficiency to little (*lit/lit*) mice (69) and to the findings in humans with Laron dwarfism (53). However, it is of considerable interest that the phenotypic consequences of GH resistance and the resulting IGF-1 deficiency in GH-R-KO mice are very mild in comparison to those observed in mice with null mutations of the IGF-1 gene and in mice with hereditary dwarfism. The IGF-1-KO mice are small at birth with poor viability, and those that survive have infantile reproductive systems totally incompatible with fertility (70). Females fail to ovulate, whereas males have very small testes and vestigial reproductive systems and apparently only one instance of pregnancy resulting from insemination of a normal female by an IGF-1-KO male has ever been recorded (A. Bellve, personal communication). From comparison of the findings in GH-R-KO and IGF-1-KO mice it must be concluded that expression of IGF-1 is absolutely required for reproductive development and function, but maternal IGF-1 that may be transmitted *via* placenta and milk plus the amounts of IGF-1 that can be produced in the absence of GH signaling are sufficient for qualitatively nearly normal, although delayed and quantitatively reduced, reproductive development and functioning in both sexes. We are currently using GH-R-KO mice to begin to explore the relative role of GH-independent (presumably mainly local) versus GH-dependent (presumably mainly hepatic and systemic) IGF-1 production in the control of reproductive development and reproductive functions in the adult.

Comparison of reproductive functions in GH-R-KO and hypopituitary dwarf mice raises a number of interesting and potentially important issues. In Snell dwarf mice (dw/dw) and Ames dwarf mice (df/df), somatotrophs, lactotrophs, and thyrotrops fail to develop as a result of mutations at the *pit-1* locus and the *prop-1* locus, respectively (71–74). In both mutants, defects in differentiation of specific cell lineages in the adenohypophysis during fetal life lead to primary deficiency of GH, PRL, and TSH (72–74). Plasma IGF-1 levels are very low, generally near or below the detectability limit of radioimmunoassays (75, 76), thus closely resembling the situation in the GH-R-KO mice (60, 61). However, both sexes are hypogonadal. Most of the females fail to undergo sexual maturation, and those that go through puberty remain sterile due to absence of adequate luteal function (71). Males have small testes and reduced numbers of germ cells with severity of these defects depending strongly on the genetic background (71, 77) and most of the males are sterile (71). Plasma gonadotropin levels are reduced and can be increased by treatment with GH or PRL (76, 78). In Ames dwarf mice, plasma glucose levels are reduced (79) again resembling the findings in

GH-R-KO mice (60; Danilovich NA, Bartke A, unpublished data), and plasma insulin levels tend to be suppressed (79) but not to the extent encountered in GH-R-KO animals (60; Danilovich NA, Bartke A, unpublished data). Similarly GH-R-KO mice, Snell and Ames dwarfs are small and obese, although the relative reduction to adult body weight is somewhat greater in the dwarfs than in the "knock-outs" (60, 71, 75). Some characteristics of GH-R-KO and Ames dwarf mice are compared and contrasted in Table I.

It is challenging to attempt to explain why absence of GH signaling, profound suppression of peripheral IGF-1 levels, and dwarf phenotype are compatible with fertility in the GH-R-KO but not in the dw/dw or df/df mice. Hypothyroidism of the dwarfs is unlikely to provide an explanation because isolated defect is thyroid function in hypothyroid (hyt/hyt) mice is compatible with fertility (80) and there is no evidence that TSH, or thyroid hormones are indispensable for gonadal function. Thus, we must suspect that absence of PRL in Snell and Ames dwarfs is responsible for their reproductive infantilism and sterility. This conclusion follows from the comparisons of reproductive phenotypes in GH-R-KO and dwarf animals and is supported by the relatively well-documented effects of PRL on gonadal function, particularly in rodents (13, 20, 81). However, recent findings in mice with targeted disruption of the *PRL* gene (PRL-KO) (82) or on the *PRL* receptor gene (PRL-R-KO) (83) contradict this interpretation. Both PRL-KO and PRL-R-KO mice undergo apparently normal sexual maturation; females cycle, ovulate, and mate, (although they do not become pregnant due to luteal deficiency); and most males are fertile with delayed fertility in some PRL-R-KO males identified as the only major defect in male reproductive function (83; Kelly PA, personal communication). Thus, isolated deficiency of either GH or PRL signaling appears to be compatible with normal (although in some cases delayed) sexual maturation in mice of both sexes and with fertility of males, whereas combined deficiency of GH and PRL is not. In this context, it is of interest that there is some overlap in the actions of GH and PRL. In mice, both hormones can stimulate somatic growth (71, 84) and various indices of immune function (85, 86). Studies in several rodent species provided evidence that both GH/IGF-1 and PRL can stimulate testicular function by a variety of mechanisms including stimulation of gonadotropin release and increasing the number of LH receptors on the Leydig cells (71, 76, 78, 87, 88). We will return to this issue below in the section dealing with broader issues of interpretation of data obtained in KO animals.

Advantages and Limitations of Studies in Knock-Out Animals

Combining the procedures for targeted *in vitro* mutagenesis and homologous recombination using embryonic stem cells allows targeted disruption (gene knock-out, KO), (i.e. induction of "null mutations" in specific genes). The resulting animals provide a unique and fascinating opportunity to study the effects of deletion of a specific gene product and make inferences about its physiological function. This allows direct verification of the suspected or inferred role of the gene in question and can lead to discovery of hitherto unsuspected functions of specific cell product. Enumeration and discussion of the existing novel findings obtained in KO animals is outside the scope of this review.

In many cases KO animals do not exhibit the expected phenotype, and it has often been proposed that this must be due to the existence of alternate ("redundant") mechanisms for the control of the same function and possibly also to the compensatory increase in the use of these alternate pathways. This interpretation is based solidly on the current understanding of the ability of complex organisms to main-

Table I. Comparison of Phenotypic Characteristics of GH-R-KO Mice and Ames Dwarf Mice

	GH-R-KO	Ames dwarfs
Genetic defect	Targeted disruption of the GH-R/GHBP gene	Mutation at the *prop*-1 locus
Primary effect	GH receptor deficiency	Absence of somatotrophs, lactotrophs, and thyrotrophs
Body weight	~1/2 of normal	~1/3 of normal
Growth hormone	Elevated	Absent
IGF-1	Nondetectable	Nondetectable
Prolactin	Elevated in males	Absent
Thyroid function	Normal*	Suppressed
LH	Normal	Reduced
Fertility: Female	Reduced	Infertile
Male	Slightly reduced	Most infertile
Glucose	Reduced	Reduced
Insulin	Greatly reduced	Reduced in males
Corticosterone	Elevated*	Normal
Longevity	Unknown**	Greatly increased

* Based on preliminary data.
** Information not available for this recently developed animal model; studies in progress suggest that longevity may be increased (Kopchick JJ, unpublished observations).
Please see text for details and references.

tain homeostasis and on the examples of multiple stimulatory and/or inhibitory mechanisms involved in the control of the same physiological function. However, this interpretation provides rationale for downplaying or discounting the unexpected phenotypic consequences of targeted gene disruption and thus complicates interpretation of results obtained in these animals. It also raises an important possibility that results obtained in KO animals may underestimate the physiological importance of targeted genes and their products.

We believe that the well-documented overlap of biological activities of GH and PRL and the results obtained in GH-R-KO, PRL-R-KO, and PRL-KO mice provide an excellent illustration of both the redundancy of physiological mechanisms and the ability of the animals to compensate for the absence of specific gene products. Thus, mild quantitative deficits in male productive functions in GH-R-KO (60, 61), PRL-R-KO (83; Kelly PA, personal communication), and PRL-KO mice (82, 89) in which signaling by either GH or PRL is disrupted, contrast with profound suppression of fertility in Snell and Ames dwarf mice that are deficient in both GH and PRL (71–74), and with significant stimulatory effects of exogenous GH, IGF-1 or PRL on testicular function in dwarf mice (69, 76, 88) and in hypophysectomized animals (81, 88, 90). Perhaps the GH-R-KO knock-outs "use" PRL to maintain normal gonadotropin release and Leydig cell function at levels compatible with fertility, whereas PRL-R-KO and PRL-KO mice rely on GH for the same purpose. It is tempting to propose that the significant increase in plasma PRL levels in GH-R-KO males (61) may represent a mechanism of physiological compensation for the inability to respond to GH. Similarly, normal or near-normal immune function in PRL-R-KO, PRL-KO (82), and GH-R-KO (Kopchick JJ, unpublished data) mice stands in sharp contrast to the well-documented ability of exogenous PRL and GH to exert major effects on immune function in different animal models (85, 86). If this interpretation is correct, we can suspect that the full range of physiological functions of the GH-IGF-1 axis may be appreciated only in animals deficient in PRL and *vice versa.* We believe that the validity of these speculations can be tested experimentally by appropriate crosses to produce "double knock-outs." Excellent discussion of compensatory mechanisms in KO animals is included in a recent article of Russell and Leng (91) concerning hormonal control of parturition.

In discussing the interpretation of findings obtained in KO animals, it is also important to mention the issue of phenotypic consequences of heterozygosity for the disrupted gene. It is reasonable to assume that the consequences of inducing a null mutation or effectively deleting a specific gene would be recessive (i.e., phenotypic consequences will become evident only in animals homozygous for the disrupted gene). This expectation is generally confirmed, and the phenotypes of homozygous wild type (+/+) animals and animals heterozygous for the null mutation (–/+) are "normal" and indistinguishable (82, 92). However, exceptions to this rule also exist. For example, body weight in female mice heterozygous for GH-R-KO (+/–) is slightly but significantly lower than the body weight of homozygous wild type (+/+) mice in the same population (60). Lactation is completely suppressed in homozygous PRL-R-KO (–/–) mice but can also be significantly compromised in the +/– animals (83). This implies that comparisons of homozygous KO mice to normal animals from the same line may underestimate the effects of gene disruption if some of the normal animals carry the disrupted gene on one of their chromosomes. This is potentially important because it is expeditious to produce KO animals by crossing the KO (i.e., –/–) mice with heterozygotes (+/–) to obtain 50% of KO pups. This mating system results in all of the "normal" progeny being heterozygous, and thus the phenotypic difference between the phenotypes of KO and "normal" animals could be potentially minimized.

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