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REVIEW

Aging-related characteristics of growth hormone receptor/binding protein gene-disrupted mice

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Abstract Since generation of the growth hormone receptor/binding protein (GHR/BP) gene-disrupted mouse nearly 10 years ago, use of this mouse model has become widespread in the elucidation of the physiological roles of GH and insulin-like growth factor-1 (IGF-1). In particular, it serves as a useful model to study mechanisms of aging. This review highlights the evidence demonstrating that the loss of GH signaling leads to lifespan extension in mice, and presents the multiple characteristics of this mouse line that suggest the life extension is due to alteration of the aging process.

Key words aging · gene disruption · growth hormone receptor/binding protein · longevity · mice

Abbreviations

+/+	wild-type
AMPK	AMP-activated protein kinase
CR	caloric restriction
CREB	cAMP response element-binding protein
Foxo1	forkhead box O1
FSH	follicle stimulating hormone
G6Pase	glucose-6-phosphatase
GHR/BP	growth hormone receptor/binding protein
GHR/BP −/−	homozygous for the GHR/BP gene disruption
GLUT4	glucose transporter 4
HI	high isoflavone
IGF-1	insulin-like growth factor-1
IR	insulin receptor
IRS-1	insulin receptor substrate-1
IRS-2	insulin receptor substrate-2
KO	knockout
LH	leutenizing hormone
LI	low isoflavone
MnSOD	manganese superoxide dismutase
MRDT	mortality rate doubling time
PEPCK	phosphoenolpyruvate carboxykinase
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α
PIN	prostatic intraepithelial neoplasia
PPAR	peroxisome proliferator-activated receptor
RXR	retinoid X receptor
T3	triiodothyronine
T4	thyroxine
Tag	large T antigen

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Growth hormone (GH) is a peptide produced and secreted predominantly by the somatotroph cells of the anterior pituitary. As the name

implies, a major role for GH is the promotion of somatic growth. It also is involved in a number of other cellular activities, including carbohydrate, lipid and protein metabolism as well as cellular differentiation (Paladini et al. 1983; Isaksson et al. 1985; Boyd and Bauman 1989; Strobl and Thomas 1994). Circulating GH is largely complexed with binding proteins (GHBP) that regulate levels of free and bound GH, prolong GH half-life and modulate GH bioactivity through competition with receptors of GH (GHR) for the ligand (Baumann 1995). GH signaling is propagated by the sequential binding of a single free GH molecule to two identical membrane-bound GHR molecules whose dimerization activates intracellular signaling cascades of tyrosine kinases, ultimately leading to the regulation of gene expression or protein activity (Argetsinger and Carter-Su 1996).

In an effort to better characterize the many roles of GH, and also as a step toward the delineation of IGF-1-dependent and -independent actions, GH signaling was abolished in mice by targeted gene disruption of the GHR/BP gene (Zhou et al. 1997). The resulting mouse serves as a mammalian model for the human autosomal recessive disease Laron syndrome, also known as primary GH resistance or insensitivity and as primary IGF-1deficiency (Zhou et al. 1997; Kopchick and Laron 1999; Laron et al. 1993).

Removal of most of the fourth exon of the murine GHR gene and replacement with a bacterial neomycin gene segment resulted in undetectable levels of both GHR and GHBP in the homozygous gene-disrupted or knockout (KO) mice (GHR/BP −/−) (Zhou et al. 1997). An unexplainable residual GH binding activity was detected in liver extracts, suggesting the possible presence of a second GH receptor (Zhou et al. 1997). Serum GH levels were significantly increased in GHR/BP −/− mice but IGF-1 levels were substantially reduced (Zhou et al. 1997). Most importantly for the impetus of this review, the GHR/BP −/− mice exhibited an increased lifespan (Coschigano et al. 2000). This review focuses on the evidence for this claim of extended longevity and examines characteristics of the mice that suggest the aging process has been affected.

Lifespan extension

Life expectancy is a parameter of GH action that is much more easily studied in mice than in humans. An examination of longevity in mice homozygous for the GHR/BP gene disruption (GHR/BP −/−) revealed a significant increase, by nearly a year, of the average lifespan of GHR/BP −/− mice as compared to normal wild-type (+/+) control mice (Coschigano et al. 2000). A subsequent study of lifespan in a more homogeneous genetic background, the long-lived C57BL/6J inbred strain widely used in aging research, revealed a similar, though less robust, increase in mean lifespan for the GHR/BP −/− mouse (Coschigano et al. 2003). In addition to an increased mean lifespan, the GHR/BP −/− mouse also displayed an increased median lifespan and an increased maximal lifespan in comparison to +/+ males (Coschigano et al. 2003). These findings have been duplicated in another genetic background and in another laboratory (Bartke et al. 2002, 2004). Thus, disruption of GH signaling in mice results in increased longevity.

In an attempt to discriminate between changes in the aging process as opposed to changes in health that appear to affect aging, a rigorous statistical evaluation of the longevity data was recently performed (de Magalhaes et al. 2005). In this study, the rate at which mortality increases with age (mortality rate doubling time or MRDT) was calculated. The MRDT increased in the GHR/BP −/− mice as compared to +/+ mice, suggesting that disruption of the GH signaling pathway influences the aging process in mice (de Magalhaes et al. 2005).

Small body size and reduced skeletal growth

Small body size is associated with a longer lifespan in many species (see references in Bartke 2005). The best studied role of GH is arguably its participation in growth. Disruption of GH signaling produced a major postnatal effect on growth of the GHR/BP −/− mice. Body weight of GHR/BP −/− mice, with measurements starting just after birth, did not differ significantly from

normal (+/+) littermates until 2–3 weeks of age (Zhou et al. 1997; Lupu et al. 2001). Rate of growth in the following weeks was significantly slower for the GHR/BP −/− mice (Zhou et al. 1997; Coschigano et al. 2000 and 2003; Lupu et al. 2001; Sims et al. 2000). Maximal adult weight of the GHR/BP −/− mice averaged ~40% the weight of the +/+ mice and was reached at an earlier age than the +/+ mice (Coschigano et al. 2000, 2003). While most organ weights were proportionately decreased in the GHR/BP −/− mice as compared to the +/+ mice, at some of the examined ages liver and kidney were found to be disproportionately smaller and brain disproportionately larger in the GHR/BP −/− mice (Coschigano et al. 2003; Lupu et al. 2001; Ng et al. 1999; Sjögren et al. 2000; Berryman et al. 2004). Body length of the GHR/BP −/− mice was also significantly less than that of the +/+ mice, although the body length difference was not as dramatic as the body weight difference (Zhou et al. 1997; Lupu et al. 2001; Sims et al. 2000; Sjögren et al. 2000). GHR/BP −/− mice exhibited disproportional skeletal growth, decreased bone mineral content and reduced bone turnover (Lupu et al. 2001; Sims et al. 2000; Sjögren et al. 2000). Lupu et al. suggested that their observations could be indicative of developmental delays due to hypoproliferation in combination with a reduced size of hypertrophic chondrocytes.

Reproductive function

Alterations in reproductive function have also been attributed to possible changes in aging (Partridge et al. 2005). Delayed or impaired reproductive function has been reported for both genders of GHR/BP −/− mice. For females, the average age for the first pregnancy of GHR/BP −/− mice was delayed, suggesting a delay in sexual maturation (Zhou et al. 1997). Subsequent analysis revealed a significant delay of the age of vaginal opening, a marker for puberty, for the GHR/BP −/− females (Danilovich et al. 1999). Estrous cycle duration was comparable in mice housed individually but was significantly longer in group-housed GHR/BP −/− females, characterized by prolonged periods of diestrus (Zaczek et al. 2002). The number of ovarian follicles was significantly reduced, especially those 200 μm in size or larger; this was not a result of increased atresia, which was unchanged (Zaczek et al. 2002; Bachelot et al. 2002). Serum estradiol levels were reduced (Zaczek et al. 2002), but the capacity of the follicles to bind lutenizing hormone (LH), follicle stimulating hormone (FSH) and IGF-1 was not diminished (Bachelot et al. 2002). As a result of the decreased number of follicles, active corpora lutea and uterine implantation sites during gestation were reduced (Zaczek et al. 2002; Bachelot et al. 2002). Fetal size in pregnant GHR/BP −/− females was significantly reduced and pregnancy prolonged while placental weight was unexpectedly increased (Danilovich et al. 1999). The changes in fetal and placental weight were related to maternal rather than fetal genotype (Danilovich et al. 1999). Litter size and body weight of the newborn pups were significantly reduced for GHR/BP −/− as compared to +/+ dams (Zhou et al. 1997; Danilovich et al. 1999). Both maternal and paternal genotype influenced the litter size (Danilovich et al. 1999).

In GHR/BP −/− males, balanopreputial separation was significantly delayed, indicating a delay in the onset of puberty (Keene et al. 2002). Absolute weights of the pituitary, testis, seminal vesicle, ventral prostate and epididymis were dramatically decreased, despite significantly increased levels of circulating prolactin (Keene et al. 2002; Chandrashekar et al. 1999 and 2001). Fertility was also decreased, perhaps as a result of the hyperprolactinemia, although the number of testicular PRL receptors was decreased (Chandrashekar et al. 1999 and 2001). The reduced testicular growth and possible impairment of spermatogenesis could be due to the reduced levels of plasma FSH seen in the GHR/BP −/− mice (Chandrashekar et al. 2001). Basal levels of plasma LH and testosterone were similar in GHR/BP −/− and +/+ male mice, but the LH and testosterone responses to administration of a single dose of gonadotropin releasing hormone were significantly attenuated in the GHR/BP −/− mice (Chandrashekar et al. 1999). Intratesticular levels of testosterone were essentially the same between GHR/BP −/− and +/+ males, but the

basal in vitro release of testosterone by saline-treated testes obtained from GHR/BP −/− mice was significantly less than for saline-treated testes obtained from +/+ mice, as was the testosterone response to LH treatment, either by the isolated testes or by the intact animal (Chandrashekar et al. 1999, 2001). The attenuated testosterone response may be due to the reduced number of LH receptors in the testes (Chandrashekar et al. 2001).

Cognitive function

A delay of cognitive aging in GHR/BP −/− mice was shown in a study led by Kinney in 2001 (Kinney et al. 2001). Using an inhibitory avoidance learning task as a measure of cognitive function, learning and retention were compared between young and old GHR/BP −/− mice and their normal siblings. While the ability to retain the learned information declined over the 28-day test period in the old normal animals, no decline in retention was seen over the same time period for the old GHR/BP −/− mice. Furthermore, the old GHR/BP −/− mice did not differ from young mice. In an assessment of locomotor behavior and emotionality (differences in which could affect the learning and retention results), both groups performed similarly. Thus, it was concluded that the absence of GH signaling may be associated with improved long-term memory in old mice, reflecting a delay in aging.

A second study further characterized the behavioral and cognitive aging of the GHR/BP −/− and +/+ mice (Kinney-Forshee et al. 2004). Results of a water maze test that assessed spatial learning and memory of young (4–6 months) versus old (12–13 months) mice demonstrated poorer performance by the old +/+ mice in comparison to their young counterparts, while both young and old GHR/BP −/− mice performed similarly to the young +/+ mice. In an open-field test, no differences in emotionality were observed for young (2–4 months) or old (12–15 months) GHR/BP −/− or +/+ mice. However, old +/+ mice showed a marked decline in activity while old GHR/BP −/− mice performed the same as young GHR/BP −/− and +/+ mice. These studies support the previous findings of delayed age-induced cognitive and behavioral decline in the GHR/BP −/− mice.

Altered carbohydrate and lipid metabolism

Although the mechanism(s) of aging are still largely unknown, several hypotheses have evolved, based mainly on studies of the anti-aging properties of caloric restriction (CR; Masoro 2000). One theory relates decreased insulin exposure and increased longevity (Parr 1997). Evaluations of glucose homeostasis in the GHR/BP −/− mice revealed severely depressed levels of circulating insulin but insignificant to moderate decreases in glucose levels in comparison to +/+ mice (Coschigano et al. 1999; Dominici et al. 2000; Liu et al. 2004; Guo et al. 2005). Serum glucagon levels were also reduced, although not as severely as insulin levels (Liu et al. 2004). Maintenance of near normal glucose levels appeared to result from an increase in tissue sensitivity to insulin (Coschigano et al. 1999; Liu et al. 2004; Guo et al. 2005). However, GHR/BP −/− mice displayed significant impairment of glucose tolerance in intraperitoneal glucose tolerance tests, suggesting reduced synthesis or release of insulin (Coschigano et al. 1999; Guo et al. 2005). Pancreatic islet size and β-cell mass were reduced, apparently due to decreases in proliferation and cell growth (Liu et al. 2004; Guo et al. 2005). Significant changes in glucose and insulin levels as well as reduced pancreatic islet size were seen in 10-day-old GHR/BP −/− pups, when growth retardation was relatively mild (Liu et al. 2004).

In addition, other parameters that may be important to the mechanisms of delayed aging in these mice were assessed (Hauck et al. 2001). Body core temperature was slightly reduced as were the levels of the thyroid hormones, triiodothyronine (T3) and thyroxine (T4).

Further evaluation of the insulin signaling pathway in liver tissue of GHR/BP −/− mice demonstrated an increase in insulin receptor (IR) expression and insulin-stimulated IR tyrosine phosphorylation as compared to +/+ mice (Dominici et al. 2000). Interestingly, no signifi-

cant changes downstream of IR were observed (e.g., efficiency of insulin receptor substrate-1 IRS-1 and Shc tyrosine phosphorylation or activation of phosphatidylinositol 3-kinase by insulin), suggesting an adaptation to the very low levels of circulating insulin and confounding the role of insulin signaling in altered longevity.

In another study, the effect of the GHR/BP deficiency on hepatic lipid metabolism was evaluated in adult male mice (Ng et al. 1999). As mentioned earlier, liver mass of the GHR/BP −/− mice was disproportionately reduced in comparison to the +/+ mice. Histological analysis demonstrated microvesicular steatosis, decreased hepatocyte mass and decreased nuclear mass with a normal nuclear:cytoplasmic ratio in the GHR/BP −/− mice. Liver phospholipid, triglyceride and cholesterol levels were all disproportionately higher in the GHR/BP −/− mice. Thus, the GHR/BP gene disruption affected liver lipid metabolism as well.

Growth hormone deficiency or resistance is characterized by a decrease in lean body mass and an increase in adiposity. Several studies have corroborated this in the GHR/BP −/− mice (Berryman et al. 2004; Egecioglu et al. 2006). Excess fat accumulation was predominantly subcutaneous (Berryman et al. 2004). Leptin levels were either unchanged or decreased in comparison to +/+ controls (Berryman et al. 2004; Egecioglu et al. 2006). Serum cholesterol, triglycerides and apoB levels as well as HDL and LDL levels were significantly reduced in GHR/BP −/− mice (Egecioglu et al. 2006).

A recent study investigated the effects of diets based on low isoflavone (LI) or high isoflavone (HI) soy protein isolates on plasma and hepatic lipid profiles as well as on longevity of the GHR/BP −/− and +/+ control mice (Bartke et al. 2004). The LI diet lowered plasma total triglyceride and fasting blood glucose levels for both GHR/BP −/− and +/+ mice and raised liver cholesteryl ester and liver total triglyceride levels adjusted for body weight of GHR/BP −/− mice. The HI diet raised the normally low level of plasma total cholesterol of GHR/BP −/− mice. The changes seen for the LI diet did not occur with the HI diet. The HI diet did improve the glucose tolerance of the GHR/BP −/− mice but negated the lifespan extension normally seen for the GHR/BP −/− mice. Overall, although the soy-based diets had some effects on lipid profiles and glucose homeostasis, these effects did not translate to an extension of the already long lifespans of the GHR/BP −/− mice.

Oxidative damage

Since improved antioxidant defenses have been suggested to effect aging, the free radical defenses of the GHR/BP −/− mice were investigated as a possible mechanism of increased longevity (Hauck et al. 2002). The activities of three antioxidant enzymes (Cu/Zn superoxide dismutase, catalase and glutathione peroxidase) and two free-radical damage markers (lipid peroxidation and protein oxidation) were measured in kidney and liver of GHR/BP −/− and +/+ males and females. While significant differences were sometimes observed, the changes seen were not consistent with the hypothesis that improved antioxidant defenses were responsible for the prolonged longevity of the GHR/BP −/− mice. The only consistent increase in free-radical defenses was seen in the kidney, perhaps explaining the resistance to renal pathologies seen in the GHR/BP −/− mice (see below). Survival following paraquat administration was also assessed to see if the GHR/BP −/− males and females exhibited altered tolerance to paraquat-mediated oxidative stress. GHR/BP −/− males appeared to be less resistant than +/+ males to the acute oxygen toxicity. No difference in survival was seen between the GHR/BP −/− and +/+ females. Overall, the results suggest that mechanisms other than improved antioxidant defenses must account for the long life of the GHR/BP −/− mouse.

Disease progression

Growth hormone has been implicated in the development of diseases and tissue damage in different organs, including the kidney and the eye. Some of these are aging-related while others are due to metabolic derangement. The following summarizes several studies of the effect of the

GHR gene disruption on the development of organ damage or disease.

A recent study demonstrated that disruption of GH signaling significantly inhibits prostate carcinogenesis (Wang et al. 2005). Prostatic intraepithelial neoplasia (PIN), thought to be a precursor to prostate cancer, was induced in mice by expression of the large T antigen (Tag) oncogene. The number of PIN lesions was assessed in GHR/BP −/− and +/+ males carrying the Tag oncogene at 9 months of age. While 7 of 8 +/+ males harbored PIN lesions of varying grades, only 1 of 8 GHR/BP −/− males developed lesions. The lack of GHR did not appear to affect Tag expression. Testosterone levels and androgen receptor expression were unaffected by lack of GHR or expression of Tag. Functional differentiation of the epithelial cells was also not compromised in the prostate. In normal appearing prostate epithelium, which had the ability to develop PIN lesions, proliferation was significantly decreased and apoptosis significantly increased in the Tag-expressing GHR/BP −/− mice as compared to Tag-expressing +/+ controls, possibly having a significant impact on prostate carcinogenesis.

Similar studies of mammary carcinogenesis in Tag-expressing females shows significant inhibition of tumor incidence, volume and burden in GHR/BP −/− as compared to +/+ mice (Swanson et al. 2005). The mammary carcinogenesis in the Tag-expressing females appears to be estrogen-independent, indicating that disruption of GH signaling may be effective at inhibiting the as yet incurable progression of estrogen-independent breast cancer in patients (Zhang et al. 2005).

Growth hormone also appears to play a role in diabetic end organ damage, including nephropathy and retinopathy (Doi et al. 1988; Yang et al. 1993; Orskov 1996; Holly et al. 1988; Smith et al. 1997). Transgenic mice expressing bovine GH develop progressive glomerulosclerosis and die prematurely, most likely of kidney failure (Doi et al. 1988; Quaife et al. 1989). Mice made diabetic by treatment with streptozotocin also develop glomerulosclerosis, but the kidney damage is prevented by expression of, or treatment with, a GH antagonist (Chen et al. 1995). A similar study to assess the role of GH in kidney damage was performed with the GHR/BP −/− mice (Bellush et al. 2000). As found with expression of a GH antagonist, disruption of the GHR gene also resulted in protection of the kidney from damage during diabetes. No lesions were seen in the glomeruli of diabetic GHR/BP −/− mice as compared to the diffuse, moderate glomerulosclerosis seen in diabetic +/+ mice. Furthermore, the glomerular volume as well as the ratio of mesangial to total glomerular surface area remained unchanged in the diabetic GHR/BP −/− mice as compared to nondiabetic GHR/BP −/− controls, in contrast to the increased values obtained for diabetic versus nondiabetic +/+ mice.

GHR/BP −/− mice are also resistant to development of high-fat diet-induced hyperglycemia and diabetes (Coschigano et al. 2002). GHR/BP −/− and +/+ males were weaned onto either a diet high in fat or onto normal chow. Mice on the high-fat diet showed a significant gain in weight and a significant increase in fasting plasma insulin levels. However, only the +/+ mice on the high fat diet showed consistent increases in fasting blood glucose levels.

Oxidative stress has been implicated in the development of age-related cataracts (see references in Wolf et al. 2005). A recent study demonstrated the presence of significantly less advanced cataracts in 20- to 24-month-old GHR/BP −/− mice as compared to +/+ controls. Only a low degree of lens opacity was observed in 6- to 7-month-old mice, with no difference seen between GHR/BP −/− and +/+ mice, indicating that cataract development was age-related and not congenital or early-adult in appearance (Wolf et al. 2005).

Caloric restriction and gene expression changes

Recent attention has focused on determining the effect of CR on the lifespan of GHR/BP −/− mice, especially in light of the lifespan extension by CR of the long-lived Ames dwarf mice (Bartke et al. 2001). Unpublished results of these studies include evidence for a very limited response of GHR/BP−/− females to CR and no effect of CR on longevity of GHR/BP −/− males

(A. Bartke and M. Bonkowski, personal communication). Several studies have been published examining gene expression in these animals. The first looked at changes in gene expression in the liver due to the gene disruption alone and then examined the effect of CR (Miller et al. 2002). Surprisingly, none of the 2,352 genes analyzed by microarray met the most stringent criteria for differential expression due to the gene disruption alone; only 10 were considered differential when the criteria were relaxed. The authors suggested that changes in gene expression related to the increase in lifespan might be more apparent at a different age or in a different tissue than was examined. Furthermore, no genes were found for which the effect of CR differed in liver between GHR/BP −/− and +/+ controls using the most stringent criteria, suggesting that CR affected the two genotypes in a similar manner. Since expression of a number of genes was affected by CR alone, the mechanism of lifespan extension may differ between CR and reduced GH signaling.

More recently, interactions between reduced GH signaling and long-term CR with regard to glucose and lipid metabolism in liver were analyzed (Al-Regaiey et al. 2005). Plasma glucose levels, which did not differ between GHR/BP −/− and +/+ genotypes in the absence of CR, decreased significantly in both genotypes as a result of CR. Insulin levels also decreased as a result of CR, even in the GHR/BP −/− mice, which already have low insulin levels in the absence of CR. Increased insulin sensitivity of the GHR/BP −/− mice, further increased by CR, was supported by homeostasis model analysis (Masternak et al. 2005a). Corticosterone, leptin and adiponectin were all significantly elevated in the GHR/BP −/− mice (Al-Regaiey et al. 2005). CR reduced the levels of leptin in both GHR/BP −/− and +/+ mice but had no effect on corticosterone or adiponectin levels. Levels of total and phosphorylated AMP-activated protein kinase (AMPK), which mediates the actions of leptin and adiponectin in promoting fat oxidation and preventing fat accumulation in tissues other than adipose tissue, were elevated in GHR/BP −/− mice but were not altered by CR. Many of the molecules involved in activation of gluconeogenesis were up-regulated in GHR/BP −/− mice, suggesting increased hepatic glucose production and fatty acid oxidation. These included forkhead box O1 (Foxo1), peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), cAMP response element-binding protein (CREB), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Increased levels of Foxo1 and manganese superoxide dismutase (MnSOD) gene expression in the GHR/BP −/− liver also suggested increased protection of the liver against oxidative stress. Several, although not all, of these genes also showed changes due to CR, revealing distinct and overlapping effects of CR and GH resistance.

Studies of the regulation of genes involved in the early steps of insulin signaling in liver and skeletal muscle were also performed (Masternak et al. 2005b). In liver, mRNA expression of IR, IRS1 and IRS2 were increased in GHR/BP −/− mice in the absence of CR, suggesting that the corresponding steps of insulin signaling may be enhanced in the liver of these insulin-sensitive mice. CR did not result in an additional increase in expression; in fact, it decreased expression of IRS1 to the level seen in +/+ mice. In contrast, mRNA levels for these three genes, as well as glucose transporter 4 (GLUT4), were not significantly altered in the skeletal muscle of GHR/BP −/− mice. Instead, CR significantly decreased their mRNA levels in both genotypes.

A third study examined the expression of genes from the peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor (RXR) families in skeletal muscle (Masternak et al. 2005a). This study first demonstrated that free fatty acid and triglyceride levels were not altered in the GHR/BP −/− muscle or plasma. CR, however, resulted in an increase in free fatty acid levels and a decrease in triglyceride levels, regardless of genotype. In contrast, plasma cholesterol levels were decreased in the GHR/BP −/− mice; CR reduced the levels in +/+ mice but had no effect in GHR/BP −/− mice. Changes in the mRNA levels of the PPAR and RXR families members (γ, α, and β/δ) were similar to those seen for the triglyceride levels. However, changes in protein levels, examined for the PPAR family members, differed from the mRNA expression. PPARα and β/δ protein levels were affected by

both GH resistance and CR while PPARγ protein levels were only affected by GH resistance. The authors suggest that these results, which are contrary to what was expected, may suggest that the whole-body insulin sensitivity seen in the GHR/BP −/− mice and increased by CR is regulated independently of the muscle PPARs and muscle insulin action.

Conclusions

Generation of the GHR/BP gene-disrupted mouse line provided a simplified model to study longevity and aging. Prior to its development, longevity and aging were studied mainly in Ames and Snell dwarf mice, which each suffered from multiple hormonal deficiencies. Use of a mouse model with only one affected hormone has clearly demonstrated involvement of GH in longevity and aging. The multiple characteristics of this mouse line suggest that the mechanisms affecting longevity and aging are complicated and far from understood.

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