AGE (2006) 28:163–171
DOI 10.1007/s11357-006-9007-7

Do long-lived mutant and calorie-restricted mice share common anti-aging mechanisms?—a pathological point of view

Yuji Ikeno · Christie M. Lew · Lisa A. Cortez · Celeste R. Webb · Shuko Lee · Gene B. Hubbard

Received: 1 December 2005 / Accepted: 15 December 2005

Abstract Rodent models are an invaluable resource for studying the mechanism of mammalian aging. In recent years, the availability of transgenic and knockout mouse models has facilitated the study of potential mechanisms of aging. Since 1996, aging studies with several long-lived mutant mice have been conducted. Studies with the long-lived mutant mice, Ames and Snell dwarf, and growth hormone receptor/binding protein knockout mice, are currently providing important clues regarding the role of the growth hormone/insulin like growth factor-1 axis in the aging process. Interestingly, these studies demonstrate that these long-lived mutant mice have physiological characteristics that are similar to the effects of calorie restriction, which has been the most effective experimental manipulation capable of extending lifespan in various species. However, a question remains to be answered: do these long-lived mutant and calorie-restricted mice extend their lifespan through a common underlying mechanism?

Y. Ikeno (✉) · C.M. Lew · L.A. Cortez · C.R. Webb
Department of Cellular and Structural Biology,
The University of Texas Health Science Center
at San Antonio,
7703 Floyd Curl Drive,
San Antonio, TX 78229, USA
e-mail: ikeno@uthscsa.edu

Y. Ikeno · C.M. Lew · L.A. Cortez · C.R. Webb
Barshop Institute for Longevity and Aging Studies,
The University of Texas Health Science Center
at San Antonio,
San Antonio, TX 78229, USA

Y. Ikeno · S. Lee
Research Service,
Audie Murphy VA Hospital (STVHCS),
San Antonio, TX 78229, USA

G.B. Hubbard
Southwest National Primate Research Center,
Southwest Foundation for Biomedical Research,
San Antonio, TX 78245, USA

Key words aging · growth hormone receptor/binding protein · knockout mouse · neoplastic disease

Abbreviations

CR	caloric restriction
GH	growth hormone
GHR	growth hormone receptor
GHR/BP	growth hormone receptor/binding protein
KO	knockout
PRL	prolactin
IGF-1	insulin-like growth factor-1
TSH	thyroid stimulating hormone
T3	triiodo-thyronnine
T4	thyroxine
AL	*ad libitum*

Introduction

Interventions of aging by various experimental manipulations could provide gerontologists with the opportunity to seek the basic mechanisms underlying aging processes. McCay et al. first reported 70 years ago that restriction of food intake of rats soon after weaning increases their lifespan (McCay et al. 1935). Since this seminal report, caloric restriction (CR) has represented the most effective intervention by which to explore underlying mechanism(s) of aging (Masoro et al. 1982; Weindruch and Walford 1988). CR attenuates much of the physiological decline associated with aging, and produces increases in both median and maximum lifespan, and suppression of many age-related diseases in rodents (Maeda et al. 1985; Masoro 1988; Weindruch and Walford 1988; Yu et al. 1982). Thereby, CR has been considered as a "gold standard" for anti-aging effects.

In 1996, Brown-Borg et al. reported that Ames dwarf mice have extended lifespan compared to their normal littermates (Brown-Borg et al. 1996). Subsequently, Snell dwarf and growth hormone receptor/binding protein (GHR/BP) knockout (KO) mice have also been shown to have a longer lifespan compared to their normal control groups (Coschigano et al. 2000; Flurkey et al. 2001). Over the past decade, these long-lived mutant mice have drawn much attention in aging research because of their marked life extension by a single gene mutation. Information obtained from these mouse models, combined with data derived from the study of calorie-restricted animals, could provide a major advantage to seek the underlying mechanism(s) of mammalian aging. Studies have demonstrated that some of the physiological characteristics of long-lived mutant mice are similar to those of CR mice (Bartke et al. 2001a). In spite of the similarity of some of the physiological characteristics, a question that still remains to be solved is whether long-lived mutant and calorie-restricted mice extend their lifespan through a common mechanism or different independent underlying mechanisms.

In this review, we will compare the pathological profile of Ames dwarf, GHR/BP KO, and calorie-restricted mice, and discuss if, from a pathological point of view, the long-lived mutant and CR mice share common underlying mechanisms to extend the lifespan.

Ames and Snell dwarf mice

Ames dwarf mice

Ames dwarf mice are the first mouse model to show a delayed aging process and a remarkable lifespan extension by a single gene mutation (Brown-Borg et al. 1996). The extension of lifespan is approximately 50% in males and more than 60% in females (Brown-Borg et al. 1996). Ames dwarf mice are very intriguing animals for aging research not only because of their remarkable lifespan extension, but also because of some of their physiological characteristics, which are similar to those of CR mice (Bartke et al. 2001a).

Ames dwarf mice are homozygous for a recessive mutation at a gene named Prophet of Pit-1 (*Prop-1*; Sornson et al. 1996), which causes developmental arrest in the anterior pituitary gland of these mice. As a result of this developmental arrest, the somatotrophs, which normally produce growth hormone (GH), the lactotrophs, which normally produce prolactin (PRL), and the thyrotrophs, which normally produce thyroid stimulating hormone (TSH), fail to differentiate. This makes Ames dwarf mice deficient in GH, PRL (Bartke 1964, 1965, 2000; Sornson et al. 1996) and TSH (Bartke 1964). These endocrine changes in Ames dwarf mice are found to lead to the various physiological changes (Bartke 2000). The physiological characteristics related to the endocrine changes in Ames dwarf mice are: (1) small body size; (2) reduced core body temperature; (3) delayed sexual maturation; (4) reduced plasma glucose levels and increased insulin sensitivity; and (5) reduced levels of various hormones and growth factors, e.g., insulin, and insulin-like growth factor-1 (IGF-1) (Bartke 1964; Bartke et al. 2001a). At birth, Ames dwarf mice are approximately normal size, but their growth rate is substantially less than normal siblings. The body weight of adult Ames dwarf mice is approximately one-third of normal sib-

lings. Deficiency of GH results in suppression of plasma IGF-1 levels, which may play an important role in their small body size. GH is also well known for its action in decreasing insulin sensitivity, and Ames dwarf mice exhibit increased insulin sensitivity (Dominici et al. 1999). Therefore, deficiency of GH could also play important roles in reduced plasma glucose and insulin. Plasma thyroxin levels are extremely low due to the reduced levels of TSH. The reduced levels of thyroid hormone could presumably lower the metabolic rate and reduce the core body temperature. PRL deficiency is associated with delayed sexual maturation and leads to infertility in female Ames dwarf mice. Interestingly, these physiological characteristics are similar to the effects of CR in rodents.

CR is well known for not only its beneficial effects on the aging process, but also on various age-related diseases, i.e., delay and/or suppression of the occurrence of both neoplastic and non-neoplastic diseases (Bronson and Lipman 1991; Ikeno et al. 2005; Iwasaki et al. 1988; Maeda et al. 1985). Because of the marked lifespan extension and similar physiological characteristics observed in both Ames dwarf and CR mice (Bartke et al. 2001a), it is of interest to examine the age-related pathological changes in Ames dwarf mice. Our group conducted pathological analyses of Ames dwarf as compared to normal mice (Ikeno et al. 2003). Our study demonstrated that the percentage of tumor-bearing mice (the percent of mice having neoplastic lesions in the group) and the tumor burden (the number of different tumors found in a mouse) were similar in Ames dwarf mice and their normal siblings. However, fatal neoplastic disease was shown to occur later in life in Ames dwarf mice, and the severity of adenocarcinoma, one of the major fatal lesions in these mice, was significantly less in Ames dwarf mice compared with their normal siblings, indicating that the mutation in Ames dwarf mice may delay the progression of fatal neoplastic disease. Ames dwarf mice also showed delayed progression of non-neoplastic lesions. The severity of glomerulonephritis, which is a major age-related non-neoplastic lesion in these mice, was also significantly less in Ames dwarf mice compared with their normal siblings, which indicates that the progression of glomerulonephritis in Ames dwarf mice is delayed. Furthermore, Ames dwarf mice showed significantly reduced disease burden and slower age-related accumulation of various pathological changes compared with their normal siblings. These findings indicate that Ames dwarf mice maintain organ and whole-body integrity during aging, thereby delaying the development of both neoplastic and non-neoplastic diseases. Delayed occurrence (progression) of both neoplastic and non-neoplastic lesions, and less disease burden are also common pathological findings in CR mice (Bronson and Lipman 1991; Ikeno et al. 2005). Therefore, Ames dwarf and CR mice exhibit similarities as well as some differences in pathological profiles (Ikeno et al. 2003).

Snell dwarf mice

In 1929, Snell (1929) reported the existence of a recessive mutation that caused dwarfism in mice, and named it “dwarf.” Snell dwarf mice are homozygous for a recessive mutation at the pituitary factor-1 (*Pit-1*) gene (Li et al. 1990). Since the *Pit-1* gene regulates the differentiation of anterior pituitary cells, the mutation in this gene, like that in *Prop-1*, causes deficiencies in GH, PRL and TSH (Li et al. 1990). This hormonal profile is identical to that of Ames dwarf mice (Bartke et al. 2001a), and phenotypes of Snell dwarf mice are very similar to the phenotypes of Ames dwarf. The data on the lifespan of Snell dwarf mice were controversial in early studies. A study by Chen et al. (1972) demonstrated that Snell dwarf mice had a shorter lifespan than normal controls, which was associated with impaired T-cell dependent function, suggesting accelerated aging in these mice. On the other hand, Silberberg (1972) referred to the “unusually long lifespan” of these mutants but provided little data and no citations to support this statement. Conclusive evidence that Snell dwarf mice live longer than their normal siblings was provided by Flurkey et al. (2001). In this study, Snell dwarf mice (mixed genders) showed a 42% longer mean lifespan compared to their normal controls (Flurkey et al. 2001). Soon after this exciting discovery, Flurkey et al. (2002)

reported detailed analyses of the effects of genetic background and gender on the lifespan of Snell dwarf mice. Their study also stated that the Snell dwarf mouse showed a delayed occurrence of age-related lesions; however, no pathology data were presented. Age-related pathological analyses of Snell dwarf mice were reported by Vergara et al. (2004). In this study, the effects of two hormonal treatments on lifespan were compared to non-treated Snell dwarf and control mice. For the pathological analyses, 45 Snell dwarf and 45 control mice were examined regardless of the hormonal regimen. Approximately 47% of the Snell dwarf mice had no obvious pathological changes, while only 7% of control mice showed no severe pathological changes. Kidney pathology also showed less advanced lesions in dwarfs compared to the control groups. These findings were similar to our pathological findings in Ames dwarf mice (Ikeno et al. 2003). Interestingly, the overall incidence of fatal neoplastic lesions seemed to be lower in Snell dwarf mice (31%) than control mice (66.7%). This interesting difference in tumor incidence may need further exploration.

Growth hormone receptor/binding protein knockout mice

After the exciting discoveries that Ames and Snell dwarf mice showed a delayed aging process and a remarkable lifespan extension from a single gene mutation, studies with Ames and Snell dwarf mice have focused on identifying the underlying mechanism(s) of the anti-aging action (Bartke 2000). Recent studies with these dwarf mice strongly suggest that reduced levels of GH and IGF-1 may have important anti-aging effects that contribute to the extended lifespan of these dwarf mice (Bartke et al. 2001a, Flurkey et al. 2001). However, the results with Ames and Snell dwarf mice cannot rule out the possibility of other hormonal changes playing an important role in the life extension exhibited by these dwarf mice because the Ames and Snell dwarf mice are also deficient in PRL and TSH due to the developmental arrest of the anterior pituitary (Bartke 1964, 2000; Sornson et al. 1996). To examine the exact role of the GH/IGF-1 axis in aging, it is of interest to conduct an aging study using an animal model in which the primary endocrine change is limited only to the GH/IGF-1 axis. In 1997, Zhou et al. (1997) successfully generated knockout (KO) mice for GH receptor/GH binding protein (GHR/GHBP). This animal model allowed gerontologists to dissect out the role of the GH-IGF-1 axis in the patho-physiology of aging from other hormonal changes seen in Ames and Snell dwarf mice. As expected, these GHR/BP KO mice showed some similarities and differences when compared to the Ames and Snell dwarf mice. Because of the absence of GHR, the biological actions of GH are completely blocked, although these mice do have elevated plasma GH levels (Zhou et al. 1997). As in dwarf mice, a deficiency in the biological actions of GH results in suppression of plasma IGF-1 levels, which presumably accounts for reduced postnatal growth (Zhou et al. 1997). These mice have small body size, with adult GHR/BP KO mice body weight being slightly greater than the body weight of Snell and Ames dwarf mice. Despite elevated plasma PRL levels (Chandrashekar et al. 1999), GHR/BP KO mice showed both delayed sexual maturation and reduced fertility (Bartke et al. 2001a, b; Chandrashekar et al. 1999; Zhou et al. 1997). Plasma levels of thyroid hormones [both thyroxine (T4) and triiodo-thyronnine (T3)] are slightly lower compared to normal siblings (Hauck et al. 2001), which may play a role in the slight decrease in core body temperature of these mice (Bartke et al. 2001a). Lack of biological actions of GH and suppression of plasma IGF-1 levels seem to have similar effects on plasma insulin and glucose levels, and on insulin sensitivity compared to the Ames and Snell dwarf mice, i.e., plasma glucose levels are normal or reduced, plasma insulin levels are reduced, and insulin sensitivity is enhanced (Bartke et al. 2001a). These mice also showed a significant life extension compared to their normal siblings (Coschigano et al. 2000, 2003; Bartke et al. 2001a), which is strong evidence that the GH-IGF-1 axis plays an important role in longevity.

Pathological analyses of GHR/BP KO mice were also conducted by our group (Y. Ikeno and G.B. Hubbard, unpublished data). Our study

demonstrated that the percentage of tumor-bearing mice (the percent of mice having neoplastic lesions in each group) was lower, and the tumor burden (the number of different tumors found in a mouse) was significantly less (approximately half) in GHR/BP KO mice compared to their normal siblings. GHR/BP KO mice also showed a significantly lower incidence of fatal neoplastic lesions compared to the normal siblings. Thus, these data indicated that GHR/BP KO mice showed an overall suppressed occurrence of tumor development compared to their normal siblings. The changes found in the neoplastic lesions in GHR/BP KO mice were more similar to the effects of CR (Bronson and Lipman 1991; Ikeno et al. 2005; Volk et al. 1994) than to the results from studies of Ames dwarf mice, in which little influence on the percentage of tumor-bearing mice, tumor burden, and incidence of fatal neoplasms was seen (Ikeno et al. 2003; Mattison et al. 2000). In addition to its suppressed occurrence, fatal neoplastic disease was shown to occur later in life in GHR/BP KO mice, and the severity of adenocarcinoma, which is one of the major fatal diseases in these mice, was significantly less in GHR/BP KO mice compared with their normal siblings, indicating that the lack of GH action in GHR/BP KO mice seemed to delay the progression of fatal neoplastic disease. GHR/BP KO mice also showed a delayed progression of non-neoplastic lesions. The severity of glomerulonephritis was significantly less in GHR/BP KO mice compared with their normal siblings, which also indicated that the progression of glomerulonephritis in GHR/BP KO mice is delayed, similar to Ames dwarf mice (Ikeno et al. 2003), possibly due to reduced GH action. Furthermore, compared with their normal siblings, GHR/BP KO mice showed a significantly lower disease burden and a slower age-related accumulation of various pathological changes, suggesting that GHR/BP KO mice maintained organ and whole-body integrity during aging similar to what is seen in Ames dwarf and CR mice (Ikeno et al. 2003, 2005). This implication is also supported by evidence showing that approximately half of GHR/BP KO mice had no obvious evidence of lethal pathology at death, which is common in Ames and Snell dwarf, and CR mice (Ikeno et al. 2003, 2005; Vergara et al. 2004). Overall, the pathological profile of the GHR/BP KO mice showed similarities to those of CR and Ames dwarf mice, but was more similar to CR mice than to Ames dwarf mice.

Do long-lived mutant and calorie-restricted mice share common anti-aging mechanisms?

Effects of CR on long-lived mutant mice

As described above, some of the physiological characteristics of long-lived mutant mice are similar to the effects of CR, e.g., small body size, less food consumption per mouse and reduced plasma IGF-1 and insulin levels. Therefore, it has been argued that these long-lived mutant mice could be mimics of CR. However, under standard housing conditions (i.e., constant access to food), Ames dwarf mice consume more food per gram of body weight than their normal siblings (Mattison et al. 2000), and frequently become obese. Relative obesity (% fat) is greater in GHR/BP KO mice than in normal siblings (Berryman et al. 2004). Thus, longevity of dwarf mice does not appear to be due to "voluntary" CR. To directly address this issue, Bartke et al. (2001b) conducted an elegant study, in which the effects of CR on lifespan were compared between Ames dwarf and their normal siblings. This study demonstrated that CR produced further extension of lifespan of Ames dwarf mice, which was also significantly longer than CR wild-type controls. Bartke et al. (2001b) have concluded that the mechanisms underlying the extended lifespan of these dwarf mice differ from those in CR for the following reasons: (1) CR Ames dwarf mice showed further life-extension compared to *ad libitum* (AL)-fed Ames dwarf mice; and (2) the shape of survival curves were different between AL-fed Ames dwarf mice and CR wild-type mice. Effects of Ames dwarfism on expression of insulin and IGF-1-related genes and on wide profiles of hepatic gene expression differed from the effects of CR on the expression of the same genes (Masternak et al. 2004; Tsuchiya et al. 2004). This conclusion was also supported by pathological analyses of

CR Ames dwarf mice conducted by our group (Y. Ikeno and G.B. Hubbard, unpublished data). Our study demonstrated that CR was associated with a significantly lower incidence of fatal neoplasms in Ames dwarf and normal mice compared to AL-fed normal siblings; although incidence of fatal neoplastic lesions in AL-fed Ames dwarf mice was similar to AL normal siblings. The fatal neoplastic disease occurred at older ages in the AL Ames dwarf and CR normal siblings compared to those of AL normal siblings. CR in Ames dwarf mice produced a further delay in the occurrence of fatal neoplasms compared to AL Ames dwarf and CR normal siblings. In addition, the group of CR Ames dwarf mice had higher incidences of death without obvious evidence of lethal pathological changes (unknown cause of death) than AL Ames dwarf mice, which indicates that CR showed additive anti-aging effects in Ames dwarf mice. The difference in the pathological profiles in AL Ames dwarf mice and CR normal mice, and additional suppression/retardation of pathology by CR in Ames dwarf mice support the conclusion by Bartke et al. (2001b) that Ames dwarf mice are not merely mimics of CR mice.

A study to test effects of CR on lifespan of GHR/BP KO mice is ongoing. The survival data from this study suggest that CR GHR/BR KO mice show similar lifespan compared to AL-fed GHR/BP KO mice (M. Bonkowski and A. Bartke, unpublished data), which also support the similarities in pathological profile between AL-fed GHR/BP KO and CR mice (Y. Ikeno and G.B. Hubbard, unpublished data).

Do long-lived mutant and CR mice extend their lifespans by common or independent mechanisms?

The longevity and pathology data obtained from studies of Ames dwarf, GHR/BP KO, and CR mice may provide us with an opportunity to dissect the possible common underlying anti-cancer and anti-aging mechanism(s) of these animal models from independent mechanisms. The longevity and pathology data suggest that Ames dwarf mice are not merely mimics of CR mice, but that Ames dwarf and CR mice share some common underlying mechanisms as well as having independent mechanisms for extended longevity. This notion is supported by a study comparing the gene expression patterns of CR and Snell dwarf mice. The data showed a partial overlap between CR and Snell dwarf mice, suggesting that there are common as well as independent mechanisms in their anti-aging action (Miller et al. 2002). On the other hand, the pathological profile of the GHR/BP KO mice showed more similarities to the effects of CR than to the pathological profile of Ames dwarf mice. However, Miller's group reported differences between GHRKO and CR mice in the hepatic profile of gene expression.

The common endocrine changes among GHR/BP KO, Ames dwarf and CR mice include suppressed levels of peripheral IGF-1 due to either GH resistance (GHR/BP KO mice), lack of GH (Ames dwarf mice), or reduced plasma GH levels (CR mice, at least initially). The changes in GH action, which are associated with reduced levels of peripheral IGF-1, seem to play important roles in the delayed occurrence of fatal neoplastic lesions, as well as in retarded somatic growth and smaller adult body size (Bartke 2000; Zhou et al. 1997), because IGF-1 is known for its potent mitogenic and anti-apoptotic effects (Bustin and Jenkins 2001). This idea was also supported by some epidemiological studies indicating correlations between plasma IGF-1 levels and some tumors (Burroughs et al. 1999; Yu and Rohan 2000), and a possible increase in the incidence of and mortality from cancer following GH treatment during childhood (Swerdlow et al. 2002).

Some non-neoplastic diseases seem to be affected by changes in GH levels or action, as was shown by the lessened severity of glomerulonephritis in both GHR/BP KO and Ames dwarf mice (Ikeno et al. 2003; Y. Ikeno and G.B. Hubbard, unpublished data). Moreover, studies of GH transgenic mice, which have pathologically high levels of GH, showed that the early death of these animals appears to be related primarily to pathological changes in the kidney (glomerulonephritis and glomerulosclerosis) (Wanke et al. 1992; Yang et al. 1993), IGF-1 transgenic mice

did not exhibit such severe pathological changes as GH transgenic mice (Doi et al. 1988), indicating the important role of GH in kidney pathology. There is also evidence that CR rats have reduced GH levels (at least initially) (Sonntag et al. 1995) and that CR F344 rats and CR C57BL/6 mice show significantly reduced severity of kidney pathology compared to their AL counterparts (Maeda et al. 1985; Ikeno et al. 2005). Therefore, reduced action of the GH/IGF-1 axis seems to play a very important role in the delayed progression of various age-related diseases and reduced disease burden in the body, which could be a major contributing factor leading to the extended lifespan observed in the long-lived mutants and CR mice.

As described above, the pathological profile of Ames dwarf mice showed some differences compared to CR and GHR/BP KO mice. CR and GHR/BP KO mice showed a significantly lower incidence of fatal neoplastic lesions compared to their normal siblings, while Ames dwarf mice showed a similar incidence of fatal neoplasms compared to their normal siblings (Ikeno et al. 2003). CR showed some additive beneficial effects on the pathology of Ames dwarf mice. These results indicate there are some independent underlying mechanisms between the Ames dwarf mice, GHR/BP KO and CR mice. The reason(s) behind these interesting differences between GHR/BP KO, CR and Ames dwarf mice remains to be identified. One possible explanation could be that primary changes in three major hormonal systems (somatotropic-, thyrotropic-, and lactotropic-axis), manifested as an "absence" of three vital pituitary hormones in the Ames dwarf mouse, may cause negative effects that counteract the anti-cancer actions commonly seen in long-lived animals. For example, GH and PRL have been shown to play important roles in intrathymic T-cell differentiation (De Mello-Coelho et al. 1998), and earlier studies showed that the immune functions of Ames dwarf mice are at subnormal levels (Esquifino et al. 1991). Snell dwarf mice also show suppressed B-cell development (Montecino-Rodriguez et al. 1996). Contrary to the latter two types of dwarf mice, GHR/BP KO mice show increased plasma PRL levels (Chandrashekar et al. 1999), which could be a compensatory mechanism because PRL and GH have some common actions. Thus, this elevated PRL level could play an important role in some of the immune functions in GHR/BP KO mice. CR is also well known for its protective roles in age-related changes in the immune system (Miller 1996). Since cell-mediated cytotoxicity by natural killer cells is known for its destructive action on cancers cells, possible changes in immune functions could explain the differences observed in the incidence of neoplastic diseases among the three long-lived animal models. Changes in immune function could also explain why CR showed additive effects in Ames dwarf mice (Bartke et al. 2001b) but not in GHR/BP KO mice (M. Bonkowski and A. Bartke, unpublished data). Further studies are necessary to uncover the possible underlying mechanisms that could explain the differences in pathology of these animal models.

Overall, the pathology data suggest that changes in the endocrine system, especially in GH and IGF-1 levels, and subsequent pathophysiological changes, play important roles in the life-extension of long-lived mutant and CR mice. We suspect therefore the presence of common underlying mechanisms in Ames dwarf, GHR/BP KO and CR mice related to functionally similar changes in the endocrine system, especially the GH/IGF-1 axis, and that the subsequent pathophysiological changes play important roles in the delay of various age-related pathologies and life extension in these animals. However, independent mechanisms that extend lifespan and change age-related pathology may also exist and remain to be identified in Ames dwarf, Snell dwarf, GHR/BP KO and CR mice.

Acknowledgments The authors wish to thank Dr. John Kopchick for providing us with the GHR/BP KO animals for this study. The authors also wish to thank Dr. Julie Mattison and Mr. Chris Wright for their contribution to the CR Ames dwarf mice survival study, Mr. Michael Bonkowski for his contribution to the GHR/BP KO survival study, and Dr. George Roth for his valuable suggestions for the CR study with long-lived mutants. This research was supported by NIH Grants AG13319 (YI) and AG19899 (AB), and VA Merit Review Grant (YI) from Department of Veteran Affairs.

References

Bartke A (1964) Histology of the anterior hypohysis, thyroid and gonads of two types of dwarf mice. Anat Rec 149:225–235

Bartke A (1965) The response of two types of dwarf mice to growth hormone, thyrotropin, and thyroxine. Gen Comp Endocrinol 5:418–426

Bartke A (2000) Delayed aging in Ames dwarf mice. Relationships to endocrine function and body size. In: Hekimi S (ed) The molecular genetics of aging. Springer, Berlin Heidelberg New York, pp 181–202

Bartke A, Coschigano K, Kopchick J, Chandrashekar V, Mattison J, Kinney B, Hauck S (2001a) Genes that prolong life: relationships of growth hormone and growth to aging and life span. J Gerontol Biol Sci 56A:B340–B349

Bartke A, Wright JC, Mattison J, Ingram DK, Miller RA, Roth GS (2001b) Extending the lifespan of long-lived mice. Nature 414:412

Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ (2004) Comparing adiposity profiles in three mouse models with altered GH signaling. Growth Horm IGF Res 14:309–318

Bronson RT, Lipman RD (1991) Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. Growth Dev Aging 55:169–184

Brown-Borg HM, Borg KE, Meliska CJ, Bartke A (1996) Dwarf mice and the ageing process. Nature 384:33

Burroughs KD, Dunn SE, Barrett JC, Taylor JA (1999) Insulin-like growth factor-1: a key regulator of human cancer risk? J Natl Cancer Inst 91:579–581

Bustin SA, Jenkins PJ (2001) The growth hormone-insulin-like growth factor-I axis and colorectal cancer. Trends Mol Med 7:447–454

Chandrashekar V, Bartke A, Coschigano KT, Kopchick JJ (1999) Pituitary and testicular function in growth hormone receptor gene knockout mice. Endocrinology 140:1082–1088

Chen HW, Meier H, Heiniger HJ, Heubner RJ (1972) Tumorigenesis in strain DW/J mice and induction by prolactin of the group-specific antigen of endogenous C-type RNA tumor virus. J Natl Cancer Inst 49:1145–1154

Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ (2000) Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 14:2608–2613

Coschigano KT, Holland AN, Riders ME, List EO, Flyvbjerg A, Kopchick JJ (2003) Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. Endocrinology 144:3799–3810

De Mello-Coelho V, Savino W, Postel-Vinay MC, Dardenne M (1998) Role of prolactin and growth hormone on thymus physiology. Dev Immunol 6:317–323

Doi T, Striker LJ, Quaife C, Conti FG, Palmiter R, Behringer R, Brinster R, Striker GE (1988) Progressive glomerulosclerosis develops in transgenic mice chronically expressing growth hormone and growth hormone releasing factor but not in those expressing insulinlike growth factor-1. Am J Pathol 131:398–403

Dominici FP, Hauck S, Argentino DP, Bartke A, Turyn D (1999) Increased insulin sensitivity and upregulation of insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2 in liver of Ames dwarf mice. J Endocrinol 173:81–94

Esquifino AI, Villanúa MA, Szary A, Yau J, Bartke A (1991) Ectopic pituitary transplants restore immunocompetence in Ames dwarf mice. Acta Endocrinol 125:67–72

Flurkey K, Papaconstantinou J, Miller RA, Harrison DE (2001) Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc Natl Acad Sci USA 98: 6736–6741

Flurkey K, Papaconstantinou J, Harrison DE (2002) The Snell dwarf mutation Pit1(dw) can increase lifespan in mice. Mech Ageing Dev 123:121–130

Hauck SJ, Hunter WS, Danilovich N, Kopchick JJ, Bartke A (2001) Reduced levels of thyroid hormones, insulin, and glucose, and lower body core temperature in the growth hormone receptor/binding protein knockout mouse. Exp Biol Med 226:552–558

Ikeno Y, Bronson RT, Hubbard GB, Lee S, Bartke A (2003) The delayed occurrence of fatal neoplastic diseases in Ames dwarf mice: correlation to the extended longevity. J Gerontol Ser A Biol Sci Med Sci 58:291–296

Ikeno Y, Hubbard GB, Lee S, Richardson A, Strong R, Fernandez E, Diaz V, Nelson JF (2005) Housing density does not influence the longevity effect of calorie restriction. J Gerontol 60A: 1510–1517

Iwasaki K, Gleiser CA, Masoro EJ, McMahn CA, Seo E, Yu BP (1988) The influence of dietary protein source on longevity and age-related disease processes of Fischer 344 rats. J Gerontol Biol Sci 43:B5–B12

Li S, Crenshaw BE III, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG (1990) Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. Nature 347:528–533

Maeda H, Gleiser CA, Masoro EJ, Murata I, McMahan CA, Yu BP (1985) Nutritional influences on aging of Fischer 344 rats: II. Pathology. J Gerontol 40:671–688

Masoro EJ (1988) Food restriction in rodents: an evaluation of its role in the study of aging. J Gerontol 43:B59–B64

Masoro EJ, Yu BP, Bertrand HA (1982) Action of food restriction in delaying the aging process. Proc Natl Acad Sci USA 79:4239–4241

Masternak M, Al-Regaiey K, Bonkowski M, Panici J, Sun L, Wang J, Przybylski GK, Bartke A (2004) Divergent effects of caloric restriction on gene expression in normal and long-lived mice. J Gerontol Ser A Biol Sci Med Sci 59:784–788

Mattison JA, Wright C, Bronson RT, Roth GS, Ingram DK, Bartke A (2000) Studies of aging in Ames dwarf

mice: effects of caloric restriction. J Am Aging Assoc 23:9–16

McCay CM, Crowell MF, Maynard LA (1935) The effect of retarded growth upon length of life span and upon ultimate body size. J Nutr 10:63–79

Miller RA (1996) Aging and immune response. In: Schneider EL, Rowe JW (eds) Handbook of the biology of aging. Academic, New York, pp 157–180

Miller RA, Chang Y, Galecki AT, Al-Regaiey K, Kopchick JJ, Bartke A (2002) Gene expression patterns in calorically restricted mice: partial overlap with long-lived mutant mice. Mol Endocrinol 16:2657–2666

Montecino-Rodriguez E, Clark R, Johnson A, Collins L, Dorshkind K (1996) Defective B cell development in Snell dwarf (dw/dw) mice can be corrected by thyroxine treatment. J Immunol 157:3334–3340

Silberberg R (1972) Articular aging and osteoarthrosis in dwarf mice. Pathol Microbiol 38:417–430

Snell GD (1929) Dwarf, a new Mendelian recessive character of the house mouse. Proc Natl Acad Sci USA 15:733–734

Sonntag WE, Xu X, Ingram RL, D'Costa A (1995) Moderate caloric restriction alters the subcellular distribution of somatostatin mRNA and increases growth hormone pulse amplitude in aged animals. Neuroendocrinology 61:601–608

Sornson MW, Wu W, Dasen JS, Flynn SE, Norman DJ, O'Connell SM, Gukovsky I, Carriere C, Ryan AK, Miller AP, Zuo L, Gleiberman AS, Anderson B, Beamer WG, Rosenfeld MG (1996) Pituitary lineage determination by the prophet of pit-1 homeodomain factor defective in Ames dwarfism. Nature 384:327–333

Swerdlow AJ, Higgins CD, Adlard P, Preece MA (2002) Risk of cancer in patients treated with human pituitary growth hormone in the UK, 1959–1985: a cohort study. Lancet 60:273–277

Tsuchiya T, Dhahbi JM, Cui X, Mote PL, Bartke A, Spindler SR (2004) Additive regulation of hepatic gene expression by dwarfism and caloric restriction. Physiol Genomics 17:307–315

Vergara M, Smith-Wheelock M, Harper JM, Sigler R, Miller RA (2004) Hormone-treated Snell dwarf mice regain fertility but remain long lived and disease resistant. J Gerontol Ser A Biol Sci Med Sci 59:1244–1250

Volk MJ, Pugh TD, Kim M-J, Frith CH, Daynes RA, Ershler WB, Weindruch R (1994) Dietary restriction from middle age attenuates age-associated lymphoma development and interleukin 6 dysregulation in C57BL/6 mice. Cancer Res 54:3054–3061

Wanke R, Wolf E, Hermannns W, Folger S, Buchmuller T, Brem G (1992) The GH-transgenic mouse as an experimental model for growth research: clinical and pathological studies. Horm Res 37:74–87

Weindruch R, Walford RL (1988) The Retardation of Aging and Disease by Dietary Restriction, Thomas, Springfield, IL

Yang CW, Striker LJ, Pesce C, Chen WY, Paten EP, Elliot S, Doi T, Kopchick JJ, Striker GE (1993) Glomerulosclerosis and body growth are mediated by different portions of bovine growth hormone: studies in transgenic mice. Lab Invest 68:62–70

Yu H, Rohan T (2000) Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 92:1472–1489

Yu BP, Masoro EJ, Murata I, Bertrand HA, Lynd FT (1982) Life span study for SPF Fischer 344 male rats fed *ad libitum* or restricted diets: longevity, growth, lean body mass and disease. J Gerontol 37: 130–141

Zhou Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Cataldo L, Coschigano K, Wagner TE, Baumann G, Kopchick JJ (1997) A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proc Natl Acad Sci USA 94:13215–13220