Altered structure and function of adipose tissue in long-lived mice with growth hormone-related mutations

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ABSTRACT
A major focus of biogerontology is elucidating the role(s) of the endocrine system in aging and the accumulation of age-related diseases. Endocrine control of mammalian longevity was first reported in Ames dwarf (Prop1\(^{df}\)) mice, which are long-lived due to a recessive Prop1 loss-of-function mutation resulting in deficiency of growth hormone (GH), thyroid-stimulating hormone, and prolactin. Following this report, several other GH-related mutants with altered longevity have been described including long-lived Snell dwarf and growth hormone receptor knockout mice, and short-lived GH overexpressing transgenic mice. One of the emerging areas of interest in these mutant mice is the role of adipose tissue in their altered healthspan and lifespan. Here, we provide an overview of the alterations in body composition of GH-related mutants, as well as the altered thermogenic potential of their brown adipose tissue and the altered cellular senescence and adipokine production of their white adipose tissue.

KEYWORDS
Ames dwarf; aging; bGH; brown adipose tissue; GHR-KO; GHA; growth hormone; Snell dwarf; white adipose tissue

Introduction
Insulin-like growth factor 1 (IGF-1)/insulin signaling (IIS) has gained considerable interest in biogerontology because the effects of IIS on longevity are highly conserved. For example, longevity can be extended in yeast (Saccharomyces cerevisiae),\(^1\) worms (Caenorhabditis elegans),\(^2\) and flies (Drosophila melanogaster)\(^3\) by reducing IIS or homologous signaling. Likewise, a reduction in growth hormone (GH) and IGF-1 signaling (related to IIS and collectively referred to as somatotropic signaling) has been shown to extend longevity in several mutant mice,\(^4-6\) and has also been implicated in human longevity.\(^7\) Since a comprehensive discussion of the mechanism(s) through which reduced IIS and somatotropic signaling increase longevity are outside the scope of this review, interested readers are referred to relevant reviews in.\(^8-10\) This article will specifically focus on how the altered structure, distribution, and function of adipose tissue in GH-mutant mice may impact their longevity.

Growth hormone mutant mice with altered longevity
Since the realization that the somatotropic axis plays a major role in mammalian lifespan, mice with altered GH signaling have been studied to elucidate the mechanisms by which GH impacts longevity. Some of the long-lived GH mutants that have been examined include Ames dwarf, Snell dwarf, and growth hormone receptor/growth hormone binding protein knockout mice (GHR/GHBP-KO, hereafter referred to as GHR-KO mice). Opposite to these dwarf mice are the short-lived bovine GH (bGH) transgenic mice which overexpress GH. In this section, we will provide a brief summary of each of these types of mutant or knockout mice. We will also provide some information on GHR antagonist (GHA) transgenic mice, which have diminished somatotropic signaling, though they have no alterations in longevity.

Ames dwarf mice (Prop1\(^{df}\)): Ames dwarf mice were first described as being dwarf, with the designated genetic symbol \(df\).\(^{11}\) These mice appear normal at birth; however, their growth is quickly retarded, and they only grow to \(\sim 1/3\) the size of their normal littermates. This reduction in growth is due to a recessive Prophet of Pituitary Factor 1 (Prop1) mutation.\(^{12}\) The end result of this mutation is a lack of differentiation of somatotrophs, lactotrophs, and thyrotrophs in the anterior pituitary which results in Ames dwarf mice having essentially no circulating levels of GH, thyroid-stimulating hormone, or prolactin. These mice are also deficient in circulating IGF-1, thyroxine (\(T_4\)), and 3',3,5-triiodothyronine (\(T_3\))\(^{12-14}\) Ames dwarf mice are exceptionally long-lived.
(approximately 40% to over 60% extension of longevity depending on sex and diet), and have measurable health benefits including improved insulin sensitivity, decreased risk of cancer, and improved energy metabolism. This improved “healthspan” (i.e. length of life lived free of frailty and disease) is arguably as important as their extended longevity.

**Snell dwarf mice (Pit1<sup>dw</sup>):** Snell dwarf mice were first described as being dwarf, with the designated genetic symbol dw. Similar to Ames dwarf mice, the Snell dwarf mouse suffers from a recessive Pituitary Factor 1 (Pit1) mutation. The Pit1 mutation results in endocrine deficits that are essentially identical to those of Ames dwarf mice described above. Because of this, Snell dwarf mice also have increased lifespan (approximately 42%) and healthspan.

**GHR-KO mice:** Since both Ames and Snell dwarf mice suffer from endocrine deficits beyond the somatotropic axis, it is important to examine a model of reduced GH signaling that leaves other hormonal axes intact. To evaluate this, Zhou et al. genetically engineered a mouse model of GH-resistance to resemble the human syndrome termed “Laron dwarfism.” As with Laron dwarfs, GHR-KO mice do not have a functional GHR due to targeted disruption of the GHR/GHBP gene. GHR-KO mice have greatly reduced levels of circulating IGF-1, and elevated levels of circulating GH, presumably due to the lack of negative IGF-1 feedback on GH production. These mice have an extension of longevity up to 40% depending on sex and genetic background. As with Ames and Snell dwarf mice, GHR-KO mice have an extension of healthspan as measured by cognition, and end-of-life pathology.

**bGH transgenic mice:** In bGH transgenic mice, over-expression of the GH transgene is typically driven by the rat phosphoenolpyruvate carboxykinase promoter (PEPCK), though other promoters (e.g. the Metallothionein 1 promoter) have been used as well. Importantly, the rate of GH secretion as a result of this transgene is not under the typical IGF-1 mediated feedback inhibition. These mice have accelerated growth, increased production of hepatic IGF-1, and live approximately 30% shorter, presumably due to the overproduction of GH.

**GHA mice:** In order to delineate which phenotypes derived from altered somatotropic signaling are important for longevity, it is important to examine a mouse model with altered somatotropic signaling which does not have altered longevity. GHA mice have greatly reduced (not absent) GH signaling due to the introduction of a mutated bGH gene. The peptide resulting from the mutated bGH gene subsequently competes with endogenous GH for a GHR binding site, but provides essentially no downstream signal. Interestingly, these mice have no alterations in their longevity despite their reduction in GH signaling.

**Alterations in body composition**

Because GH is a major lipolytic hormone, it is of little surprise that GH-deficient and GH-resistant mice have an increase in percentage of body fat. The most drastic increase in percentage of body fat is seen in GHR-KO mice, which can be observed at essentially all ages. Remarkably, the absolute weight of fat depots in male GHR-KO mice has been reported to be equal to that of their normal littermates, despite their diminutive size. It is worth mentioning the effect of GH-resistance on adiposity was shown to be organ-dependent since adipose-specific ablation of the GHR gene results in a fatty phenotype, while liver-specific ablation of the GHR gene does not. Interestingly, despite lacking both GH and thyroid hormone, Ames dwarf mice appear to have an increase in adiposity that is less pronounced and largely age-dependent. It is worth mentioning that while GHR-KO and Ames dwarf mice gain considerable adiposity, they do not naturally become as obese as GHA mice, which become exceptionally obese. As expected, bGH mice have a decrease in adiposity; however, it appears that this is only true in adult mice. The same relationship between GH and adiposity exists in GH-deficient and GH-resistant humans, as well as patients with acromegaly (overproduction of GH), meaning that the findings in GH-mutant mice described below are likely to be applicable to humans. This is very important because studies focused on bioenergetics and metabolism are of increasing interest due to the growing obesity epidemic worldwide, and particularly in the United States.

**Effects of altered adipokine production on insulin sensitivity**

Ames dwarf mice have low levels of circulating glucose and insulin, while GHR-KO mice have profoundly reduced levels of circulating insulin and a moderate decrease in glucose, indicating these animals are insulin sensitive. The insulin sensitivity of Ames dwarf and GHR-KO mice has been demonstrated through the so-called “intraperitoneal insulin tolerance test,” and has been further demonstrated in Ames dwarf mice through the use of a hyperinsulinemic euglycemic clamp. Importantly, it is believed that their improved insulin sensitivity is critical to their extended longevity. Thus, it has been a central focus of our laboratory to understand how a variety of tissues, including adipose tissue, impact the insulin signaling
in Ames dwarf and GHR-KO mice. It has previously been established that visceral fat negatively impacts insulin sensitivity through the secretion of pro-inflammatory cytokines such as IL-6 and TNF-α. The effect of visceral fat on insulin signaling is best exemplified by the effect of surgically removing visceral fat, which ultimately leads to improved insulin sensitivity, presumably through a reduction in circulating pro-inflammatory cytokines. Interestingly, removal of most of the epididymal and perirenal WAT in Ames dwarf and GHR-KO mice promotes insulin resistance rather than insulin sensitivity. It appears this is mainly due to a shift from pro- to anti-inflammatory cytokine production in their visceral WAT (where IL-6 and TNF-α production is downregulated, and adiponectin production is upregulated). It is worth noting that adiponectin is typically negatively correlated with adiposity, which is the opposite of what is observed in these mice. It does, however, appear that adiponectin levels are positively associated with longevity and negatively associated with GH signaling. Results of an elegant studies of the relationship(s) between GH, adiposity, and adiponectin levels (including the bioactive high molecular weight variant of adiponectin) can be found in. The epididymal WAT depot in Ames dwarf mice also has an upregulation in genes that are beneficial for metabolism, including insulin receptor and PGC-1α.

Interestingly, removal of visceral WAT in Ames dwarf mice downregulated genes in subcutaneous WAT (sWAT) that are involved in insulin signaling. This is important, since sWAT is traditionally considered to be "good" adipose tissue in terms of its impact on insulin sensitivity. Also noteworthy is that the circulating levels of leptin are increased in Ames dwarf (including animals on a high fat diet or calorie restriction) and GHR-KO mice, compared to their respective controls. As expected, circulating levels of leptin are decreased in bGH mice compared to their controls. Interestingly, both circulating leptin and adiponectin levels are increased in GHA mice despite no alterations in longevity.

**Reduced senescent cell burden**

A study by Stout et al. used a variety of GH-related mutant mice to establish the role of GH in age-related adipose tissue dysfunction and redistribution. Their study demonstrated that 18-month-old Ames dwarf, Snell dwarf, and GHR-KO mice retain a higher ratio of extra- to intra-peritoneal WAT than their respective controls. The reason these mutant mice maintain a high ratio of extra- to intra-peritoneal WAT may be due in part to the retained capacity of their preadipocytes to differentiate, which has been shown to play a role in age-related ectopic lipid redistribution. Importantly, this suggests that GH action contributes to the natural age-related accumulation of intraperitoneal adiposity, which is a predictor of future pathologies. In addition, acromegaly patients have also been shown to ectopically redistribute lipids, thus strengthening the argument that GH action impacts lipid redistribution.

The same study by Stout et al. also showed that the natural age-related accumulation of senescent cells in adipose tissue was delayed in the absence of GH action. Both Snell dwarf and GHR-KO mice have a lower senescent cell burden, along with lower expression of several molecular markers of senescence including p16 and p21, compared to their respective controls. Importantly, bGH mice and Ames dwarf mice treated with GH during the early postnatal period have an increased senescent cell burden, further illustrating the relationship between GH and adipose-specific cellular senescence. These findings are of particular interest because Ames dwarf, Snell dwarf and GHR-KO mice are long-lived, while bGH and GH-injected Ames dwarf mice are short-lived, thus indicating that the GH-dependent acceleration of cell senescence and redistribution of lipids may play a pivotal role in longevity.

**Improved thermogenesis in brown adipose tissue**

Work in our laboratory indicates that improved energy metabolism may be a “biomarker,” and likely a mechanism, of extended healthspan and lifespan of GH-related mutant mice. GH-deficient mice have improved energy metabolism as measured by an increase in oxygen consumption and heat production per gram body weight, as well as a decrease in respiratory quotient. Because brown adipose tissue (BAT) aids in regulating these parameters, it has become a tissue of interest for our laboratory. The first indication that GH action may alter BAT physiology was observed in a study conducted by Li et al. which showed that GHR-KO mice have an increase in the relative weight of the interscapular BAT (iBAT) depot. Moreover, GHR-KO mice have an increase in the expression of UCP-1 (both protein and mRNA levels) in iBAT. This study also demonstrated that GHA mice show a similar phenotype to GHR-KO mice, while bGH mice have a decrease in both the relative weight of the iBAT depot and UCP-1 mRNA expression. The increased expression of UCP-1 observed in GHR-KO mice may in part be due to these mice having an increase in the BAT mRNA expression of FGF-21, which has been shown to stimulate UCP-1 expression.

Recently, we demonstrated that similar to GHR-KO mice, Ames dwarf mice have BAT that is more active than that of their normal littermates. As seen in GHR-KO mice, Ames dwarf mice have an increase in the
relative weight of the iBAT depot, as well as increased expression of UCP-1 mRNA. Moreover, iBAT from Ames dwarf mice has an increase in the expression of other genes related to thermogenesis (e.g., PGC-1α, ADRβ3, and DIO2) and genes related to fatty acid metabolism (e.g., FAS, HSL, and LPL). Alterations in BAT fatty acid metabolism are important because oxidation of fatty acids plays a major role in BAT-thermogenesis. Further, examination of Hematoxylin/eosin stained iBAT cross-sections revealed that Ames dwarf mice have depleted lipid vacuoles and an increase in the number of nuclei per field, which is indicative of increased BAT activity. Finally, our study showed that surgical removal of the iBAT depot in Ames dwarf mice resulted in an unexpected physiological response: namely, a reduction in the relative weight of the subcutaneous, epididymal, and perirenal adipose depots (in contrast to the increase in relative weight observed in their normal littermates). Moreover, iBAT removal in dwarf mice resulted in a reduction in the size of adipocytes in these WAT depots, while iBAT removal in normal mice resulted in an increase in adipocyte size. Our laboratory interprets this unique physiological response in dwarf mice to possibly be due to the thermal stress caused by the iBAT removal surgery, where the already low body temperature of the dwarf mice (approximately 1.5°C lower than their normal littermates) dropped even lower, resulting in an increased demand for thermogenesis, and therefore, an increased utilization of stored lipids. Because the studies mentioned above were conducted at standard temperature, future studies involving thermoneutral housing (30°C) is an area of extreme interest to our laboratory to delineate the role(s) of body temperature and BAT-thermogenesis in the extended longevity of Ames dwarf mice.

**Concluding remarks**

Numerous studies involving adipose tissue are related to obesity and metabolic syndromes, both of which greatly increase the risk of developing diabetes and other chronic diseases. While these studies are obviously of extreme importance, data from several laboratories (including our own findings) indicate adipose tissue function and distribution plays a major role in the aging process. Studies conducted to date of adipose tissue in GH-deficient mice. Moreover, preliminary studies from our laboratory, along with studies done by a collaborating laboratory, indicate that some of the observed alterations in adipose tissue may be dependent on ambient temperature. We have found that when long-lived GH mutant mice are housed in thermoneutral conditions, the phenotype of their adipose tissue begins to resemble that of their respective controls (Bartke and Masternak, unpublished observations). Overall, we are intrigued by the prospect that adipose tissue may prove to play a determinate role in the extended longevity of GH-mutant mice. Readers that are interested in learning more about age-related changes in adipose tissue, as well as more detail on the possible mechanisms by which adipose tissue alters longevity, are referred to.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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