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REVIEW ARTICLE

Growth Hormone's Impact on Adipose Tissue and Aging

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Abstract

A reduction in growth hormone has repeatedly been shown to improve healthspan and lifespan in mice and attenuate age-related conditions in subsets of comparable clinical populations. While aging results in progressive physiological changes in many tissues that leads to declines in biological function, this review will focus on the role of growth hormone in adipose tissue with respect to the aging process. Growth hormone dramatically and uniquely alters adipose tissue mass, composition, function and distribution, with decreases in hormone action resulting in a counterintuitive "healthy obese" state. As clinical studies are somewhat limited, much of our understanding of this hormone's unique effect on adipose tissue and aging comes from mouse lines with specific alterations to the growth hormone axis. Thus, this review will provide an overview of the healthspan and lifespan consequences of growth hormone action in mouse lines and briefly describe comparable clinical conditions. The review will also summarize the general changes in adipose tissue with normal aging as well as the unique changes in this tissue in response to growth hormone.

Introduction

Aging and the multitude of age-associated diseases are among the greatest challenges in our healthcare system. Thus, finding interventions that prevent, postpone, or even reverse age-related phenotypes and pathologies are urgently needed. Decreasing growth hormone (GH) action is one promising 'intervention' worthy of further exploration. In mice, the most potent method for lifespan extension is growth hormone receptor (GHR) gene disruption as the GHR knock-out (KO) mice are recognized as the world's longest-lived laboratory mouse, winning the Methuselah mouse prize for longevity[1]. These mice are well-studied and protected from many pathologies, exhibiting resistance to various age-associated diseases including numerous cancers (natural or induced), type 2 diabetes, kidney damage, neuromusculoskeletal frailty, and cognitive decline[2-5]. Remarkably, humans with Laron Syndrome (LS) - a genetic condition of GH insensitivity usually caused by inactivating mutations of the GHR gene - share some of the same health benefits seen in the GHRKO mouse. That is, individuals with LS have reductions to key age-associated signaling pathways and are resistant to cancer, diabetes, and cognitive decline[6]. These findings are not limited to GHR disruption as other mouse lines with reduced GH action and clinical conditions of inherited isolated GH deficiency show similar health benefits[7], sparking interest in evaluating the unique characteristics associated with reduced GH activity in the context of aging.

It is of interest that aging and age-related diseases mirror the comorbidities commonly associated with obesity. That is, obesity (excess adipose tissue) is linked to increased risk of common chronic diseases such as cardiovascular disease, hypertension, type 2 diabetes, cancer, etc[8]. Therefore, adipose tissue appears to accelerate or, at minimum, contribute to the aging process. Adipose tissue, which is a crucial energy reserve and endocrine organ, is also dramatically altered by GH. As such, a deeper understanding of the unique adipose tissue phenotype in response to GH is warranted and may provide insight into as to why decreased GH activity attenuates aging. Interestingly, a reduction in GH action, despite improved health and lifespan, is accompanied by a counterintuitive – but intriguing – increase in adipose tissue mass or obesity. This review aims to briefly summarize the healthspan and lifespan data in mouse lines and clinical conditions associated with the effects of GH. An overview of the role of adipose tissue in aging and the unique properties of adipose tissue in mice and humans with decreased GH action is also discussed, which may provide clues for future pharmacological interventions targeting age or age-related diseases.

Overview of growth hormone

GH is a 22kDa peptide hormone secreted in a pulsatile manner from somatotrophs in the anterior pituitary gland. Release is controlled, in part, by the balance of two hypothalamic hormones: 1) growth hormone releasing hormone (GHRH), which promotes GH release; and 2) GH inhibitory hormone or somatostatin, which prevents GH release. As an endocrine hormone, GH is

released from the somatotrophs into the bloodstream and acts on distant cells throughout the body via binding to the growth hormone receptor (GHR). Since GHR expression is present on most cells, many different organs and tissue systems including bone, kidney, muscle, liver, and adipose tissue are affected by GH action[9]. To initiate its action on cells, GH binds to the GHR, the receptor undergoes a conformational change, resulting in the activation of the tyrosine kinase, Janus kinase 2(JAK2), which initiates signaling via multiple pathways including the JAK/signal transducer and activator of transcription (STAT) signaling pathway [10] While JAK/STAT is not the only signal transduction pathway initiated by the binding of GH to its receptor, it is a key pathway that regulates growth and metabolism [11]

As the name implies, GH plays a critical role in somatic growth. In addition to supporting growth, GH is important in the regulation of nutrient metabolism, including glucose homeostasis, proteolysis, and lipid and mineral metabolism. In adipose tissue, GH increases lipolysis, causing release of free fatty acids (FFA), and inhibits lipogenesis, resulting in a net loss of adipose tissue mass[12]. In other tissues, such as muscle, GH causes a rise in protein synthesis and promotes insulin resistance, sparing glucose as an energy source. Thus, individuals with higher GH levels are relatively lean with an increase in muscle tissue and a decrease in fat mass. It is important to note that for adipose tissue, GHR receptors are present on most cell types found in the tissue including adipocytes[13], preadipocytes[14], assorted immune cells[15] and other cell types within the tissue. Although most attention has been given to the role of GH on adipocyte morphology and function, the response of adipose tissue to GH is a result of the collective signaling of all the cells within the tissue.

Growth hormone and aging

Circulating GH levels decrease with advanced age in mammalian species[16]. This has led many to postulate that GH replacement therapy may provide anti-aging benefits. However, there is significant evidence, especially in rodents and in humans with extremes in GH action, that GH does not prevent aging but rather accelerates the aging process by increasing several aging associated pathways. Below we summarize longevity data and the basic phenotypic consequences of select mouse lines with altered GH action (also summarized in Table 2). Although aging studies are scarce, we will also provide general characteristics of several clinical populations with alterations in the GH/IGF-1 axis.

Mouse lines with altered growth hormone and aging

GH action (both increased and decreased) has been extensively studied in mice[9,17]. Mouse models with alterations to the GH axis include mice with spontaneous mutations that inhibit GH production (Ames and Snell mice), transgenic expression of bovine GH or a GH antagonist (bGH and GHA mice), germline knockouts of the GH gene or GHR gene (GH-/- and GHR-/- mice), and induced disruptions of GH or the GH receptor (AOiGHD, 1.5mGHRKO, 6mGHRKO, and

AdGHRKO mice). Many other mouse lines with GH alterations have been developed but are outside the scope of this manuscript. A detailed summary of the various mice with alterations in the GH/IGF1 family either upstream or downstream of GHR or to the GHR itself are provided in a recent review[9].

Snell and Ames mice: The first mice with GH alterations to be described were two separate spontaneous mutations that result in mice with decreased body size. Snell mice, first reported in 1929[18], have a recessive mutation in the Pou1f1 gene which disrupts the function of the somatotrophs, lactotrophs, and thyrotrophs, resulting in deficiencies in GH, prolactin (PRL), and thyroid stimulating hormone (TSH). Ames mice have a similar deficiency in GH, PRL, and TSH, caused instead by a recessive mutation in the Prop1 gene. Both Ames and Snell mice have greatly reduced circulating GH, and consequently reduced IGF-1. This results in mice with decreased body size and weight, but improved insulin sensitivity[19]. Both of these models also exhibit 40-50% increased lifespan[20], indicating that GH may have a negative impact on lifespan.

Growth hormone receptor knock-out and growth hormone knock-out mice: One disadvantage of using the Snell or Ames mice to study GH is the decreases in PRL and TSH that occur in tandem with decreased GH in these mice. To provide GH specific models, two mouse lines were subsequently generated with gene disruptions that are specific to GH and its receptor, GH knockout (GHKO) mice and GH receptor knockout (GHRKO) mice. GHRKO mice lack functional GH receptor and serve as a mouse model of LS[21]. These mice were developed in the 1990s by the Kopchick laboratory and have some notable phenotypes. Body size and weight are considerably decreased in GHRKO mice, but these mice have increased fat mass, as will be more fully detailed below[22]. Despite their obesity, GHRKO mice are extremely insulin sensitive, resistant to cancer[3], and are extremely long-lived[4], being the longest-lived laboratory mouse. Likewise, a GHKO mouse line was more recently reported by List et al[20,23] that has decreased GH secretion similar to Ames and Snell mice, but without disruptions to PRL or TSH. GHKO differ from GHRKO mice in that they are GH deficient rather than GH resistant. However, like GHRKO mice, GHKO mice have decreased IGF-1 and decreased body size and weight[23]. They also have increased insulin sensitivity despite their increased fat mass and decreased lean mass[23]. Although lifespan has not yet been reported in these mice, the similarity of their phenotype to that of GHRKO mice indicates that their healthspan and lifespan might be similarly increased.

Bovine growth hormone (bGH) and growth hormone receptor antagonist (GHA) transgenic mice: Prior to the development of GHRKO mice, transgenic mice including bGH and GHA mice were developed to study GH in rodents. The bGH transgenic mice were among the first transgenic mouse lines to be generated in the early 1980's[24]. These mice serve as a mouse model of acromegaly, with extremely high circulating GH and IGF-1 levels and increased body size and weight[25]. bGH mice have increased lean mass and

decreased fat mass[26], but are unhealthy, due, in part, to increased tumor incidence, insulin resistance, and greater cardiac, vascular and kidney damage than littermate controls[9,27]. Lifespan is markedly reduced in bGH mice (~50% the lifespan of controls)[27], cementing the negative impact that GH has on lifespan. In addition to bGH transgenic mice, a mouse line expressing a growth hormone antagonist (GHA mice) has also been reported[28]. The growth hormone antagonist, Pegvisomant approved for use in patients with acromegaly, binds to the GH receptor but does not activate its intracellular signaling pathways and was discovered using this mouse line. GHA mice have a phenotype that is similar to GHRKO but less extreme. GHA mice have increased serum GH, decreased serum IGF-1 and decreased body length and weight. They are also obese, with increased fat mass and decreased lean mass, and have increased insulin sensitivity [29]. GHA mice have decreased cancer incidence but were previously reported not to have a statistically significant increase in lifespan[4]; however, there is a clear trend towards an increase in lifespan for female mice. As the only study reported on lifespan in GHA mice, it is likely that a sufficiently powered study could determine lifespan extension to be significant and unpublished data from our laboratory indicate that the female GHA mice indeed have extended longevity.

Mice with temporal disruption of growth hormone action: To go along with lifelong, systemic GH disruptions, mouse lines have also been generated with GH disrupted temporally (AOiGHD, 1.5mGHRKO, 6mGHRKO) or locally (AdGHRKO). Adult-onset isolated GH deficiency (AOiGHD) mice, first reported in 2011 by the Kineman Lab[30], utilize an inducible diptheria toxin receptor and Cre/lox system to destroy somatotrophs at 10-12 weeks of age. Thus, these mice are a model of adult GH deficiency, with decreased serum GH and IGF-1. AOiGHD mice have a normal body size and weight but have increased fat mass and decreased lean mass. Unsurprisingly, these mice have increased insulin sensitivity to go along with the decreased GH and IGF-1. Female AOiGHD mice have a 13% increase in mean lifespan with no change in median or maximal lifespan[31]. Also utilizing the Cre/lox system, GHR knockouts at two different ages (6 weeks/1.5 months and 6 months) have been reported. Both lines are GH resistant, with increased serum GH and decreased serum IGF-1, but the 1.5mGHRKO[32] mice have decreased body length and weight, while the 6mGHRKO mice[33] have normal body weight and only show a decreased body length at advanced age. Both lines have increased fat mass, decreased lean mass, and improved insulin sensitivity, similar to GHRKO mice, and 6mGHRKO males also have decreased cancer incidence, which has not been studied in 1.5mGHRKO mice. The lifespan phenotype of these lines, however, is less striking than that seen in GHRKO mice, as increased lifespan in both lines is limited to females. The 1.5mGHRKO mice have an 18% increased maximal lifespan in females, while 6mGHRKO mice females exhibit increased mean, median (20%), and maximal (15%) lifespan.

GHR has also been knocked out specifically in adipocytes, highlighting the role GH has on the function of adipose tissue. Adipocyte-specific GHRKO (AdGHRKO) mice[34] have normal body length and weight and increased fat mass. AdGHRKO mice also have increased insulin sensitivity, and males exhibit a 6% increase in lifespan[35]. The improvements in glucose homeostasis and lifespan, despite only having GHR knocked out in a specific cell (adipocytes) in a single tissue, demonstrates the importance of GH signaling in adipose tissue to the aging process.

Humans with altered growth hormone signaling

Inherited growth hormone receptor defects: There are two localized groups of people with LS: an Israeli cohort and an Ecuadorian cohort. Both groups lack normal GH signaling due to abnormal GH receptors (GHRs), resulting in IGF-1 deficiency[36], and these changes have intriguing health outcomes. Many different GHR variations have been found to result in LS, but the vast majority of the Ecuadorian cohort has been found to have the E180 splice mutation in exon 6 of the GHR gene[37]. In contrast, the Israeli cohort is more ethnically heterogenous, resulting in various GHR defects [6,38]. Importantly, no incidences of cancer are found using epidemiological data that included patients from the Ecuadorian and Israeli cohort as well as people with LS outside of the major cohorts[39]. Patients from the Ecuadorian cohort have no instances of diabetes and show increased insulin sensitivity[38], while those from the Israeli cohort develop insulin resistance, and a few members have experienced complications from diabetes. Ultimately, despite their obesity—and likely due to their resistance to cancer—people with LS appear to have typical lifespans, with most living into their seventies and beyond[40].

Inherited growth hormone deficiency: Isolated GH deficiency (IGHD) in humans results from mutations in genes that encode GH [41]. Of the various types, untreated individuals with IGHD from Brazil (IGHD type 1B, owing to a mutation of the GHRH receptor gene), have profoundly reduced IGF-1 and GH levels[7]. The Brazilian IGHD type 1B adults are among the best studied due to the larger cohort of individuals with the condition. They have reduced β-cell function, but do not have a high prevalence of diabetes[42]. Overall, patients with untreated IGHD have normal lifespans once they reach adulthood, but they have a higher frequency of death before 20 years of age[7]. Interestingly, a number of individuals within the Brazilian cohort are centenarians, which is not common in this population. Further, there is indication of improved healthspan and protection from aging-related diseases such as cardiovascular disease and cancer (with the potential exception of skin cancer)[7].

Acromegaly: Acromegaly is a condition in which the body produces excess GH in adulthood most commonly due to a pituitary adenoma. Patients with acromegaly often express cardiovascular biventricular

hypertrophy, hypertension, and metabolic abnormalities—all of which contribute to mortality[43]. There appears to be an increased cancer incidence in people with acromegaly (particularly, elevated risk of thyroid, colon, and breast cancer), although whether this contributes to an increased risk of death is debated[44]. Without treatment, patients with acromegaly have an expected lifetime reduction of 10 years[45]. However, when GH and IGF-1 are restored to normal levels, people with this disease live as long as the general population[46]. From this, one can gather that chronic GH exposure can reduce longevity in humans.

Adipose tissue overview

The obesity epidemic has spawned greater interest in adipose tissue. This once relatively simple tissue (predominantly thought to function as a passive energy reservoir) is now appreciated to be more dynamic, influential in metabolism through assorted secretory products, and highly responsive to various metabolic signals. Additionally, adipose tissue represents the largest organ in humans, suggesting it could have profound impact on age and metabolic health. As summarized in Table 1, adipose tissue is complex, serving more than just as an energy storage site with distinct anatomical locations, secretory products, cell composition, nerve innervation, and blood/lymphatic vasculature. This review will focus mainly on white adipose tissue (WAT) although loss of brown adipocytes in brown adipose tissue (BAT) and beige adipocytes in WAT as well as their activity occurs with aging and may also be an important consideration for therapeutic targeting. To add to the complexity, tissue morphology, location and function are altered in response to aging and GH as described in subsequent sections.

Table 1: Complexity of adipose tissue		
Functions of adipose tissue	Triglyceride energy reservoir Endocrine organ Thermoregulation Appetite regulation Cushion vital organs Immune functions Reproduction Hematopoiesis Lymphopoiesis	
Subtypes of adipose tissue	White adipose tissue (WAT) – unilocular lipid droplets Brown adipose tissue (BAT) – multilocular lipid droplets Beige/Brite adipose tissue – within WAT depots, multilocular lipid droplets	
Main depot locations of adipose tissue	**Mouse** Visceral: mesenteric, retroperitoneal, perirenal, perigonadal (epididymal in males/paraovarian in female), epicardial Subcutaneous: cranial, subscapular, intrascapular BAT, inguinal, gluteal Other sites: bone marrow, intramuscular, ectopic	**Human** Visceral: omental, mesenteric, retroperitoneal, perirenal, epicardial Subcutaneous: abdominal (deep and superficial), gluteal, femoral Other sites: bone marrow, intramuscular, ectopic
Common secretory products of adipose tissue	Adipokines: Leptin, adiponectin, visfatin, resistin, chemerin, apelin, vaspin, omentin, FGF21, RBP4, irisin, etc. Cytokine/cytokine-like proteins: TNFα, MCP1, IL-1β, IL-4, IL-9, IL-10, IL-15, etc.) Lipids: fatty acids, cholesterol, retinol, prostaglandins, leukotrienes, lysophospholipids, sphingolipids Extracellular matrix: collagens, fibronectin, laminin, heparan sulfate, hyaluronan, etc. Other: non-coding RNAs, assorted metabolites, exosomes, etc	
Cell types of adipose tissue	Adipocytes (distinct subpopulations) Preadipocytes Mesenchymal stem/stromal cells Endothelial cells Fibroblasts PericytesLeukocytes: Macrophages, Mast cells, Dendritic cells, Neutrophils, Eosinophils, B cells, T cells	

White adipose tissue changes in normal aging

Changes in mass and distribution of WAT with age - Adipose tissue undergoes dramatic changes in mass, anatomical location, cell composition and function throughout adult aging, which may drive the pathophysiology of some age-related diseases. As for mass, WAT tends to increase with age, peaking often around 50-70 years in humans and decreasing at more advanced ages[47-49]. Rodents show a similar trend of peak WAT mass at approximately 19 months of age with decline thereafter, which varies based on strain and sex[50] and is accompanied by decreased insulin sensitivity and increased oxidative damage in the tissue[51]. WAT mass in both humans and rodents is thought to redistribute from subcutaneous sites to intra-abdominal/visceral sites with advancing age[52-54]. It is noteworthy that the methods used to assess fat redistribution show distinct results in rodents. That is, weights of specific fat depots in mice consistently show a trend towards reduced subcutaneous fat mass and increased intra-abdominal depots[50,55,56]; however, when assessing whole-body adiposity using MRI, the opposite trend is seen (increase in the subcutaneous to visceral fat ratio with advancing age due to loss in visceral fat mass)[57]. Regardless, this redistribution is accompanied by an increase in ectopic deposition of fat in non-adipose tissues, such as liver and muscle. Collectively, these age-associated shifts in fat amount and distribution predispose the individual to metabolic dysfunction and related health complications.

Cellular changes with age - Aging also influences adipose tissue cell abundance and cell function. In terms of abundance, there are greater numbers of immune cells[54] (especially macrophages, T and B cells) and senescent cells with aging[58,59]. In fact, a recent single cell RNA sequencing study shows that the age-related, widespread increase and activation of T cell and B cell subsets in many tissues is first detectable in adipose tissue[60], suggesting WAT as an early aging target. In addition to increases in immune cells with age, there are also unique subsets that emerge with age. For example, a resident population of aged adipose B cells (AABs) are localized to fat-associated lymphoid clusters[61] – a non-classical lymphoid tissue cluster in adipose tissue[62]. AABs are distinct from age-associated B cells in spleen and are regulated by the Nlrp3 inflammasome[61].

Senescent cells are also abundant in WAT with age. WAT senescence is best studied in preadipocytes, and senescence impairs not only their proliferation and differentiation but also reduces the adipogenic potential of neighboring cells through the release of the senescence-associated secretory phenotype (SASP)[59]. Other cells in WAT, such as macrophages[58], adipocytes[63] and endothelial cells[64], also undergo senescence and contribute to the dysfunction of the tissue with advancing age. Many other functional or molecular changes in cells occur with age as is reflected in recent transcriptomics studies[60,65]. For example, 1) preadipocytes exhibit lower ability to replicate and differentiate, which prevents adipogenesis[66], 2) brown adipocytes have

decreased UCP-1 expression and, thus, reduced thermogenic capacity, and 3) macrophages upregulate V-set immunoglobulin-domain-containing 4, an immune checkpoint protein involved in cancer and inflammatory diseases, which may further drive agerelated changes[67]. This adipose field continues to rapidly evolve, and these examples highlight the need to consider all cells and not just the adipocytes in aging adipose tissue.

Structural and depot-dependent changes with Age - Other structural features of adipose tissue are altered with aging. For example, aging induces an increase in WAT fibrosis, characterized by an increase in extracellular matrix deposition that limits the remodeling potential and promotes inflammation. Mice of advanced age (~30 months) show a striking seven-fold increase in fibrosis within epididymal WAT[68]. In this same study, vascularity is reduced and angiogenic capacity by ~50% These structural changes result in functional changes. That is, adipose tissue hypoxia occurs with aging, which has been demonstrated not only by immunohistochemistry but also by direct measurements of interstitial partial pressure of oxygen[69]. This can, in part, be explained by the reduced vascularity in adipose tissue as well as the hypertrophy of adipocytes of aged mice[68]. Collectively, these changes contribute to the metabolic dysfunction of adipose tissue with age. Finally, the age-associated changes in adipose tissue are mediated in a depot-specific manner. For example, the capacity of preadipocytes to differentiate and express adipogenic properties are depot-specific with epididymal showing lower ability than subcutaneous or perirenal[70] with age, and senescence, of some WAT cell subsets, is depot dependent[58].

Although beyond the scope of this review, fat storage, distribution, cell composition, and some functional characteristics with age, such as in vivo triglyceride synthesis and breakdown[71], differ by sex and racial/ethnic group[72,73]. Overall, adipose tissue is highly dynamic, which likely reflects a tissue that needs to adapt to frequent changes in nutrient availability, hormonal status and cold-exposure for optimal function. Undoubtedly, the changes in WAT with age contribute to age-related metabolic dysfunction and promote a state of chronic, systemic, low-grade inflammation.

Growth hormone and white adipose tissue

GH has a pronounced effect on the amount of WAT (and BAT). In humans, circulating GH levels are strongly and inversely correlated with WAT mass. That is, GH deficiency in adults causes increased fat mass[74], which somewhat reverses when treated with recombinant human GH[75]. Likewise, the absence of or reduction in GH action in patients with LS and Brazilian adults with IGHD type 1B, respectively, increases central and total adiposity[76]. In contrast, acromegaly is associated with marked reductions in total body fat[77], and treatment of acromegaly to normalize GH levels increases body fat[77].

As most clinical studies focus on WAT mass with respect to GH, there are many unresolved questions as to the function and

quality of the tissue. Mice provide an opportunity to do longitudinal and more invasive measures associated with WAT, providing a deeper understanding of the molecular and cellular changes in response to GH (and aging). As summarized in Table 2, studies to date suggest that GH action provides a unique perspective on AT quantity versus quality. That is, GH reduces AT mass, which is commonly considered favorable for overall metabolic health. However, decreases in GH activity, which increase WAT mass or obesity, result in an overall improvement in metabolic health and lifespan – not commonly associated with obesity – and improve the "quality" of WAT. Understanding how reductions in GH uncouple obesity from its metabolic complications provides an intriguing means to evaluate not only how GH impacts AT, but to better understand the characteristics of AT that contribute to aging.

Excess growth hormone action: The short-lived, bovine GH (bGH) transgenic mice are commonly used to assess WAT in the context of excess GH. Like humans with acromegaly, adult bGH mice have less total body fat[80]. However, longitudinal measures of body fat show that young bGH mice have greater adiposity but, unlike control mice, have no significant gains in fat mass throughout adult life[26]. Even when provided a high fat diet, bGH mice remain relatively protected from significant gains in fat accumulation[81,82]. While decreased fat mass is typically considered favorable for metabolic health, studies characterizing the cellular and molecular changes in bGH WAT suggest a highly dysfunctional tissue, which likely contributes to the early demise of these mice. For example, WAT of bGH mice has notable fibrosis with the subcutaneous depot most impacted[83]. Likewise, immune cell analyses by flow cytometry to characterize the leukocyte population show greater macrophage and regulatory T-cell infiltration in subcutaneous and mesenteric depots of bGH WAT depots as compared to littermate controls[84]. RNA sequencing analyses of bGH WAT reveal depot specific alterations in genes associated with lipid metabolism and immune cell activation, notably B and T-cells[85]. Like humans with acromegaly, the adipokine profile associated with excess GH is also less favorable, including decreased levels of adiponectin[86]. Finally, subcutaneous WAT-derived mesenchymal stem cells isolated from bGH mice have impaired differentiation relative to controls[87] and most WAT depots show marked increases in senescent cell accumulation[88]. Collectively, these studies suggest that the decrease in fat mass due to increased GH action may result in WAT of poorer quality that may contribute to the poor health outcomes of both bGH mice and in humans with acromegaly.

Reduction in growth hormone action: In search of characteristics that may improve healthspan or lifespan, greater attention has been given to the features of adipose tissue of mice with decreased GH activity. The various changes in WAT and BAT due to reduction in GH action are summarized in Table 2. Regardless of the mechanism by which the effects of GH are reduced in mice, all mice share an increase in WAT mass and most an increase in BAT mass. In fact, high fat

diet feeding of GHRKO, AOiGHD, or GHA mice results in even greater fat mass gains although these mice remain relatively protected from impairments in glucose homeostasis with high fat diets[29]. The obese state of the mice is either accompanied by a counterintuitive increase in lifespan (GHRKO, Ames, Snell), which may be sex specific (AOiGHD, AdGHRKO, 1.5mGHRKO, 6mGHRKO), or no change in lifespan. As increased fat mass typically comes with assorted negative metabolic perturbations, the fact that decreased GH action results in no reduction in lifespan and improvement in several healthspan parameters despite marked obesity is noteworthy and suggests inherent benefits to WAT with reduced GH action.

Key to the healthy obesity phenotype in these mice is that the WAT is not uniformly distributed, but rather predominantly isolated to the subcutaneous (Sc) depots and there is a resistance to the redistribution to visceral depots with advancing age in these mice[86]. Sc WAT is well established to be biologically distinct from visceral and intra-abdominal depots and its accumulation has a have a more favorable risk profile in terms of metabolic disease as has been elegantly and recently reviewed[87,88]. In most of these mice, the Sc depot is also the depot that exhibits the most dramatic changes in gene expression[83,89], the most significant change in adipocyte size[23,34,90], the most protection from fibrosis[23,81,91], greater adipogenic capacity[86] and the most protection from WAT and BAT inflammation[89,92]. Thus, the changes and preservation of specifically in Sc WAT in response to GH may be a critical factor that preserves metabolic health in these animals.

While the most dramatic changes can be seen in Sc depots, the positive changes in WAT with reductions in GH action are not limited to Sc depots. The circulating levels of adipokines/cytokines, which reflect the collective secretory profile of all WAT depots, from WAT in mice (and humans) are also favorable, such as the higher levels of adiponectin[84]. Visceral WAT from GHRKO mice also show lower inflammatory cytokine production[93]. Surgically removing epididymal and perirenal depots of both GHR-/- and Ames dwarf mice also show that these depots have distinct beneficial effects as compared to these same depots in control mice[93,94]. Likewise, visceral depots of GHRKO mice have been shown to exhibit deactivation of Nlrp3 inflammasome and have increased naïve T cells in aged mice[95]. Finally, despite the fact that obesity and aging increase cellular senescence[59,96], the increased WAT mass in these mouse lines is associated with lower senescent cell burden[86]. That is, GHRKO, Ames, and Snell mice have lower levels of senescence in most depots of WAT in comparison to age-matched controls, which may be attributed to their delayed aging[86]. Interestingly, the intermediate phenotype of GHA mice does not result in reduced WAT senescence; however, considering the marked increase in adiposity with age, this could be considered a positive phenotype[91].

Table 2: Phenotype of mice with decreased growth hormone action

		GHRKO	GHKO	AOiGHD	AdGHRKO	1.5mGHRKO	6mGHRKO	GHA	Ames	Snell
	Primary Defect	Disruption of the *Ghr* gene	Disruption of the GH gene	Inducible ablation of somatotrophs	Disruption of the GHR gene within adipocytes	Inducible knockdown of the *Ghr* gene	Inducible knockdown of the *Ghr* gene	Transgenic for GHR antagonist gene	Spontaneous mutation in *Prop1*	Spontaneous mutation in *Pou1f1*
GH/IGF-1 Axis	GH	↑	↓	↓	↔	↑	↑	↑	↓	↓
	IGF-1	↓	↓	↓	↔	↓	↓	↓	↓	↓
Aging	Maximum, median, or mean lifespan	↑	n.d.	↑♁, ↔♂	↔♁, ↑♂	↑♁ Maximal lifespan, ↔♂	↑♁ median and maximal lifespan, ↔♂	↔	↑	↑ mean and maximal
WAT	WAT Mass	↑	↑	↑	↑	↑	↑	↑	↑	↑
	WAT Depot Location	Sc	Sc, retro, ♂ mes	Sc, retro	Sc, retro, mes, ♁ epi	Sc at 9 mo., ♂ peri; ♂ Sc and mes at 19 mo.	Sc, ♂ mes at 12 mo.	Sc, Ing	Sc, Epi, Retro perirenal, visceral	Sc
BAT	BAT Mass	↑	↑	n.d.	↑♁	↑ ♁	↔	↑	↑	n.d.
	BAT Activity	↑	n.d.	n.d.	↓	n.d.	n/a	↑	↑	↑
Adipokines /Cytokines	Leptin	↑	n.d.	↑	↔	↔	↓♁ at 12 mo., ↑ ♂	↑	↑	↑
	Adiponectin	↑	n.d.	↔	↓	↑	↔	↑	↑	↑
	TNF-α	↔	n.d.		↔	n.d.	↔	n.d.	↓	↓
	IL-6	↓	n.d.	↔ ♁, ↑ ♂	↔	↑♁, ↔ ♂	↔	n.d.	↓	↓
	Resistin	↑	n.d.	n.d.	↓	↑♁	↑♂	n.d.	↔	n.d.
	FGF21	↔	n.d.	n.d.	↔	n.d.	n.d.	n.d.	n.d.	n.d.
	Other	↔MCP-1, ↓ IL-1β	n.d.	n.d.	↔MCP-1	↔MCP-1	↔ MCP-1	n.d.	n.d.	n.d.
	Fibrosis	n.d.	↓ in Sc	n.d.	↓	n.d.	n.d.	↓ in Sc	n.d.	n.d.

		GHRKO	GHKO	AOiGHD	AdGHRKO	1.5mGHRKO	6mGHRKO	GHA	Ames	Snell
	Primary Defect	Disruption of the *Ghr* gene	Disruption of the GH gene	Inducible ablation of somatotrophs	Disruption of the GHR gene within adipocytes	Inducible knockdown of the *Ghr* gene	Inducible knockdown of the *Ghr* gene	Transgenic for GHR antagonist gene	Spontaneous mutation in *Prop1*	Spontaneous mutation in *Pou1f1*
Other Features	**Senescence**	↓	n.d.	n.d.	n.d.	n.d.	n.d.	↔	↓	↓
	Misc.	↑ potential for adipogenesis and differentiation in AT-MSC in Sc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Low body temp	n.d.
	References	9,22,85,90,97-104	9,23	30,31,84,90	9,34,35,102,105	9,32,35,90,99	9,33,106	9,12,81,90,91,97,101,107	9,12,98,108-110	9,86,94,98,111,112

↑increase, ↔ no change, ↓decrease, n.d. indicates no data, ♁ female, ♂ male; if no sex is indicated, sex differences were not reported or there were no sex differences.

Conclusion

Collectively, these data suggest that efforts to identify effective anti-aging strategies should include means to reduce GH action. Indeed, a consensus statement from aging researchers at the "Interventions to Slow Aging in Humans" workshop lists pharmacological inhibition of the GH/IGF-1 axis as the first of six strategies with the greatest promise[113]. Among the many target tissues of GH that could contribute to the enhanced longevity phenotype, WAT shows significant promise due to the dramatic modifications in this tissue that occur with aging and to the unique preservation and anti-aging properties of Sc WAT by GH as described in this review. Many questions linking GH-induced WAT changes to the aging phenotype remain unresolved. For example, the dramatic Sc deposition and alteration in composition and function in WAT with reduced GH action is evident in almost every mouse line assessed. However, clinical studies have not usually had the ability to discern among individual depots or evaluate adiposity in a longitudinal manner. Thus, it remains to be seen whether the depot-specific phenotypic changes seen in mice have relevance to humans. Likewise, our understanding of normal adipose tissue with aging continues to evolve, especially with the availability of newer methods that allow for evaluation of cell-specific contributions via single cell transcriptomics. Most data on GH and WAT from mice and humans, to date, use whole tissue that might mask the contribution of individual cells to the observed phenotype. Finally, data described in this review focus on extremes in GH action due to genetic alterations, which does not represent interventions that could involve pharmacological anti-aging strategies. These future studies will be key to understanding the impact of GH on aging and the role of adipose tissue.

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I certify that neither I nor my co-authors have a conflict of interest as described above that is relevant to the subject matter or materials included in this work.

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