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COMMENTARY

Functionally enhanced brown adipose tissue in Ames dwarf mice

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ABSTRACT

Reduced insulin-like growth factor 1/insulin signaling (IIS) has been linked to extended longevity in species ranging from yeast to mammals. In mammals, this is exemplified in Ames dwarf (*Prop1*$^{df/df}$) mice, which have a 40%–60% increase in longevity (males and females, respectively) due to their recessive Prop1 loss-of-function mutation that results in lack of growth hormone (GH), thyroid-stimulating hormone and prolactin. Our laboratory has previously shown that Ames dwarf mice have functionally unique white adipose tissue (WAT) that improves, rather than impairs, insulin sensitivity. Because GH and thyroid hormone are integral to adipose tissue development and function, we hypothesized that brown adipose tissue (BAT) in Ames dwarf mice may also be functionally unique and/or enhanced. Here, we elaborate on our recent findings, which demonstrate that BAT is functionally enhanced in Ames dwarf mice, and suggest that BAT removal in these mice results in utilization of WAT depots as an energy source. We also discuss how our findings compare to those in other long-lived dwarf mice with altered IIS, which unlike Ames dwarf mice, are essentially euthyroid. Lastly, we provide some insights into the implications of these findings and discuss some of the necessary future work in this area.

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Introduction

A reduction in insulin-like growth factor 1 (IGF-1)/insulin signaling (IIS), as well as homologous signaling in lower species, has been shown to extend longevity in yeast (*Saccharomyces cerevisiae*),[1] worms (*Caenorhabditis elegans*),[2] flies (*Drosophila melanogaster*),[3] mice (*Mus musculus*)[4] and also, likely in humans.[5] While the mechanisms by which reduced IIS impacts aging are outside the scope of this article, interested readers are referred to relevant reviews in refs. 6 and 7 Our laboratory has focused on several types of mice with depleted somatotropic (growth hormone/IGF-1) signaling to further understand the underlying mechanisms of their extended longevity. In doing so, several key mechanisms have emerged, including improved insulin signaling[4] and improved energy metabolism.[8] Focusing on metabolic tissues involved in insulin signaling, our laboratory demonstrated that long-lived dwarf mice with reduced somatotropic signaling have functionally unique white adipose tissue (WAT) (details later in the commentary).[9,10] Extrapolating from the idea that adipose tissue in these mice is altered, and energy metabolism greatly depends on brown adipose tissue (BAT),[11] we hypothesized that BAT function in these mice may also be altered and/or enhanced. Our results, which show that BAT function is enhanced in Ames dwarf mice, were recently published in Endocrinology,[12] and are discussed later in this commentary. Along with this discussion, we will also provide necessary background information and an interpretation of the significance and overall future directions of our work.

Life extension in dwarf mice

In 1996, Brown Borg et al. showed that Ames dwarf (*Prop1*$^{df/df}$) mice live 40–60% longer than their normal littermates (males and females, respectively).[13] Ames dwarf mice suffer from a recessive Prophet of Pituitary Factor 1 (Prop1) loss-of-function mutation, which results in lack of differentiation of somatotrophs, lactotrophs and thyrotrophs in the anterior pituitary.[14] This, in turn, leads to depleted levels of growth hormone (GH), thyroid-stimulating hormone (TSH) and prolactin, with secondary effects including greatly reduced circulating levels of thyroid hormones (TH) and IGF-1. These mice appear normal at birth, however, sexual maturation is delayed and their growth is retarded, resulting in these mice only growing to ~30% the size of their normal littermates. Snell dwarf (*Pit1*$^{dw/dw}$) mice, that suffer from an endocrine defect essentially identical to that of

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Ames dwarf mice (though from a mutation on a different chromosome), are also long-lived.[15] While these mutants are deficient in several hormonal axes, Coschigano et al. demonstrated that growth hormone receptor/growth hormone binding protein knockout mice (GHR/GHBP-KO, hereafter referred to as GHRKO mice) are long-lived and share several metabolic phenotypes with Ames dwarf and Snell dwarf mice which are likely responsible for their extended longevity.[16] Opposite of what is found in mice lacking GH action, bovine growth hormone (bGH) transgenic mice overexpressing GH are short-lived,[17] leading to the conclusion that reduced activity of the somatotropic axis is the major mechanism of extended longevity in dwarf mice.

Unique white adipose tissue in dwarf mice

One of the most striking metabolic phenotypes of long-lived dwarf mice is their improved insulin signaling and glucose homeostasis. In Ames dwarf mice, both peripheral insulin and glucose are greatly reduced, while in GHRKO mice, peripheral insulin is greatly reduced and glucose is moderately reduced.[4] The concurrent reduction in both insulin and glucose levels suggests improvement in the ability of insulin to clear glucose, and presents a phenotype opposite of metabolic syndrome. This has been proven by measuring glucose levels following intraperitoneal injection of insulin (typically referred to as an intraperitoneal insulin tolerance test), and through the use of a hyperinsulinemic euglycemic clamp.[4] The reasons for their increased insulin sensitivity may, in part, be due to alterations in the expression levels of hepatic genes related to glucose metabolism,[18] a reduction in pancreatic β-cell formation,[19] and reduced peripheral GH which correlate with improved insulin sensitivity.[4] Interestingly, Ames dwarf and GHRKO mice have a phenotype opposite of metabolic syndrome despite having increased adiposity, including epididymal WAT (eWAT), which typically promotes insulin resistance. Moreover, peripheral adiponectin levels in these mice are increased, rather than reduced,[18] as typically occurs with increased adiposity. The increased adiponectin production observed in Ames dwarf and GHRKO mice is of particular importance due to its ability to promote insulin sensitivity (possibly by increasing AMPK activity), as well as its anti-inflammatory and anti-atherogenic properties.

Together, this led to the hypothesis that eWAT in GHRKO and Ames dwarf mice may have altered functionality from that of their respective controls. Since eWAT promotes insulin resistance through production of pro-inflammatory cytokines, surgical removal of eWAT results in increased insulin sensitivity in normal rats.[20] However, surgical removal of eWAT in GHRKO and Ames dwarf mice results in decreased insulin sensitivity.[9,10] This unique finding following eWAT removal is at least partially due to GHRKO and Ames dwarf mice upregulating gene expression of insulin receptor, adiponectin and peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) which promote insulin sensitivity, and downregulating pro-inflammatory cytokines, such as TNF-α and IL-6, which decrease insulin sensitivity.[9,10]

Age-related WAT redistribution and senescent cell burden are also altered in these long-lived mice. Both Ames dwarf and GHRKO mice retain a higher extra- to intraperitoneal ratio of WAT distribution as they age,[21] contrary to the typically observed age-related visceral redistribution of WAT. Moreover, dwarf mice do not show the typical age-related accumulation of senescent cells in the major adipose tissue depots.[21] However, Ames dwarf mice treated with exogenous GH accumulate more senescent adipocytes, thus showing that GH action is directly responsible for cellular senescence in adipose tissue.[21] The mechanism(s) by which GH alters cellular senescence in these mice has not yet been elucidated.

Improved energy metabolism in dwarf mice

Ames dwarf mice and GHRKO mice have lower core body temperatures (T_{co}) than their normal littermates ($\sim$1.5°C and $\sim$0.4°C, respectively)[22,23] most likely due to the lack of GH action (as well as TH in Ames dwarf mice). Because of the lower T_{co} and lack of action of these metabolic hormones in dwarf mice, it was unexpected when Westbrook et al. demonstrated these mice have higher metabolic rates as measured by oxygen consumption (VO_2) and heat production per gram body weight through whole-body indirect calorimetry.[8] Moreover, these mutant mice have a reduced respiratory quotient (RQ), indicating preferential usage of lipids as an energy source over carbohydrates.[8] Interestingly, the short-lived bGH transgenic mice trend in the opposite direction for all 3 previously listed indirect calorimetry output parameters.[8] This led our laboratory to hypothesize that the metabolic phenotype of dwarf mice may be a "biomarker" of longevity. Along with an increased metabolic rate, GHRKO mice have increased relative BAT weight and BAT-specific mRNA and protein levels of uncoupling protein 1 (UCP-1),[24] which is responsible for uncoupling the electron transport chain to produce heat. Since Ames dwarf mice have an altered WAT phenotype, differences in energy metabolism and T_{co}, and since studies have shown that GHRKO mice have an increase in BAT UCP-1, we hypothesized that BAT in Ames dwarf mice may vary from that of their normal littermates. The findings supporting this hypothesis are described in detail below.

Altered gene expression and morphology in Ames dwarf mouse brown adipose tissue

In our study,[12] we reported that Ames dwarf mice have an increase in relative BAT weight, along with an increase in both thermogenic and lipid metabolism genes. Importantly, we found that Ames dwarf mice have increased UCP-1 mRNA expression. Moreover, transcriptional coactivators of UCP-1, peroxisome proliferator-activated receptor gamma (PPARγ) and PGC-1α, were also increased. PPARγ and PGC-1α also have additional functions in adipose tissue, including adipocyte differentiation and mitochondrial biogenesis, respectively. Type II iodothyronine deiodinase (DIO2) mRNA was also increased, which has previously been reported in hypothyroid rats.[25] DIO2 is responsible for converting thyroxine (T_4) to the bioactive form of triiodothyronine (T_3), which potentiates the effects of norepinephrine (norepinephrine regulates many aspects of BAT-thermogenesis).[11] Further, β_3 adrenergic receptor (ADRβ3, norepinephrine receptor) mRNA was also increased. Lastly, genes involved in the uptake and breakdown, as well as the de novo synthesis of triglycerides, were also upregulated, including Acetyl-CoA carboxylase (ACC1), fatty acid synthase (FAS), hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL).

The most striking difference in BAT between Ames dwarf mice and their normal littermates was the morphological alterations observed through H&E staining. The normal littermates exhibited typical BAT cross sections, with fibrous tissue surrounding small lipid vacuoles and nuclei. However, in dwarf mice, the lipid vacuoles were mostly depleted, and more nuclei per field were visible. This phenotype is typically apparent in mammals exposed to lower ambient temperatures.[26] Taken together, we hypothesize that the increased heat loss in these diminutive animals leads to a decrease in T_{co} and upregulation of thermogenic genes, as well as depletion of local lipid stores in BAT. This may also explain the unexpected increase in VO_2 observed in these mice. Unfortunately, the relationship between body size, lack of metabolic hormones, and lower T_{co} is complex, and further studies are needed to fully understand the relationship between these parameters and BAT function. For example, whether Ames dwarf mice are hypothermic or anapyrexic has yet to be determined. This is because lack of TH action typically leads to a lower "set-point" in T_{co} (anapyrexia);[27] however, an anapyrexic mammal would not have an increase in heat production, which is the case in Ames dwarf mice. Further complicating our understanding of dwarf thermoregulation is the small stature of these mice, which increases their surface area to body mass ratio, thereby increasing their heat production as a separate phenomenon from their lower T_{co}. Studies in thermoneutrality (discussed later in the commentary) or with hormone replacement therapy may provide insight into the specific mechanism(s) altering Ames dwarf BAT.

Brown adipose tissue and metabolism in Ames dwarf mice

To further understand how BAT in Ames dwarf mice impacts their metabolism, we surgically removed the interscapular BAT (iBAT) depot from both Ames dwarf mice and their normal littermates. As expected, surgical removal of the iBAT depot resulted in a decreased T_{co} in both normal and dwarf mice. Interestingly, this decrease was larger in dwarf mice. We did not see any significant impact on glucose tolerance or insulin sensitivity (as measured by a glucose and an insulin tolerance test, respectively) following iBAT removal, which was unexpected since BAT has been suggested to play a role in glucose homeostasis.[28] Even more unexpected was the finding that iBAT removal did not significantly impact VO_2 or heat production in normal mice, though a slight numerical trend toward impairment (i.e. decrease) was visible. We did, however, see a significant impairment in VO_2 and heat production in dwarf mice following iBAT removal, indicating BAT may play a larger role in their whole-body energy metabolism than in their normal littermates. Further, iBAT removal did not impact overall sympathetic outflow in normal or dwarf mice as measured by a norepinephrine challenge, where anesthetized mice are injected with a bolus of norepinephrine, and their VO_2 and heat production are measured by indirect calorimetry. It is worth noting that we did observe increased sympathetic outflow in dwarf mice compared with their normal siblings, which is not surprising considering the increase in BAT thermogenic genes, particularly ADRβ3.

Brown adipose tissue removal in Ames dwarf mice results in decreased weight of white adipose tissue depots

Indirect calorimetry measurements revealed opposite changes in RQ in Ames dwarf mice and their normal littermates following iBAT removal. Normal mice had an increased RQ over their "sham" controls (indicative of burning less fat), while dwarf mice had a decreased RQ over their "sham" controls (indicative of burning more fat). We believe the observation in normal mice was expected because a major fat burning tissue was removed. However, we were initially perplexed by the decreased RQ observed in dwarf mice. Supporting the decreased RQ in dwarf mice, we observed a decrease in relative weight of

the major WAT depots (epididymal, perirenal and subcutaneous), which was accompanied by a decrease in adipocyte size. Visually, there were signs of beiging in these depots, although more studies are needed to definitively support this statement. In normal mice, we observed opposing alterations (increased relative adipose tissue weight and adipocyte size), which supports the observed increase in RQ. We believe the unique physiologic response seen in dwarf mice may be because the already low T_{co} in dwarf mice is at a critical threshold, and when their normal thermogenic mechanisms are compromised by iBAT removal, dwarf mice will begin to burn any fat available to maintain a viable body temperature. It is worth mentioning that possible alterations in shivering thermogenesis in muscle between normal and dwarf mice might have played a role in the opposite physiologic responses following iBAT removal, however, we have not yet conducted studies to examine this effect.

Concluding remarks

Our recent report in *Endocrinology* demonstrated that Ames dwarf mice have functionally enhanced BAT. This was illustrated through altered BAT gene expression, altered BAT morphology and increased sympathetic outflow. Moreover, iBAT removal in these mice resulted in a unique physiologic response where Ames dwarf mice appear to utilize lipids more readily as an energy source. We believe this is due to a critical drop in their T_{co}, following the loss of a major thermogenic tissue. Our work aligns with the narrative that diminished somatotropic signaling leads to functionally enhanced BAT, which has been demonstrated in GHRKO mice.[24] These mice have increased relative BAT weight, increased BAT UCP-1 mRNA and protein levels, and a shift in their BAT-specific transcriptome toward increased cellular metabolism.[29] To date, however, thermogenic-specific genes and sympathetic outflow have not been analyzed in these mice, nor has the effects of BAT removal. In our study, we also demonstrated that growth hormone releasing hormone knockout (GHRHKO) mice have an increase in BAT UCP-1 mRNA expression; however, other thermogenic genes were not altered, as was observed in Ames dwarf mice. Since GHRKO mice are essentially euthyroid and do not have a T_{co} that is as drastically altered as Ames dwarf mice,[23] further studies in GHRKO mice are needed to delineate the specific roles of body temperature and TH action in regulating BAT phenotypes. Similar studies in GHRHKO mice would be of interest.

Collectively, our study raises several interesting questions, namely, is the increased BAT function of Ames dwarf mice influencing their longevity, and are the findings in these mutants due mostly to the major drop in their T_{co}. Data from our laboratory, where Ames dwarf mice are housed in thermoneutral conditions (30°C), and also at standard housing conditions (23°C), indicate that ambient temperature is a determining factor in their energy metabolism. We have shown that the differences in VO_2 and RQ between Ames dwarf mice and their normal littermates are diminished at thermoneutrality.[30] Since our laboratory considers elevated VO_2 and reduced RQ as possible biomarkers of longevity, we suspect that significant alterations in longevity in Ames dwarf mice housed under thermoneutral conditions should be observed. Our laboratory has already started preliminary longevity studies at thermoneutrality to test this hypothesis. We also have plans to further study in Ames dwarf mice involving thermoneutral housing to elucidate the role of ambient temperature in their enhanced BAT.

Traditionally, anti-obesity research has focused on decreasing caloric intake, while increasing energy expenditure has not been a major focus because of the difficulty in maintaining exercise regimens, and the belief that humans possess BAT only in infancy (i.e., after infancy, BAT is not present). However, with the discovery of BAT in adult humans,[31] increasing BAT activity as a means to increase energy expenditure has become a new target for combating obesity.[32] Most research in this area has focused on pharmacological modulation of BAT function. Our research indicates that alterations in ambient temperature, and other factors affecting heat loss, may be sufficient to increase BAT activity.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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