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Translational Article

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Translational

# GH and IGF1: Roles in Energy Metabolism of Long-Living GH Mutant Mice

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Of the multiple theories to explain exceptional longevity, the most robust of these has centered on the reduction of three anabolic protein hormones, growth hormone (GH), insulin-like growth factor, and insulin. GH mutant mice live 50% longer and exhibit significant differences in several aspects of energy metabolism as compared with wild-type mice. Mitochondrial metabolism is upregulated in the absence of GH, whereas in GH transgenic mice and dwarf mice treated with GH, multiple aspects of these pathways are suppressed. Core body temperature is markedly lower in dwarf mice, yet whole-body metabolism, as measured by indirect calorimetry, is surprisingly higher in Ames dwarf and Ghr–/– mice compared with normal controls. Elevated adiponectin, a key antiinflammatory cytokine, is also very likely to contribute to longevity in these mice. Thus, several important components related to energy metabolism are altered in GH mutant mice, and these differences are likely critical in aging processes and life-span extension.

**Key Words:** Hormones—Aging—Mitochondria—Body temperature—Inflammation.

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MANY theories have been presented over the last several decades to explain exceptional longevity in animals and humans. Perhaps, the most robust of these to date focuses on three interrelated endocrine systems: the growth hormone (GH), insulin and its hormone homologs, and insulin-like growth factor (IGF) pathways. As far down the evolutionary ladder as yeast, there is strong evidence that a carbohydrate regulatory system exists and that if perturbed, life span extension is observed. As one moves up the ladder to nematodes and fruit flies, both insulin and IGF become important in the maintenance of metabolism. Similar to yeast, if ligand or receptor expression or signaling factors in these pathways are disrupted (ie, nematode *daf-2*; Drosophila *chico*), an extension of life span is observed. In the mammalian system, the endocrine system becomes more complex with the addition of GH, a hormone that controls circulating IGF1 levels and thus has somatic actions yet also exhibits key metabolic functions that are independent of IGF1. Disturbing the GH pathway either by severely reducing plasma levels or by receptor disruption significantly extends health span and life span in mice.

## Key Characteristics of Long-Lived GH-Related Mutants

The Ames dwarf, Snell dwarf, and GH receptor knockout (Ghr–/–) mice are the longest living mouse mutants discovered to date (1–3). The Ames and Snell dwarfs are phenotypically identical with similar hormone deficits caused by loss-of-function mutations that affect proper differentiation of the same pituitary cell types. Mutations in Prop-1 and Pit-1 result in deficient circulating GH, prolactin, and thyrotropin in Ames and Snell mice, respectively (4,5). As a consequence of the lack of plasma GH stimulation, plasma IGF1 levels are barely discernable in these mice (6). On average, Ames mice live 49%–69% longer (males and females, respectively), whereas Snell dwarfs live nearly 50% longer than their normal counterparts (1,2,7). The differences in life span between these dwarf mice result from differences in genetic background rather than the pituitary deficiencies. The other long-living GH mutant, the Ghr–/– mouse, was generated by targeted disruption of the GH receptor and GH-binding protein (8). These mice live up to 46% longer than wild-type siblings and also exhibit undetectable circulating levels of IGF1 owing to dysfunctional GH

receptors (no liver IGF1 stimulus; (3)). The current record for the longest-lived Ghr–/– mouse is 1,819 days (Bartke, personal communication 2003). Hence, the major physiological difference between these long-living mice is the presence (Ames and Snell) and absence (Ghr–/–) of an intact GH signaling system. This difference, in turn, results in some heterogeneity in downstream targeting of GH and IGF1 between these mice (comprehensive review in (9)).

Several aspects of GH and IGF status as they relate to energy metabolism have been explored in GH mutant mice and include insulin signaling, adipose tissue metabolism/inflammation, body temperature, and mitochondrial and oxidative pathways. The mitochondria play a central role in energy metabolism through oxidative phosphorylation and ATP synthesis, apoptosis, and in the generation of free radicals (produced as byproducts). These reactive oxygen species induce oxidative stress but have also been shown to regulate cellular signaling and integrate energy state, stress signaling, and survival (10). The oxidative damage resulting from reactive oxygen species and incurred by mitochondria and other cellular components results in disturbed energy budgets at the cellular and tissue levels and likely contributes to the aging phenotype (11–13). Moreover, it has been shown that defects in the electron transport chain (ETC) contribute to the etiology of several disease states (reviewed in (14)). The mitochondria thus drive energy metabolism in the cells and tissues and likely contribute to cellular aging.

### Mitochondrial Function

Examination of individual oxidative phosphorylation (OXPHOS) components has the potential to uncover areas of altered function that may result from differences in circulating levels of hormones and contribute to longevity differences between mutant and wild-type mice. Complex I of the OXPHOS system is the largest of the multimeric ETC proteins and contributes significantly to the generation of free radicals, respiration rate, and overall control of the ETC in mammalian species (15,16). Inhibition of complex I (as little as 25%) can profoundly impact energy metabolism and contribute to less efficient energy production in aging (17–19). Many reports documented significant declines in complex I activity with aging (20–23). In GH-deficient Ames mice, several alterations in OXPHOS complexes have been observed. Increased complex I activity and protein levels have been demonstrated in liver tissues from healthy old dwarf mice in comparison to age-matched wild-type mice (20 months of age; (24)). In addition, greater declines in activity with age were observed in GH-sufficient wild-type mice versus Ames mice in liver, skeletal muscle, and kidney tissues (24). The liver tissue is a key player in metabolism, as it orchestrates the supply of energy substrates to other tissues. Thus, increased liver complex I activity and protein in old dwarf mice suggest that mitochondrial function is better preserved in these long-living dwarf mice at old ages.

Considering that complex I governs overall ETC function, then the differences observed in complex I in the dwarf mice may underlie the elevated levels of other downstream enzymes in the OXPHOS system (III, IV, and V) of these mice. However, ATP synthase (complex V) is not coupled to ETC processes, so the higher level of complex V in dwarf tissues is not a result of a generalized upregulation of OXPHOS proteins. Instead, it may be indicative of ample energy availability versus energy deficits observed in diseases with decreased ATP synthase protein levels and/or mutations in genes encoding components of the ATP synthase complex (25,26). Ames mouse tissues also exhibited elevated levels of the adenine nucleotide translocator (messenger RNA or protein), a protein involved in both the transport of ATP and the maintenance of the mitochondrial permeability transition pore complex (and thus, apoptosis). If complex I is the major controller of overall ETC function as reported (21) and a decline in activity contributes to decreased energy production and aging processes, then Ames mice exhibit an advantage in many tissues over wild-type mice. This advantage may contribute to the delayed aging phenotype enjoyed by Ames mice (24). A major regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1-alpha, has also been shown to be upregulated in tissues of Ames mice (and Ghr–/– mice; (24,27–29)). However, an increase in mitochondrial numbers is not evident as indicated by similar mitochondrial DNA:nuclear DNA ratios between dwarf and wild-type mice (24). Therefore, this nuclear hormone activation likely contributes to other aspects of metabolism including antioxidant defense, insulin sensitivity, and β-oxidation.

It has been reported that overall mitochondrial protein synthesis decreases by middle age in humans (30), a finding associated with decreasing plasma GH concentrations (31). Factors that regulate the synthesis and activities of the mitochondrial ETC complexes, however, are largely unknown (32). Cellular metabolism is stimulated by anabolic hormones, increasing metabolic activity (oxygen consumption and glucose oxidation), oxidative phosphorylation, and reactive oxygen species production. However, neither GH nor IGF1 have been shown to directly modulate the expression or activities of the OXPHOS proteins. Thus, the prediction that anabolic hormones stimulate mitochondrial function via oxidative phosphorylation activities is tenuous. To examine this assertion directly, we treated Ames mice with GH (25 μg porcine GH/injection, subcutaneous; two times daily; (33)) for 7 days, a treatment regimen has been shown to significantly increase circulating levels of IGF1 (6,33). Body and liver weights increased in response to GH injection ($p < .0001$; Figure 1A and B), indicative of IGF1 action. We observed a significant suppression in liver complex I protein (12-month-old GH-treated dwarf mice $-1.989 \pm 0.39$ versus saline-treated dwarf mice $4.034 \pm 0.79$ relative optical density units; $n = 6$ per treatment; $p = .0408$) suggesting that GH influences OXPHOS and thus mitochondrial metabolism. Although the electron flow through

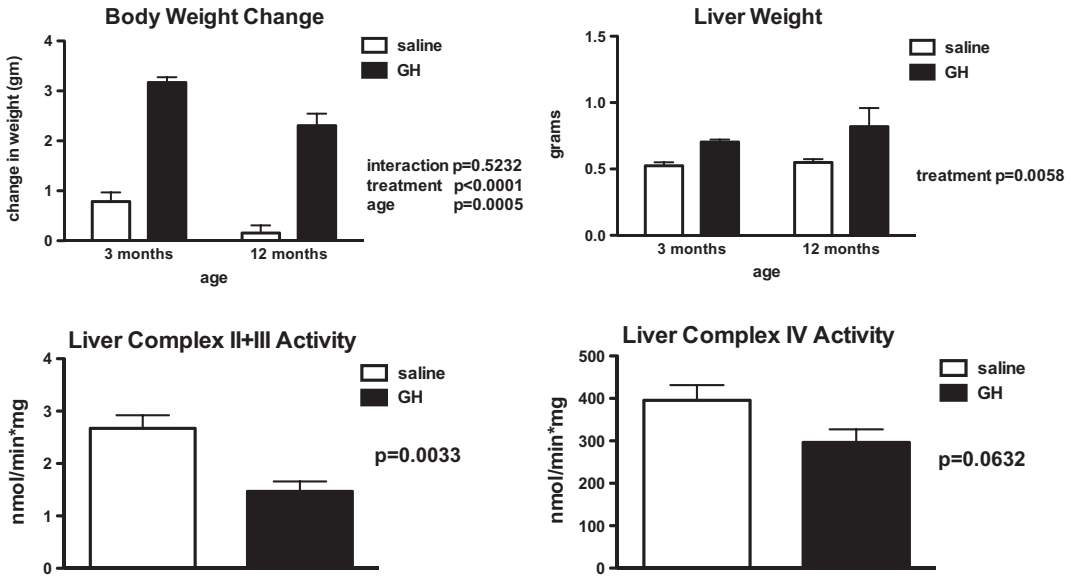


Figure 1. Body and liver weights and complex enzyme activities in liver tissue from Ames dwarf mice following a short-term 7-day treatment with growth hormone (GH; 25 µg porcine GH per injection × two injections per day for 7 days). Top left: Body weight change (g); top right: Liver weights (g); bottom left: Liver complex II + III activity (ng/min × mg protein) in 12-month-old Ames mice; and bottom right: Liver complex IV activity (nmol/min × mg protein) in 12-month-old Ames mice. Details of enzyme assays described in (24). Values represent means ± *SEM*, $n$ = 7–8 mice per treatment.

complexes I and III (I + III activity) was not altered significantly by GH treatment in 12-month-old dwarf mice, electron flow through complexes II + III (II + III activity) was suppressed 45% in comparison to dwarf mice treated with saline ($p < .01$; Figure 1C). GH treatment also decreased the activity of cytochrome c oxidase (complex IV) by 25% when compared with the saline-treated mice ($p = .06$; Figure 1D). No differences in complex protein levels were observed in 3-month-old dwarf mice similarly treated with GH. Furthermore, levels of gene expression for complexes II, III, and V as well as protein levels of complexes I, II, and V are markedly decreased in mice with high circulating levels of GH (GH transgenic mice; Figures 2 and 3) and shortened life spans. Taken together, these data are compelling and suggest that the metabolic actions of GH decrease the expression and activities of OXPHOS components. There is also the possibility that GH's actions are secondary to increased insulin (as insulin promotes mitochondrial activity) in these mice. Nevertheless, Ames mice exhibit negligible levels of plasma GH and IGF1 and reduced levels of insulin, and it is this lack of anabolic activity that may result in the upregulation in OXPHOS and mitochondrial metabolism, potential underlying factors in their long life.

Extremely low thyroid hormone concentrations in Ames dwarf mice also preclude the assertion that enhanced metabolic activity may be responsible for the elevated OXPHOS output in older dwarf mice. We found that treatment of Ames mice with thyroxine (2 µg in 0.9% saline) every other day for 1 week did not affect liver gene expression of the OXPHOS complexes but did reduce the activity of liver complex II + III by 43% ($p < .0001$; Figure 4). In contrast to Ames mice, hypothyroid rats exhibit reduced complex V activity compared with euthyroid animals and short-term 3,5-l-diiodothyronine injection enhanced this activity (34). As additional information, Panici and coworkers (35) reported that short-term (6-week) T4 administration to Ames mice had no affect on longevity. Although lifelong treatment of Snell dwarf mice with thyroxine reduced their life span (36).

### Antioxidative Defenses and Oxidative Damage

Oxidative metabolism is closely linked to mitochondrial metabolism as this system counters the metabolic by-products (free radicals) produced during the oxidative phosphorylation process. Moreover, oxidative stress underlies mitochondrial dysfunction and impaired energetics. Several lines of evidence demonstrate that GH plays a role in antioxidative defense. The high plasma GH and IGF1 concentrations found in short-living GH transgenic mice are strongly associated with increased superoxide radicals, increased oxidative damage, and significantly suppressed tissue antioxidative enzyme levels (MnSOD, CuZnSOD, catalase, and GPX; (37–40)). Both direct and specific effects of GH and IGF1 in vitro (primary hepatocytes) support the in vivo evidence demonstrating that these two hormones downregulate the expression of antioxidative enzymes (41). Therefore, the significant downregulation of oxidative defense capacity and the multiple indices of physiological decline associated with premature aging (early reproductive senescence, glomerulonephritis, glomerulosclerosis, early onset, and increased incidence of tumors, etc) likely lead to the reported 50% reduction in life span in animals with pharmacological levels of plasma GH (42). In comparison, IGF1 transgenic mice do not experience this severe renal pathology suggesting that GH is the main culprit (43).

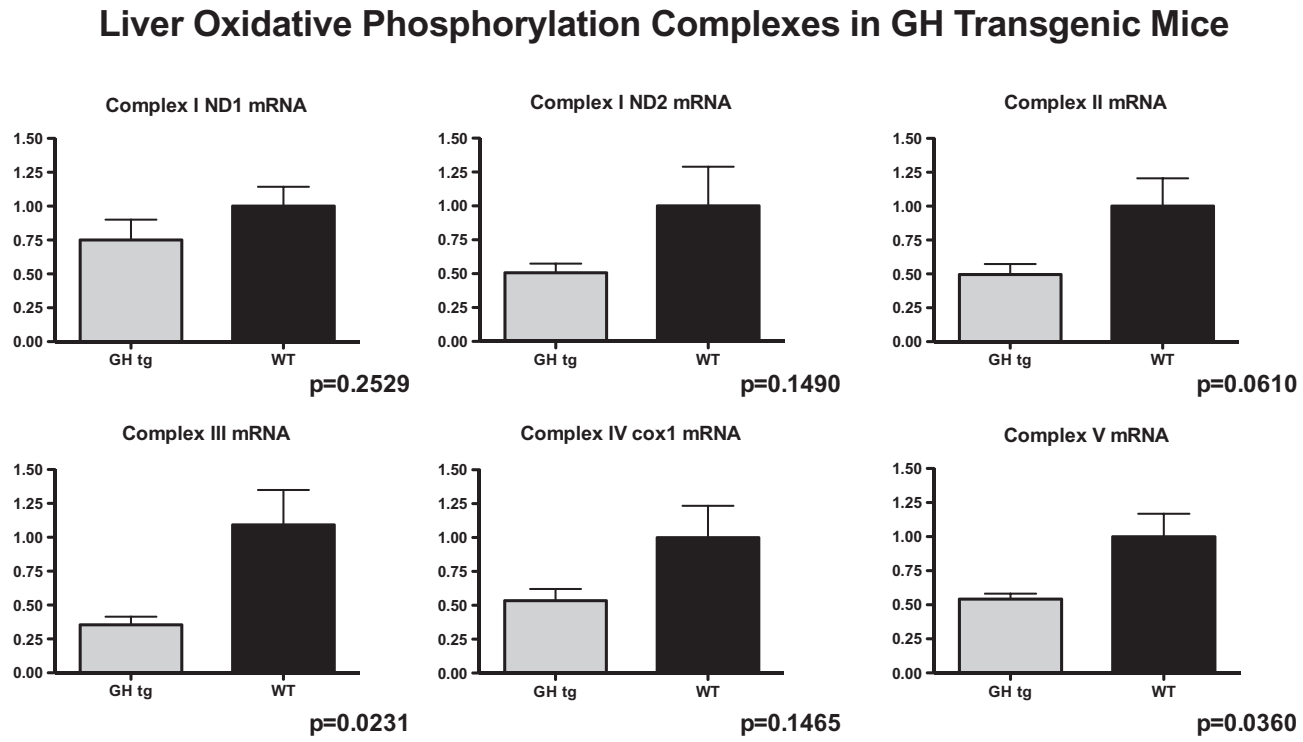


Figure 2. Gene expression of complex enzymes in 3-month-old male liver tissue from GH transgenic and wild-type control mice. Primer pairs and real-time reverse transcription-PCR conditions described in (24). Values represent means ± *SEM*, *n* = 6–7 mice per genotype.

In striking contrast, GH deficiency in dwarf mice results in significantly enhanced antioxidative defense capacity. Ames mice exhibit elevated catalase, SOD, and GPX in multiple tissues (activities, protein and/or messenger RNA; (38–40,44)). GH administration to dwarf mice suppresses these same enzymes when compared with saline-injected

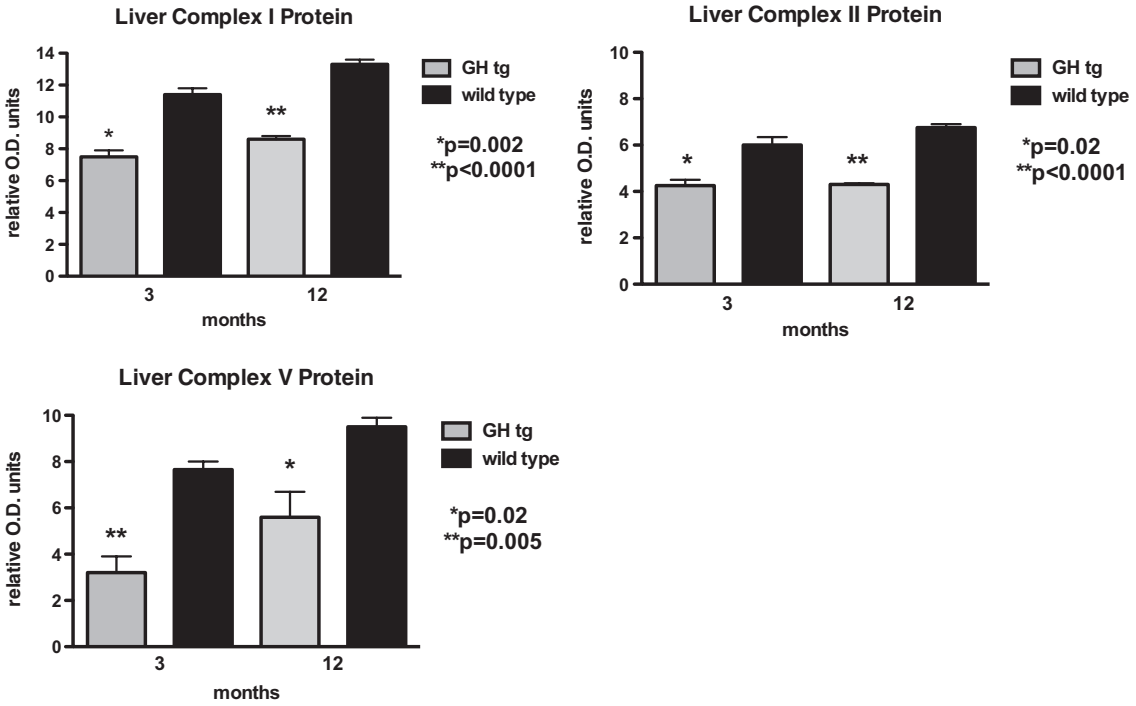


Figure 3. Liver protein levels of complexes I, II, and V in 3- and 12-month-old male tissue from growth hormone transgenic and wild-type control mice. Protein extraction and immunoblotting assays and antibodies are described in (24). Values represent means ± *SEM*, *n* = 7–8 mice per age per genotype.

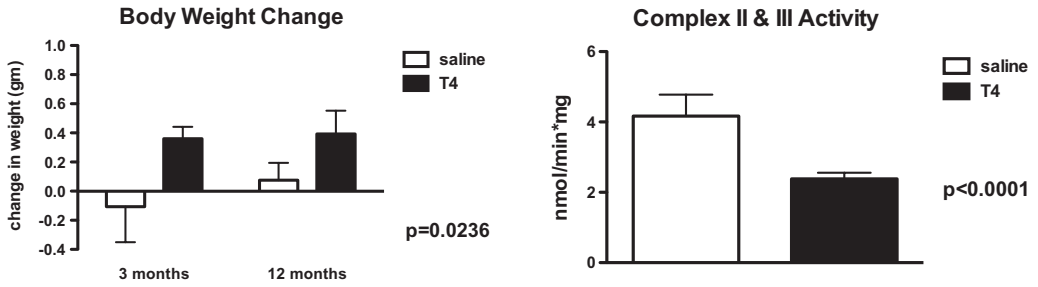


Figure 4. Body weight change (g) and liver complex II + III activity (nmol/min × mg) in Ames dwarf mice following short-term treatment with thyroxine or saline. Details of complex II + III activity assay described in (24). Values represent means ± *SEM*, $n$ = 6–8 mice per treatment.

dwarf mice (33). Nonenzymatic antioxidative defense mechanisms such as glutathione and metallothionein are also elevated in dwarf mice. The lack of anabolic stimulation of mitochondrial metabolism and the elevated oxidative defense mechanisms in these mice result in lower liver hydrogen peroxide generation and lower oxidative damage to nuclear and mitochondrial DNA, proteins, and lipids in several tissues (45–48).

Mitochondria play an important role in metabolic rate and energy metabolism as oxidative phosphorylation is responsible for the majority of whole-animal oxygen consumption and in the control of cellular respiration (49). Mitochondrial metabolism is suppressed in many mammals (including mice) during fasting and daily torpor and is mechanistically linked to reductions in reactive oxygen species production and body temperature (50).

### Body Temperature

Hunter and his colleagues (51) reported in 1999 that core body temperature (*T*co) was significantly lower in Ames dwarf mice than in normal animals from the same strain. In this study, *T*co was continuously monitored in singly housed animals at an ambient temperature of 26°C using intraperitoneally placed transmitters and telemetric recording. The difference in *T*co between normal and dwarf animals was large, approximately 1.6°C. In addition, some dwarf animals in this study exhibited periods of hypothermia with *T*co dropping to values near or below 33°C for periods of up to 6 hours (51). Subsequently, we reported that *T*co in Ghr–/– animals was also reduced, although the reduction was much smaller than in Ames dwarf mice (approximately 0.4°C) and statistically significant only at some phases of the 24-hour *T*co rhythm (52). Major reduction of *T*co in Ames dwarfs and a more modest effect in Ghr–/– can be readily related to the reduced action of calorigenic hormones, thyroxine, GH, and insulin in these mutants: Ames dwarfs are severely hypothyroid due to thyroid stimulating hormone deficiency and do not produce GH; Ghr–/– mice are GH resistant and both are hypoinsulinemic.

We propose that reduced *T*co contributes to extended longevity of Ames dwarf and Ghr–/– mice. In an elegant study of Conti and colleagues (53), reducing body temperature of mice by hypothalamic overexpression of uncoupling protein 2 led to a significant increase of life span. Association of reduced body temperature with increased longevity was also reported in the human (54). Forced restriction of food intake (calorie restriction, CR) leads to reductions in the levels of thyroid hormones and in body temperature. In mice, CR can induce periods of torpor with associated hypothermia (55,56). Additionally, an enlargement of interscapular brown adipose tissue was observed in Ghr–/– mice over that of wild-type mice as well as elevated levels of uncoupling protein-1 in this tissue (57).

### Oxygen Consumption and Respiratory Quotient

Although lower *T*co would seem to imply a downward shift in energy and metabolism, studies by indirect calorimetry revealed that oxygen consumption per unit of body mass is significantly increased in both Ames dwarf and Ghr–/– mice in comparison to the corresponding normal controls (58). This finding was not expected. Animals subjected to CR show an initial decrease in metabolic rate but after a period of adaptation and weight loss, consume the same amount of oxygen per unit of lean body mass as controls with unlimited access to food (59). Reporting results of studies of energy metabolism in terms of lean body mass is supported by evidence that metabolic rate scales more closely with this value (generally calculated as body weight$^{0.67}$ or body weight $^{0.75}$) rather than with body weight. However, the differences between findings in long-lived mice with GH-related mutations and in genetically normal animals subjected to CR are not a simple reflection of different ways of presenting the results because the adjustments to "lean body mass" are based on the assumption that smaller animals are leaner, whereas adiposity (body fat as percent of body weight) in long-living dwarf mice is either significantly increased or near normal (60,61). The interpretation of findings from studies of energy metabolism in mice and common laboratory rodents is further complicated by the fact that basal or resting metabolic rates in these animals are difficult to measure, and the average metabolic rate that can be fairly precisely determined over periods of 12 or 24 hours includes energy used for thermogenesis and maintaining body temperature. In mice, thermoneutral environmental temperature is approximately 30°C (86°F) (62),

and thus, standard housing conditions (usually about 22°C) represent a thermal stress. For dwarf mice, which are much smaller than normal mice and thus have higher body surface to body mass ratio, the loss of heat by radiation can be assumed to be greater (in spite of increased or normal insulation by subcutaneous fat), and thus, the energy demand for thermogenesis is presumably further increased at "room temperature."

An increase in oxygen consumption per gram body weight in dwarf mice was associated with a significant reduction in respiratory quotient (RQ; calculated as a ratio of carbon dioxide output to oxygen consumption). RQ provides an estimate of the use of metabolic fuels to generate energy with an RQ of 1.0 representing exclusive reliance on carbohydrates and RQ of 0.7 representing reliance on fats. Increased reliance on "fat burning" or, more precisely, β-oxidation of fatty acids for satisfying energy needs is a normal response to reduced availability of nutrients during fast or long between-meal intervals (such as sleep in the human) and has been associated with improved metabolic homeostasis and extended longevity in a variety of situations (63–65). A shift from carbohydrate to fatty acid utilization in response to CR is believed to represent an important metabolic adaptation of mitochondrial function and one of the key mechanisms of extended longevity (66).

Recent findings in our laboratory indicate that differences in oxygen consumption ($VO_2$) and RQ between normal mice and long-lived mutants can be eliminated or greatly reduced by several days of exposure to thermoneutral temperatures. This raises an intriguing possibility that the metabolic characteristics of GH-related mouse mutants detected by indirect calorimetry at ambient temperatures of 22°C (increased $VO_2$/g and reduced RQ) represent an exaggerated response of these diminutive animals to cold stress and that the resulting phenotype is one of the mechanisms of their extended longevity. In indirect support of this hypothesis, Koizumi and his colleagues (56) reported that maintaining CR mice at a thermoneutral temperature eliminates some of the beneficial effects of CR. This included a significantly shorter survival of calorically restricted C57BL/6 mice at 30°C versus 20–22°C. The authors ascribed these findings to elimination of torpor, which was a daily occurrence under the employed CR protocol (56).

## Adipose Tissue, Adipokines, and Inflammation

The loss of GH's somatic effects, the majority of which are due to IGF1, results in animals of small size but extended longevity. However, as mentioned previously, GH has direct metabolic effects that are IGF1-independent, affecting lipids via lipolysis, lipid oxidation and lipid mobilization, and further regulating body composition (67,68). In addition, levels of adipokines, factors produced by adipocytes, are affected by fat mass and composition and have been shown to affect diverse processes such as energy expenditure, carbohydrate and lipid metabolism, and inflammation (69). Circulating levels of adiponectin, an important antiinflammatory adipokine, are elevated (70,71) in long-lived GH-related mouse mutants, whereas the expression of proinflammatory cytokines, interleukin 6 and tumor necrosis factor alpha, are reduced. Increased adiponectin secretion undoubtedly reflects the absence of negative control of its secretion by GH, along with differences in the size and distribution (72) of adipocytes.

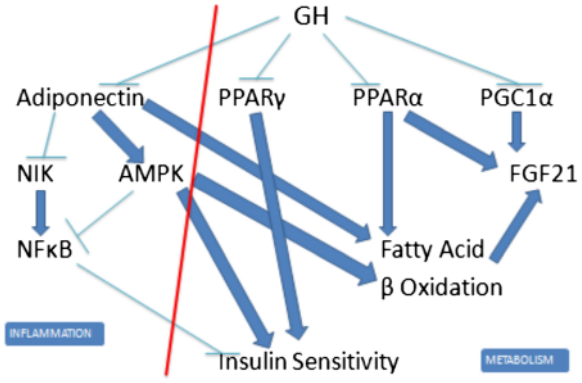


Figure 5. Interactions of pathways mediating effects of growth hormone (GH) on metabolism and inflammation. GH inhibits adiponectin, peroxisome proliferator-activated receptor γ, PPARα, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha expression and thus promotes inflammation, insulin resistance, and reduced oxidation of fatty acids. Suppression of GH signaling in long-lived mutants removes these inhibitory effects and thus reduces inflammation and promotes insulin sensitivity and fatty acid oxidation. (This diagram is greatly simplified and is not intended to present all pathways and mediators involved).

We believe that extended longevity of Ames dwarf and Ghr–/– mice is causally related to phenotypic characteristics induced by elevated adiponectin levels (Figure 5). Adiponectin reduces proinflammatory nuclear factor kappa-light chain-enhancer of activated B cells signaling by reducing expression of NFκB-inducing kinase, an upstream regulator of nuclear factor kappa-light chain-enhancer of activated B cells, and by adenosine monophosphate–activated protein kinase-mediated inhibition of this pathway. Adiponectin activates adenosine monophosphate–activated protein kinase, promotes β-oxidation of fatty acids, and enhances insulin sensitivity. Reduced nuclear factor kappa-light chain-enhancer of activated B cells signaling may contribute to improved responses to insulin, although inflammation and insulin resistance have been dissociated in transgenic mice (73). We have recently shown that removing most of the epididymal and perinephric adipose tissue from adult Ghr–/– mice reduces adiponectin levels, increases RQ, and promotes insulin resistance, thus leading to normalization of the phenotypic characteristic believed to be causally linked to longevity (74). Metabolic characteristics of transgenic mice overexpressing adiponectin are remarkably similar to those of Ames dwarf and Ghr–/– mice, except for the percentage of body fat that is reduced in the transgenics (75,76). Transgenic mice overexpressing human adiponectin in the liver have an increased life span (77).

In the human, there is considerable evidence for antiinflammatory and antiatherogenic effects of adiponectin

(78–80). Circulating adiponectin levels are increased in centenarians and in offspring of exceptionally long-lived individuals (81–83). Laron dwarfs, which share GH resistance and many of the resulting phenotypic characteristics with Ghr–/– mice, have elevated adiponectin levels (84) and are remarkably protected from age-related disease, including type 2 diabetes and cancer (85,86). It should be pointed out that the average longevity of Laron dwarfs does not differ from that of the normal individuals from the same population, but this may reflect the increased incidence of deaths resulting from accidents and alcohol-related causes in individuals affected by this syndrome (85).

Finally, there is considerable evidence that low-grade inflammation, other inflammatory processes, as well as infections and the resulting "inflammatory load" can profoundly influence longevity and health span in mammals (87,88). GH was reported to exert antiinflammatory effects in experimentally induced sepsis by promoting secretion of interleukin 10 and reducing the levels of tumor necrosis factor alpha (89,90). Moreover, the levels of an important marker of inflammation, C reactive protein, in GH-deficient participants were reduced by GH therapy (91). In contrast to these observations, inflammatory processes and levels of proinflammatory cytokines are increased in transgenic mice overexpressing GH (92,93). Because circulating levels of GH in these animals are extremely high, differences between the results obtained by GH injections and by overexpression of the GH gene may reflect a nonlinear likely biphasic dose–response relationship between GH levels and inflammation. In clinical studies, biphasic inverted U dose response was reported for the effects of GH on cardiac function (94) and for relationship of circulating IGF1 levels to all cause mortality (95).

## Conclusion

Considering the components of energy metabolism presented (mitochondrial and oxidative metabolism, body temperature, RQ, and adiponectin), the conclusion that in the long-lived mutants, GH deficiency throughout life is beneficial in terms of health span and life span is difficult to debate. In addition, a very recent publication demonstrated that male IGF1 receptor heterozygous mice do not live longer than wild-type mice (nor do they exhibit differences in end-of-life pathology), and the extension of longevity in females was very modest (less than 5%; (96)). These investigators conclude that a reduction in circulating IGF1 levels in CR and dwarf mice plays little if any role in delayed aging and longevity. This evidence supports our own work in dwarf mice that strongly proposes that reduced GH rather than the secondary reduction of IGF1 is the key to longevity in mammalian systems. Further study is warranted regarding the apparent importance of timing of hormonal perturbations (life long vs neonatally or adult only; (35)) and consequent effects on energy metabolism as they relate to aging processes and longevity.

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