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Original Article

Life-span Extension With Reduced Somatotrophic Signaling: Moderation of Aging Effect by Signal Type, Sex, and Experimental Cohort

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Abstract

Reduced somatotrophic signaling through the growth hormone (GH) and insulin-like growth factor pathways (IGF1) can delay aging, although the degree of life-extension varies markedly across studies. By collating data from previous studies and using meta-analysis, we tested whether factors including sex, hormonal manipulation, body weight change and control baseline mortality quantitatively predict relative life-extension. Manipulations of GH signaling (including pituitary and direct GH deficiencies) generate significantly greater extension in median life span than IGF1 manipulations (including IGF1 production, reception, and bioactivity), producing a consistent shift in mortality risk of mutant mice. Reduced Insulin receptor substrate (IRS) expression produces more similar life-extension to reduced GH, although effects are more heterogeneous and appear to influence the demography of mortality differently. Life-extension with reduced IGF1 signaling, but neither GH nor IRS signaling, increases life span significantly more in females than males, and in cohorts where control survival is short. Our results thus suggest that reduced GH signaling has physiological benefits to survival outside of its actions on circulating IGF1. In addition to these biological moderators, we found an overrepresentation of small sample sized studies that report large improvements in survival, indicating potential publication bias. We discuss how this could potentially confound current conclusions from published work, and how this warrants further study replication.

Keywords: IGF1—Growth hormone—Longevity—Mice

Extension of life span through mammalian genetic mutation was first demonstrated in Ames Dwarf mice (1). These animals have a pituitary deficiency, resulting in extremely low levels of growth hormone (GH), prolactin and thyroid stimulating hormone. This initial finding generated a strong interest in the role of the GH signaling pathway in aging, and downstream effects of reduced somatotrophic signaling, particularly changes in insulin and insulin-like growth factor I (IGF1) signaling. A possible link between aging and IGF1/insulin signaling in mammals was particularly exciting because related signals in invertebrates also influence aging (2), indicating evolutionarily conserved molecular mechanisms (3). Furthermore, polymorphisms in these pathways in humans have been associated with long life (4).

Manipulations that reduce GH signaling at a number of different levels have been shown to extend mouse life span. Snell Dwarf mice have a similar deficiency in pituitary development to Ames Dwarf mice and can live longer than controls in particular conditions (5). Targeted disruption of the GH receptor can also extend mouse life span (6), as can disruption of growth-hormone releasing hormone production (7). While interference in several aspects of GH signaling appear to consistently affect mouse life span, manipulations of insulin and IGF1 signaling have produced mixed life-span effects. The majority of circulating IGF1 is produced in the liver, and exerts its cellular effects through binding to the IGF1 receptor, although there is substantial cross-reactivity between IGF1 and insulin with each binding to the other's receptor, albeit at lower

affinities. Heterozygous deletion of the IGF1 receptor (IGF1R+/–) was initially shown to extend life span (8), but only significantly in female mice. However, later replication of this work by both the same group (9) and a different group (10) reported a much milder life-span phenotype (although still significant in females) using different genetic backgrounds. Mice with adult liver-specific inactivation of IGF1 production show some extension in life span (11), again, only significantly in females, as do animals with loss of pregnancy-associated plasma protein-A expression (12), a zinc metalloproteinase that enhances the bioactivity of IGF1. Genetic inhibition of insulin receptor substrate proteins (IRS I and IRS II), downstream of IGF-IR, can also extend life span: homozygous deletion of IRS I extends life span (13,14), although heterozygous deletion of IRS II was reported to extend life span in one study (15) but not another (13).

The inconsistency of life-span extension with these genetic models has been a subject of much discussion, with authors arguing that differences in macronutrient composition of diet, body weight, sex, health status, and survival profile of control groups could explain variation in life-span extension (9,10,16–20). However, most discussions have centered on qualitative comparisons between two single studies, limiting the ability to ascertain whether differences in results are a consequence of specific differences in study design. Life span responses to manipulations can differ considerably between laboratories even when the same strain and source of mice is used, and explicit effort has been made to match variables such as light cycle, housing temperature and environmental enrichment (21,22). Differences between laboratories in life-span responses with the same genetic manipulation might therefore simply be the result of unrelated laboratory specific variation and/or represent sampling error or measurement errors.

In an effort to better explain variation in life-span responses to GH-IGF1-IRS manipulation, we conducted a meta-analysis. We collated data from available published studies that examined life span in animals with genetic manipulations of these signaling pathways in comparison to unmanipulated controls. Our data collection protocol resulted in 42 different control-treatment survival comparisons, and cumulated survival data from 2,460 individuals. This allowed us to provide a quantitative test of whether life-span extension is greater with particular types of manipulation, and whether variation in responses is consistently related to previously suggested variables such as sex, survival of control groups, or relative changes in body weight. We were unable to look at effects of dietary composition on life-span extension in these models, due to inadequate information in some studies, and we urge authors to provide such information in future experiments. By collating data from different studies, we were also able to provide an insight into the demographic causes of life-span extension. It has been suggested that life-span extension with reduced GH signaling delays the onset of aging, but does not impact its rate once it starts (23). The problem is that quantifying such a change requires a large sample size, usually not possible with individual mouse studies. Our meta-analytic approach provides the power to access how life-span extension through altered GH-IGF1-IRS signaling arises demographically.

Results

Increased Survival With Reduced Somatotrophic Signaling

Across all studies reduced pituitary, GH, IGF1, and IRS signaling extended median life span by on average 157 days, 21.6%, with the overall meta-analytic hazard rate at median life span for mutant animals significantly lower than that of controls (the log hazard ratio, $\ln HR_{50} = -0.87$, 95% confidence interval, CI [–1.17, –0.57], $z = -5.65$, $p < .0001$). While this effect is substantial and significant, there is a high level of heterogeneity between studies ($Q_{41} = 78.6$, $p = .0004$, $I^2 = 56.0\%$). Part of this heterogeneity is explained by splitting animals according to the underlying signals/hormones that are manipulated (test of moderator: $Q_{M2} = 10.53$, $p = .005$, marginal R^2 or $R^2_{[m]} = 55.5\%$, which is heterogeneity accounted by the three signaling categories, *sensu* (24)). Animals with mutations that predominately alter GH signaling (also including other pituitary hormones in Ames and Snell Dwarf mice; see Table 1 for models included in different moderator groups) show greater relative reductions in mortality (Figure 1A) when compared to the reduction in mortality generated by mutations that interfere with either IGF1 or IRS. Notably, the degree of heterogeneity in response to manipulation further differed among the three subgroups, with GH/pituitary manipulations also showing the least heterogeneity in life-span response, while IRS manipulations show the highest (GH: 5.6%, IGF: 34.1%; IRS: 47.2%; Figure 1A). We note here that data from IRS manipulations is derived from only three different studies. The substantial heterogeneity observed within this category might partly result from a lack of concordance between two studies for one particular mouse model (IRS2+/–), and there has been less independent replication with each mouse model than those GH/pituitary studies. We also tested whether strain (seven different strain variants in the full set) explained variance across the dataset, but the proportion of variance explained was negligible (6.7% for $\ln HR_{50}$ and ~0% for the models analyzing demography presented below).

It has previously been qualitatively noted that life-span extension in Ames-Dwarf mice occurs predominately through a shift in the age at which age-associated mortality becomes apparent (25). A more recent study of mortality changes with reduced IGF1 signaling, however, suggested a decrease in the rate of aging when serum IGF1 levels are lowered (26). To understand whether these effects are consistent across different genetic models of reduced somatotrophic signaling, we assessed alterations in Gompertz parameters of mortality across available mouse data. Fitting Gompertz ($m(t) = a + \exp(bt)$) models to life-span data separates parameter b, the "aging rate" of the population (the rate of increase in mortality over time, t), and parameter a, which describes the vulnerability to dying from ageing related causes (27,28).

Table 1. Animal Models Included in Each Moderator Category

Moderator Category	Animal Models Included
Growth hormone/pituitary manipulation	Ames & Snell Dwarfs, GH Receptor –/–, GH releasing hormone –/–, GH antagonist
Insulin like growth factor 1 (IGF1)	IGF1R+/–, liver-specific IGF1–/–, Pregnancy associated plasma protein A (PPAP-A), IGF1 hypomorphic allele
Insulin receptor substrate (IRS)	IRS1–/–, IRS1+/–, IRS2+/–

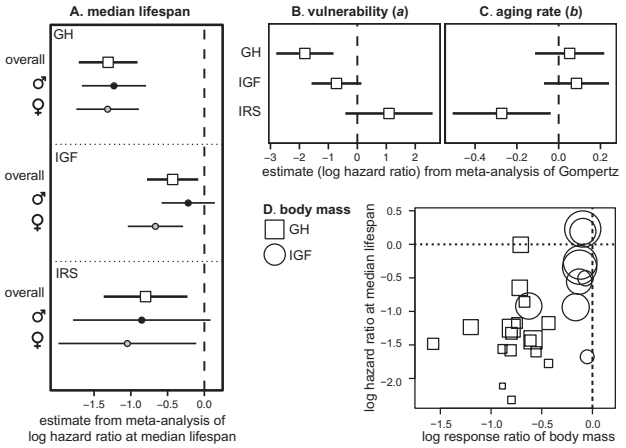

Figure 1. Life-span extension with reduced somatotrophic signaling. (**A**) The log-hazard ratio for median life span in each of the three moderator groups. Negative values indicate increased survival in the mutant group. (**B** & **C**) The log-hazard ratio for Gompertz parameters in each moderator group. (**D**) The relationship between change in body mass and change in life span for GH/pituitary and IGF1 mutant animals.

There was a significant overall change in parameter *a* in mutant mice compared to controls across all animal models ($\ln HR_a$ = −0.88, 95% CI [−1.63, −0.13], z = −2.30, p = .02), although mice with mutations disrupting different hormonal signals showed different changes in this demographic parameter (test of moderator: Q_{M2} = 10.11, p = .006, $R^2_{[m]}$ = 65.1%, Figure 1B). Considering only mice with manipulations of pituitary/GH signaling, parameter a is substantially reduced (Figure 1B, p < .01), generalizing the effect previously noted by Bartke et al. (25). Mice with manipulations in IGF1 signaling show a similar change although the effect across all studies is non-significant (Figure 1B, p = .05). When IRS signaling is manipulated, however, this parameter is significant with a sign in the opposite direction (Figure 1B, p = .047; see also Supplementary Table S1). We therefore might expect a modulation of ageing rate with reduced IRS signaling to be the demographic cause of life-extension. When examining changes in the rate of aging—parameter b—there is a significant reduction in this parameter with reduced IRS signaling (p < .01), with no change in this parameter in GH and IGF1 mutant mice (Figure 1C, Supplementary Table S1). There also appears to be lower heterogeneity surrounding the Gompertz estimates for reduced IRS signaling that those other manipulations, which could indicate a more consistent effect on the demography of mortality. However, we are again cautious of interpretation of this result given the lower level of replication for these models.

The Sex-Specificity of Life-span Extension

There has been growing recognition that interventions extending life span have sex-specific effects (17,29). Reduced IGF1 signaling has been reported to have a stronger life-extending effect in females than males in several studies (8,10). Reduced pituitary/GH signaling has also been qualitatively noted to extend life span to a greater extent in females (1,30). To understand whether any of these sex-specific effects are a consistent feature of life-span extension through altered somatotrophic signaling, and/or differ in severity across different manipulation types, we tested whether sex was a significant predictor of life-span extension. When including all studies life-span extension is significantly stronger in females than males (while including hormonal manipulation type as moderator, see above: $\ln HR_{[male]}$ = 0.31, 95% CI [0.04, 0.57], z = 2.24, p = .025).

However, this sex effect was only significant with IGF1 manipulations ($\ln HR_{[male]}$ = 0.45, 95% CI [0.09, 0.81]) but not those of pituitary/GH signaling or IRS ($\ln HR_{[male]}$ = 0.09, 95% CI [−0.43, 0.62], $\ln HR_{[male]}$ = 0.07, 95% CI [−0.54, 0.68], respectively, Figure 1A).

The Relationship Between Life-span Extension and Body Mass

Most interventions that extend life span in mice typically reduce body weight, and this reduced growth allocation may contribute to life-span extension (31). To quantify how relative changes in body weight relate to life-extension we extracted data on body mass from the papers used in our longevity analyses above, and from associated publications. Most data was available for young animals (~3 months; although little was available for IRS manipulated animals and these were excluded from this analysis), so we restricted our analysis to that period, but note that these data are well correlated to measurements at 12 months of age (for the data available: r_s = .73, n = 15, p = .002). Manipulations that reduce IGF1 signaling tended to reduce body weight (the log response ratio, lnRR = −0.23, 95% CI [−0.49, 0.03]), although to a lesser degree (test between both categories: z = 3.29, p < .01) than with reduced GH signaling (lnRR = −0.78, 95% CI [−0.97, −0.57]).

To understand whether the degree of life-span extension is related to the change in body mass, both across and within these different manipulation types, we tested if the change in body mass with a particular manipulation predicts the degree of life-span extension. This test would hint that life-span extension in these mouse models represents a direct trade-off with body size (as highlighted in (31)). When considering all data across all GH-IGF1 manipulations, the change in body weight in a particular model was a significant predictor of the degree of life-span extension ($\ln HR_{[slope\ of\ lnRR]}$ = 1.01, 95% CI [0.41, 1.62], Z = 3.28, p = .001, Q_{M1} = 10.7, p = .001, $R^2_{[m]}$ = 71.97%; Figure 1D). However, this predictive effect of change in body weight was fully explained by the differences that occur between the different manipulation types (Figure 1D). When body mass change was included in a model that also includes a treatment category (either pituitary/GH or IGF1), the slope was substantially reduced and was no longer significant ($\ln HR_{[slope\ of\ lnRR]}$ = 0.35, 95% CI [−0.65, 1.36], z = 0.70, p = .49). Indeed, within each treatment category change in body mass had no discernable effect (centered log response ratio: 0.03 ± SE = 0.90, z = 0.03, p = .97). The initial significant relationship between body weight change and degree of life-extension, when different hormonal manipulations are ignored, appears to be an example of Simpson's paradox (32): when a trend appears across a broad set of data but this effect disappears (or in other situations can be reversed) when effects within different subgroups are taken into account. In this situation, while manipulations of reduced pituitary/GH signaling have greater effects on both body mass and life span than reduced IGF1 signaling, within either grouping there is no relationship between these two variables.

Life-span Extension and Baseline Cohort Life Span

Another quantitative measure that has been suggested to be important in determining treatment effects in longevity studies is the life span of the control animals; for example, whether the average life span of the control group is small (eg, 500 days) or large (eg, 1,000 days). It has been suggested that particular manipulations might rescue the high mortality effects of poor housing conditions (eg,

infections or extreme temperature/humidity variation), or genetic backgrounds that have an increased susceptibility to particular diseases, rather than generally impacting aging. It has also been highlighted that strains can differ in their activity of particular pathways that regulate the aging process and influence life span, including the GH-IGF1 axis (33), and thus manipulations of these pathways will have stronger effects in some backgrounds than others. Under these scenarios, larger life-span effects of a manipulation are expected in short-lived controls (10).

Xu et al. (9) provided a qualitative comparison of changes in life span with IGF1 manipulation in four different studies where each cohort differed in its control group life span, and suggested that reduced IGF1 signaling preferentially extends life span in short-lived strains. Simply examining the relationship between control group life span and degree of life-extension across studies, however, fails to account for regression to the mean. That is, if the life span of long and short-lived control cohorts differs purely because of random sample variance, then we would expect that subsequent treatment group life spans would be closer to the average (34), generating such a relationship as a statistical artifact. It is notable that for two strains examined by Xu et al. (9). differences in control strain life span were correlated with baseline levels of IGF1 signaling, and partial inhibition of IGF1R had a greater inhibitive effect on IGF1 and IRS activation in the strain that showed greatest life-extension. This finding suggests that these strain life spans could be a consequence of underling differences in physiology rather than random sample variance. Nonetheless, because regression to the mean can lead to substantial artifactual relationships that have no biological meaning (34,35), we sought to account for this while exploring further the relationship proposed by Xu et al. (9).

If reduced IGF1 signaling preferentially extends the life span of short-lived strains we would expect a difference in variance for life span between control and treatment groups (35). Therefore we tested for differences in equality of variance for life span between treatment groups in each of our hormonal signaling categories. We note here that these tests assume underlying normality and a true estimator of repeatability. While median life span is usually close to normally distributed (as in the Gompertz), more sophisticated and accurate statistical methods are available for accounting for regression to the mean (36) but require a greater sample size than is available in this analysis. Such approaches in situations of greater sample size may help to validate or refute these hypotheses.

Within GH and IRS signaling groups we find no statistical evidence of a relationship between the control group life span and the degree of life-span extension. There is no significant difference in the equality of variance between control and treatment groups (Supplementary Table S2) and no significant relationship is found even when simply regressing median life span of the control group against the change in median life span induced by the genetic manipulation (GH: $r_s = -.41$, $p = .10$, IRS: $r_s = .54$, $p = .13$). In contrast, for the IGF1 grouping the variance is lower in the treatment groups' median life spans compared to controls, in both males and females (Supplementary Table S2). The negative correlation between control group median life span and the increase in life span with IGF1 reduction is negative (correlation: $r_s = -.63$, $p = .01$), which, when viewed along-side the reduction in variance, suggests that reduced IGF1 signaling may preferentially extend life span in short-lived control cohorts that are exposed are exposed to some kind of stress (10) or have higher levels of IGF1 signaling (9).

Publication Bias

We examined publication bias through rank correlations of the summed sample size in a study against $\ln HR_{50}$, the life-span effect measured at median life span. Across the whole set this indicated potential bias ($r_s = .75$, 95% CI 0.41–0.91), meaning that small studies tended to find a larger effect at median life span (Supplementary Figure S1). Within each treatment category there are also relationships between sample and effect size, although this appears weaker for the IGF1 group, where sample sizes are generally larger (Supplementary Figure S1; IRS [$r_s = 1$, but note there were only three studies for this subgroup]; GH [$r_s = .79$, 95% CI 0.08–0.97]; IGF1 [$r_s = .41$, 95% CI –0.60–0.92]). Unfortunately, the nature of this relatively small set precludes any imputation of bias to correct for this possible effect, especially while analyzing the moderating variables.

Such publication bias suggests that the data included in this meta-analysis, and thus that published in the literature, may not provide an accurate representation of the true biological effect, and that tests of moderating factors across these studies (eg, sex, type of manipulation) could also be influenced by this or *vice versa*. We note, however, that the detection of publication bias in statistical terms is not proof that there was actual bias in publication (where studies that find smaller effects are less likely to be published), and we might speculate that expensive mouse life-span studies would be published regardless of their outcome. The relationship between sample size and effect size could instead be influenced by unknown moderating variables. For example, follow-up studies on mouse models like the Ames and Snell Dwarf mice are based on citations of previous work where dwarfs have been reported to live up to 50% longer (eg, see for an example of this citation (37)), and therefore in these follow-up studies authors begin with a smaller sample size as they expect a big effect. In follow-up studies exploring the role of IGF1 in mouse longevity, by contrast, where results have been less consistent, authors have specifically stated that they design their experiments with larger sample sizes because of this (10). These components of study design could explain differences in both sample size and effect size and crucially associated known and unknown moderating variables across the main treatment groups (Supplementary Figure S1), and similar effects within the GH grouping when considering studies on Ames and Snell Dwarfs compared to other GH manipulations (Supplementary Figure S2). However, such effects would not explain any relationships between sample size and effect size within treatment groups, and/or where experiments have been designed without prior knowledge of potential effect sizes.

Overall, the effects reported in this article represent the current state of published knowledge on GH-IGF1-IRS signaling and longevity, but the publication bias highlights that this published knowledge may not represent the true biological effect of these manipulations, across all laboratories and conditions. Our caution on publication bias warrants independent replication of these studies to alleviate concerns about such bias.

Discussion

Our results indicate that reduced somatotrophic signaling extends life span in mice. The degree of this life-extension, however, is dependent on the type of signal that is manipulated, with life-span extension (as assessed at median life span) across studies being greater and more consistent (lower heterogeneity) when GH/general pituitary signaling is reduced, rather than when IGF1 or IRS signaling components are manipulated directly. Reduced GH/pituitary signaling robustly increases median life span, has a clear effect on vulnerability

to die from ageing related causes (Gompertz parameter a), and is not dependent on sex or the control group's median life span. Life-span extension with reduced IGF1 signaling, by contrast, is sex-specific, with females showing greater life-span extension than males, and in both sexes greater life-span extension is observed when the control cohort has a shorter life span. Our study of IRS mutants highlights substantial heterogeneity in the change in median life span, which is not explained by sex or the life span of the control group, although might be a consequence of lower replication across models and laboratories compared to the data available for GH and IGF1.

Our analysis of life-extension via reduced IRS signaling (through genetic inhibition of IRS1 or IRS2 protein expression), also highlights that the mortality effects of this manipulation appear distinct, demographically, from life-span extension through reductions in GH and IGF1. Reduced IRS signaling reduces Gompertz parameter b and hence slows the rate of aging. We first note that our sample size for this analysis is small, with data comprised from three different studies and nine control-treatment survival comparisons. Additional replication of this reduction in the rate of aging with considerable sample size within a single study may help to confirm this result, particularly in light of the potential publication bias that we highlight. Nonetheless, there are several reasons why reduced IRS signaling may affect mouse mortality differently to GH/IGF1 signaling. Insulin receptor substrates are intra-cellular signaling kinases that are phosphorylated by signals from both the insulin and IGF1 receptors. Genetically reduced IRS signaling will therefore decrease, or inhibit, some signaling responses that occur with IGF1R activation. However, since IRS proteins also promote various aspects of the insulin signaling cascade after phosphorylation by the insulin receptor, including activation of Akt/PKB, reduced IRS signaling will also interfere with insulin signaling and glucose homeostasis (13). The direct role of alterations in insulin signaling in life-span extension is poorly understood: mouse models of life-extension can show both heightened and reduced insulin sensitivity and glucose homeostasis (38). Mice that are insulin sensitive can be short-lived, and mice that have reduced expression of the insulin receptor (IR), and are insulin insensitive, have similar life spans to wild-types (although the study acknowledges a small sample size and a possible interactive effect with sex) (39). Another possibility is that reducing IRS signaling increases mortality rates slightly early in life, but slows mortality late in life, which would appear to modulate the rate of aging. In line with this suggestion, a life-span study of mice with a genetic reduction of p110α PI3K activity, which is downstream of both the IR and IGF1 receptors, reported that this manipulation reduced mouse survival slightly early in life but increased survival after 500 days (40).

Results on the demography of mortality that we reveal in this study can also be qualitatively compared to results from several recent meta-analysis on life-span extension through reduced mTOR signaling (41) and dietary restriction (27,42). In relation to the slowed rate of aging observed with IRS signaling, the only other manipulation shown to consistently reduce the rate of aging in mice is dietary restriction. The similarity between these two manipulations in patterns of mouse mortality might suggest that alterations in aspects of the insulin-signaling cascade could contribute to slowed aging with dietary restriction (27), and/or that these manipulations have similar effects on some causes of mouse death. Our results for changes in demography with reduced GH/pituitary signaling point to a consistent lowering of the mortality risk of mutant mice across life. We recently demonstrated that reduced mTORC1 signaling, through rapamycin or genetic manipulation, results in similar demographic responses (41). Concordantly, both pituitary and GH impaired mutants show reduced mTORC1 signaling (43,44), in fasted and fed states (43), and it has been suggested that this may contribute to life-span extension in these models. The similar resultant demographic responses to these treatments would support this hypothesis.

Using meta-analysis, we also conducted a test of the hypothesis that body size trades-off against life span, and that relative changes in growth may relate to degree of life-span extension. If greater body weight change leads to incremental increases in life span, we would expect the two factors to correlate. Although there is substantial variation in the degree of body weight change within each of these hormonal manipulation groups, we find no statistically significant relationship between body weight change and degree of life-span extension—once the broad type of manipulation is taken into consideration. This is consistent with two manipulative studies in Ames (45) and Snell (46) Dwarf mice, where early life treatment with both GH and T4 combined substantially increased the body mass of these dwarfs but had relatively minor effects on the life-span phenotype (although see (37) for an example of a GH manipulation that did reduce life span in Ames Dwarf mice). These results suggest that life-span extension with these manipulations is not simply a consequence of reducing costs associated with the anabolic actions of growth, and that manipulations of the GH-IGF1 axis are providing other anti-aging benefits, perhaps linked to their effects on stress resistance (37), insulin sensitivity (47), or inflammation (48)—traits not directly linked to growth and body size.

It's further notable that while reduced IGF1 signaling has a smaller effect on body mass than reduced GH signaling, these differences in effects on body mass do not seem to explain why we observe a smaller degree of life-span extension when only IGF1 signal is reduced. Thus, the effects of reduced GH signaling, specifically, may provide life-span benefits outside of its effects in reducing IGF1 production. The different effects of GH and IGF1 on pathways and mechanisms of aging might also be sex-specific, as reduced IGF1 signaling provides a greater life-span benefit to females, while reduced GH signaling has a similar life-span effect in both sexes. Interestingly, partial inhibition of IGF1R enhances resistance to oxidative stress only in females (8,10), not males, suggesting that IGF1 signaling may differently regulate some of the cellular processes linked to aging in females only. A greater understanding of the cellular processes that are differentially regulated by either GH or IGF1 manipulation, particularly in each sex, may provide mechanistic insights into the regulation of life span by somatotrophic signaling.

We hope that our review will stimulate future experimental and comparative research on the physiological causes for life-span variation across mutant somatotrophic signaling models, and animals showing life-span extension. For example, data from tissue-specific GH receptor knockout animals is already revealing that GH's life-span benefits may be generated independently of effects on circulating IGF1. GH's main effects on circulating IGF1 occur through its stimulatory effects on IGF1 secretion in the liver. Liver-specific GH receptor knockout mice show greatly reduced levels of circulating IGF1 (~90%) and reductions in body mass similar to that seen in IGF1 mutant mice (49). Yet they do not live longer than controls (43), suggesting that the life-span benefits of GH occur outside of liver-derived IGF1. A contrasting example, brain-specific inhibition of either IGF1R (50) or IRS2 (15) has been reported to extend life span, suggesting that central actions of these signaling pathways may be important in the regulation of life span. Once sufficient data is available, meta-analytic comparative assessments of life-span extension in tissue-specific and

inducible GH, IGF1, and IRS mice will help to reveal the specific tissues and life-periods contributing to life-span extension in the mouse models reviewed here.

Experimental Procedures

Search Protocol

We began by searching for published research on life-span extension with reduced GH, IGF1 or IRS signaling in mice by checking several published reviews that provide comprehensive summaries of life-span extension with altered activity of these pathways (19,51,52). We then conducted additional searches using Web of Science and Google Scholar.

We included studies that manipulated the activity of somatotrophic signaling axis globally, that is, without inhibition of gene expression in a particular tissue. This allowed us to use an unbiased search protocol and also provided opportunity to split animals into broad moderator groups accorded to the type of signal manipulated. We also only included data from studies where data from the sexes was separated, because we were interested in the effects of sex, and in how these treatments influence demography. Inclusion of studies where data from the sexes are combined, even when they do not statistically differ in median life span, might skew the demographic data. We included manipulations of somatotrophic signaling at a number of different levels (Supplementary Data). We collected data from models with impaired pituitary signaling (ie, the Ames and Snell Dwarf mice) and GH signaling (either by inhibition of its production [eg, disruption of growth-hormone releasing hormone], or its reception [eg, targeted GH receptor disruption]). We further included studies that reduced IGF1 signaling, either by reducing IGF1 detection (eg, IGF1R +/– mice), its bioactivity (eg, through knockout of IGF1 binding protein PAPPA), or it's circulating production. We note here that we included data from liver-specific IGF1 knockout mice, since this reduces the majority (75%–80%) of IGF1 in circulation. We further collected data from studies of life span where expression of either insulin receptor substrate protein was impaired (eg, IRS1 and IRS2). We excluded IRS2–/– animals from this analysis because males die prematurely (13), from diabetes (53), and not directly from aging.

Data Extraction and Analysis

Raw individual survival data was used whenever possible. When unavailable, mortality was measured from survival curves. Gompertz models were then fitted using maximum likelihood estimation (54), and estimates of sampling variances were obtained from the CIs around the parameters fitted on the individual level data. For one study where there was some ambiguity in date of death for a small proportion of individuals (26) sampling variance was estimated through simulation (27). To assess overall effects on life span a hazard ratio at median life span was calculated using the number of individuals died and at risk in both experimental groups, for which sampling variances are known (42). Meta-analyses were run on these hazard ratios at median life span and the hazard ratio of the two Gompertz parameters with treatment group (reduced GH signaling) over the control group—negative hazard ratio estimates indicate improved survival with the manipulation. Mixed effects (multilevel) meta-analyses were conducted using the package "metafor" (55) in R (56), with study identifiers included as a random term. Heterogeneity in meta-analyses was assessed by a multilevel version of I^2 (57). We tested for publication and reporting bias using rank tests of sample size against effect size.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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References

1. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature*. 1996;384:33. doi:10.1038/384033a0.
2. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science*. 2003;299:1346–1351. doi:10.1126/science.1081447.
3. Barbieri M, Bonafè M, Franceschi C, Paolisso G. Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. *Am J Physiol Endocrinol Metab*. 2003;285:E1064–E1071. doi:10.1152/ajpendo.00296.2003.
4. Bonafè M, Barbieri M, Marchegiani F, et al. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocrinol Metab*. 2003;88:3299–3304. doi:10.1210/jc.2002-021810.
5. Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci U S A*. 2001;98:6736–6741. doi:10.1073/pnas.111158898.
6. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology*. 2000;141:2608–2613. doi:10.1210/endo.141.7.7586.
7. Sun LY, Spong A, Swindell WR, et al. Growth hormone-releasing hormone disruption extends lifespan and regulates response to caloric restriction in mice. *eLife*. 2013;2:e01098.
8. Holzenberger M, Dupont J, Ducos B, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature*. 2003;421:182–187. doi:10.1038/nature01298.
9. Xu J, Gontier G, Chaker Z, Lacube P, Dupont J, Holzenberger M. Longevity effect of IGF-1R(+/-) mutation depends on genetic background-specific receptor activation. *Aging Cell*. 2014;13:19–28. doi:10.1111/acel.12145.
10. Bokov AF, Garg N, Ikeno Y, et al. Does reduced IGF-1R signaling in Igf1r+/- mice alter aging? *PLoS One*. 2011;6:e26891. doi:10.1371/journal.pone.0026891.
11. Svensson J, Sjögren K, Fäldt J, et al. Liver-derived IGF-I regulates mean life span in mice. *PLoS One*. 2011;6:e22640. doi:10.1371/journal.pone.0022640.
12. Conover CA, Bale LK. Loss of pregnancy-associated plasma protein A extends lifespan in mice. *Aging Cell*. 2007;6:727–729. doi:10.1111/j.1474-9726.2007.00328.x.
13. Selman C, Lingard S, Choudhury AI, et al. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J*. 2008;22:807–818. doi:10.1096/fj.07-9261com.

14. Selman C, Partridge L, Withers DJ. Replication of extended lifespan phenotype in mice with deletion of insulin receptor substrate 1. *PLoS One*. 2011;6:e16144. doi:10.1371/journal.pone.0016144.
15. Taguchi A, Wartschow LM, White MF. Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science*. 2007;317:369–372. doi:10.1126/science.1142179.
16. Taguchi A, White MF. Response to comment on "Brain IRS2 Signaling Coordinates Life Span and Nutrient Homeostasis". *Science*. 2008;320:1012.
17. Austad SN, Bartke A. Sex differences in longevity and in responses to anti-aging interventions: a mini-review. *Gerontology*. 2015;62:40–46. doi:10.1159/000381472.
18. Selman C, Lingard S, Gems D, Partridge L, Withers DJ. Comment on "Brain IRS2 signaling coordinates life span and nutrient homeostasis". *Science*. 2008;320:1012; author reply
19. Sonntag WE, Csiszar A, deCabo R, Ferrucci L, Ungvari Z. Diverse roles of growth hormone and insulin-like growth factor-1 in mammalian aging: progress and controversies. *J Gerontol A Biol Sci Med Sci*. 2012;67:587–598. doi:10.1093/gerona/gls115.
20. Bartke A. Aging: all in the head? *Cell Metab*. 2007;6:153–154. doi:10.1016/j.cmet.2007.08.007.
21. Harrison DE, Strong R, Allison DB, et al. Acarbose, 17-α-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. *Aging Cell*. 2014;13:273–282.
22. Miller RA, Harrison DE, Astle CM, et al. An Aging Interventions Testing Program: study design and interim report. *Aging Cell*. 2007;6:565–575.
23. Bartke A, Wright JC, Mattison JA, Ingram DK, Miller RA, Roth GS. Extending the lifespan of long-lived mice. *Nature*. 2001;414:412. doi:10.1038/35106646.
24. Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol*. 2013;4:133–142.
25. Bartke A, Wright JC, Mattison JA, Ingram DK, Miller RA, Roth GS. Longevity: extending the lifespan of long-lived mice. *Nature*. 2001;414:412.
26. Lorenzini A, Salmon AB, Lerner C, et al. Mice producing reduced levels of insulin-like growth factor type 1 display an increase in maximum, but not mean, life span. *J Gerontol A Biol Sci Med Sci*. 2014;69:410–419.
27. Simons MJ, Koch W, Verhulst S. Dietary restriction of rodents decreases aging rate without affecting initial mortality rate – a meta-analysis. *Aging Cell*. 2013;12:410–414. doi:10.1111/acel.12061.
28. Kirkwood TB. Deciphering death: a commentary on Gompertz (1825) 'On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies'. *Philos Trans R Soc Lond B Biol Sci*. 2015;370.
29. Maklakov AA, Lummaa V. Evolution of sex differences in lifespan and aging: causes and constraints. *Bioessays*. 2013;35:717–724. doi:10.1002/bies.201300021.
30. Flurkey K, Papaconstantinou J, Harrison DE. The Snell dwarf mutation Pit1(dw) can increase life span in mice. *Mech Ageing Dev*. 2002;123:121–130.
31. Bartke A, Sun LY, Longo V. Somatotropic signaling: trade-offs between growth, reproductive development, and longevity. *Physiol Rev*. 2013;93:571–598. doi:10.1152/physrev.00006.2012.
32. Wagner CH. Simpson's paradox in real life. The American Statistician. 1982;36:46–48.
33. Yuan R, Meng Q, Nautiyal J, et al. Genetic coregulation of age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. *PNAS*. 2012;109:8224–8229.
34. Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. *Int J Epidemiol*. 2005;34:215–220. doi:10.1093/ije/dyh299.
35. Kelly C, Price TD. Correcting for regression to the mean in behavior and ecology. *Am Nat*. 2005;166:700–707. doi:10.1086/497402.
36. Ghidey W, Stijnen T, van Houwelingen HC. Modelling the effect of baseline risk in meta-analysis: a review from the perspective of errors-in-variables regression. *Stat Methods Med Res*. 2013;22:307–323. doi:10.1177/0962280211412244.
37. Panici JA, Harper JM, Miller RA, Bartke A, Spong A, Masternak MM. Early life growth hormone treatment shortens longevity and decreases cellular stress resistance in long-lived mutant mice. *FASEB J*. 2010;24:5073–5079. doi:10.1096/fj.10-163253.
38. van Heemst D. Insulin, IGF-1 and longevity. *Aging Dis*. 2010;1:147–157.
39. Nelson JF, Strong R, Bokov A, Diaz V, Ward W. Probing the relationship between insulin sensitivity and longevity using genetically modified mice. *J Gerontol A Biol Sci Med Sci*. 2012;67:1332–1338. doi:10.1093/gerona/gls199.
40. Foukas LC, Bilanges B, Bettedi L, et al. Long-term p110α PI3K inactivation exerts a beneficial effect on metabolism. *EMBO Mol Med*. 2013;5:563–571.
41. Garratt M, Nakagawa SN, Simons MJ. Comparative idiosyncrasies in life extension by reduced mTOR signalling and its distinctiveness from dietary restriction. 2016;15:737–743.
42. Nakagawa S, Lagisz M, Hector KL, Spencer HG. Comparative and meta-analytic insights into life extension via dietary restriction. *Aging Cell*. 2012;11:401–409. doi:10.1111/j.1474-9726.2012.00798.x.
43. Dominick G, Berryman DE, List EO, et al. Regulation of mTOR activity in Snell Dwarf and growth hormone receptor gene disrupted mice. *Endocrinology*. 2014;156:565–575.
44. Sharp ZD, Bartke A. Evidence for down-regulation of phosphoinositide 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR)-dependent translation regulatory signaling pathways in Ames dwarf mice. *J Gerontol A Biol Sci Med Sci*. 2005;60:293–300.
45. Do A, Menon V, Zhi X, et al. Thyroxine modifies the effects of growth hormone in Ames dwarf mice. *Aging (Albany NY)*. 2015;7:241–255.
46. Vergara M, Smith-Wheelock M, Harper JM, Sigler R, Miller RA. Hormone-treated snell dwarf mice regain fertility but remain long lived and disease resistant. *J Gerontol A Biol Sci Med Sci*. 2004;59:1244–1250.
47. Masternak MM, Panici JA, Bonkowski MS, Hughes LF, Bartke A. Insulin sensitivity as a key mediator of growth hormone actions on longevity. *J Gerontol A Biol Sci Med Sci*. 2009;64:516–521. doi:10.1093/gerona/glp024.
48. Sadagurski M, Landeryou T, Cady G, et al. Growth hormone modulates hypothalamic inflammation in long-lived pituitary dwarf mice. *Aging Cell*. 2015;14:1045–1054.
49. List EO, Berryman DE, Funk K, et al. Liver-specific GH receptor gene-disrupted (LiGHRKO) mice have decreased endocrine IGF-I, increased local IGF-I, and altered body size, body composition, and adipokine profiles. *Endocrinology*. 2014;155:1793–1805.
50. Kappeler L, De Magalhaes Filho C, Dupont J, et al. Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism. *PLoS Biol*. 2008;6:e254.
51. Berryman DE, Christiansen JS, Johannsson G, Thorner MO, Kopchick JJ. Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. *Growth Horm IGF Res*. 2008;18:455–471. doi:10.1016/j.ghir.2008.05.005.
52. Junnila RK, List EO, Berryman DE, Murrey JW, Kopchick JJ. The GH/IGF-1 axis in ageing and longevity. *Nat Rev Endocrinol*. 2013;9:366–376. doi:10.1038/nrendo.2013.67.
53. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature*. 1998;391:900–904.
54. Pletcher. Model fitting and hypothesis testing for age-specific mortality data. *J Evol Biol*. 1999;12:430–439.
55. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Software*. 2010;36:1–48.
56. Team R. A language and environment for statistical computing. *Austria R Foundation for Statistical Computing*. 2006.
57. Nakagawa S, Santos EA. Methodological issues and advances in biological meta-analysis. *Evol Ecol*. 2012;26:1253–1274.