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

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Received July 26, 2016; Editorial Decision Date January 6, 2017

Decision Editor: Rafael de Cabo, PhD

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
insulin receptor substrate (IRS) IRS1−/−, IRS1+/−, IRS2+/−

insulin like growth factor 1 (IGF1) IGF1R+/−, liver-specific IGF1−/−, pregnancy associated plasma protein A (PPAP-A), IGF1 hypomorphic

growth hormone/pituitary manipulation Ames & Snell Dwarfs, GH receptor −/−, GH releasing hormone −/−, GH antagonist

table 1. animal models included in each moderator category

<table>
<thead>
<tr>
<th>Moderator Category</th>
<th>Animal Models Included</th>
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<tr>
<td>growth hormone/pituitary manipulation</td>
<td>Ames &amp; Snell Dwarfs, GH Receptor −/−, GH releasing hormone −/−, GH antagonist</td>
</tr>
<tr>
<td>Insulin like growth factor 1 (IGF1)</td>
<td>IGF1R+/−, liver-specific IGF1−/−, pregnancy associated plasma protein A (PPAP-A), IGF1 hypomorphic allele</td>
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There was a significant overall change in parameter \(a\) in mutant mice compared to controls across all animal models (lnHR \(a\) = −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 than 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: lnHR\[male\] = 0.31, 95% CI [0.04, 0.57], \(z\) = 2.24, \(p\) = .025).

However, this sex effect was only significant with IGF1 manipulations (lnHR\[male\] = 0.45, 95% CI [0.09, 0.81]) but not those of pituitary/GH signaling or IRS (lnHR\[male\] = 0.09, 95% CI [−0.43, 0.62], lnHR\[male\] = 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: \( r = .73, n = 15, p = .002 \)). Manipulations that reduce IGF1 signaling tended to reduce body weight (the log response ratio, \( \ln RR = -0.23, 95\% \text{ CI } [-0.49, 0.03] \)), although to a lesser degree (test between both categories: \( z = 3.29, p < .01 \)) than with reduced GH signaling (\( \ln RR = -0.78, 95\% \text{ CI } [-0.97, -0.57] \)).

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 (\( \ln \text{HR}_{\text{slope of lnRR}} = 1.01, 95\% \text{ CI } [0.41, 1.62], Z = 3.28, p = .001, Q_{\text{adj}} = 10.7, p = .001, R^2_{\text{adj}} = 71.97\% \); 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 (\( \ln \text{HR}_{\text{slope of lnRR}} = 0.33, 95\% \text{ CI } [-0.65, 1.36], z = 0.70, p = .49 \)). 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 group 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 underlying 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: \( r = -.41, p = .10 \), IRS: \( r = .54, p = .13 \)). 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: \( r = -.63, p = .01 \)), which, when viewed alongside 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 \( \ln \text{HR}_{\text{treatment}} \), the life-span effect measured at median life span. Across the whole set this indicated potential bias (\( r = .75, 95\% \text{ CI } 0.41–0.91 \)), 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 \( [r = 1, \text{ but note there were only three studies for this subgroup}] \); GH \( r = .79, 95\% \text{ CI } 0.08–0.97 \); IGF1 \( r = .41, 95\% \text{ CI } -0.60–0.92 \)). 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 somatotropic 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 b), 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 300 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—one 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 (multi-level) 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 I² (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.

Funding

S.N. is supported by an ARC Future Fellowship (Australia; FT130100268). M.J.P.S. is supported by a Sir Henry Wellcome and a Sheffield Vice Chancellor’s Fellowship and the Natural Environment Research Council (J024597,
M005941, and N013832 to Terry Burke). M.G. acknowledges support of a fellowship from the Michigan Society of Fellows.

Acknowledgments
We thank Christian Sell and Colin Selman for providing additional information on two studies.

References