Probing the Relationship Between Insulin Sensitivity and Longevity Using Genetically Modified Mice

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Interference in insulin and/or insulin-like growth factor 1 (IGF-1) signaling can extend invertebrate life span, and interference in IGF-1 signaling can extend murine life span. Whether interference with murine insulin signaling, which can be diabetogenic and pathological, is also life-extending is controversial. We therefore measured life span in 3 murine strains genetically modified to reduce or increase insulin sensitivity. Mice with reduced insulin sensitivity were hemizygous for a null mutation in the insulin receptor (insulin receptor knockout mice; IRKO+/−). Mice with increased insulin sensitivity either had a null mutation of protein tyrosine phosphatase 1B (PTP-1B−/−) or overexpressed Peroxisome proliferator-activated receptor-α coactivator (PGC)-1α (PGC-1αTG). Life span of insulin insensitive IRKO+− mice was increased (males) or unaffected (females). Life spans of mice with increased insulin sensitivity were shortened overall (PTP-1B−/− mice) or partially (PGC-1αTG: survival at the 25th percentile was reduced). These results show that insulin sensitivity in some murine genotypes is inversely related to longevity and provide further evidence for evolutionary conservation of this pathway as a modulator of longevity.

Key Words: Insulin sensitivity—Life span—Longevity—Mice.

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In invertebrates, interfering with the activity of intermediates orthologous to proteins in the mammalian insulin/insulin-like growth factor 1 (IGF-1) signaling pathway often leads to extended longevity and retarded aging (1). Strains carrying mutations of daf2 in Caenorhabditis elegans and Chico in Drosophila melanogaster (2,3), the orthologs of the mammalian insulin/IGF-1 receptors and a signaling intermediate, respectively, show lengthened life. An associated phenotype in these long-lived mutants is resistance to oxidative and other stressors, suggesting that oxidative stress limits life span in these species (4–6).

An important question is whether the insulin/IGF-1 signaling pathway have extended life span. These include the Ames and Snell dwarf mice (9,10) as well as Insulin Receptor Substrate 1 (IRS1) knockout (KO) mice (11), brain-specific Insulin Receptor Substrate 2 (IRS2) KO mice (12), Growth Hormone Releasing Hormone (GHRH) KO mice (13), and strains that are hemizygous for a null mutation of IGF-1 receptor (14,15). Insulin Receptor (IR) intermediates are shared by insulin and IGF-1 signaling pathways, and dwarf mice show altered insulin as well as IGF-1 signaling. Thus, whether life spans of dwarf or IR KO mice are modulated solely by IGF-1 signaling, or by insulin signaling as well, is unclear.

Evidence that impairment specific to insulin signaling can extend mammalian life span is weaker. First, a number of mammalian models with extended life span appear to have increased rather than decreased insulin sensitivity. Mice and rats under dietary restriction, one of the more robust means of extending life span (16), have markedly reduced serum insulin levels, often exhibit increased glucose tolerance, and show other evidence of enhanced insulin sensitivity (17–19). Long-lived Ames and Snell dwarfs also have reduced.
circulating insulin levels and evidence of increased insulin sensitivity (20–22). In addition, reduced insulin sensitivity is a risk factor for diabetes. Given the pathological sequelae of diabetes, it seems counterintuitive that reducing insulin sensitivity on mammals would increase life span. However, some models of impaired insulin signaling are associated with increased longevity or resistance to life-threatening oxidative stress. The fat-specific insulin receptor knockout mouse, which carries a fat-specific null mutation of the insulin receptor, is reported to have increased life span (23). However, although fat-specific IRKO mice have de facto impaired insulin signaling in adipose tissue, they are protected from age- and obesity-related insulin insensitivity and glucose intolerance (24). Mice carrying a mutation in the insulin receptor orthologous to one of the life-extending daf2 mutations in C. elegans have impaired insulin sensitivity but exhibit increased resistance to oxidative stress and longer survival following paraquat administration (25). Recently, it was reported that life spans of these insulin-insensitive mice were neither shortened nor lengthened (26).

With the exception of this recent report, experiments to test directly whether variation in insulin sensitivity modulates longevity in mammals are lacking. Here, we measured longevity in mice that were genetically modified to either increase or reduce insulin sensitivity, by either enhancing or reducing the ability of insulin to activate the downstream intermediates of the insulin receptor signaling cascade. The two models with increased insulin sensitivity either had a null mutation in PTP-1B (protein tyrosine phosphatase 1B, PTP-1B−/− [27]) or were transgenic for PGC-1α (PGC-1αTG) (28). The model with reduced insulin sensitivity was hemizygous for a null mutation in the insulin receptor (insulin receptor knockout mouse; IRKO−/− [29,30]).

METHODS

Mouse Strains

IRKO−/− breeding pairs were obtained from Dr. Dominic Accili. Details of their development are published (29). PTP-1B−/− breeders were obtained from Brian Kennedy (27) and PGC-1αTG mice were developed by one of us (31). All strains had been backcrossed to C57BL/6J for greater than six generations. Breeders used to generate the mice, used in this study, were hemizygous for the null allele or transgene of each strain so that wild-type (WT) littermates could be obtained and used as controls.

Maintenance of Mice

All mice were maintained under pathogen-free barrier conditions using microisolator cages in a temperature-controlled environment. Mice were housed in groups up to five per cage on ventilated racks at 60 air changes per hour. Bedding was Harlan Tek-Fresh and was changed weekly. The animal room was maintained at 23°C with a 12 hour light/12 hour dark cycle. Mice were fed irradiated Harlan Teklad LM-485 Rodent diet, ad libitum, and provided with acidified water. Cages were inspected twice daily (AM and PM) on weekdays and once daily on weekends and holidays for morbidity and mortality.

The mice used in this study were genotyped at 4–5 weeks of age by PCR analysis of DNA obtained from tail clips. Mice were assigned to survival groups at 2 months of age and allowed to live out their entire life span, that is, there was no censoring of the mice when measuring survival. All procedures followed the guidelines approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio.

Analysis of life Span

Mice were allowed to live out their life, and the life span for individual mice was determined by recording the age of spontaneous death. The survival curves were compared statistically using the log-rank test. The significance and direction of the effect of each mutation on longevity was analyzed by fitting a Cox proportional hazards model to the data along with other, experiment-specific explanatory variables (described in the Results section). The 25th percentile (when 25% of the mice died), median, mean, 90th percentile, and maximum survivals were calculated for each group. Mean survivals (±SEM) for each experimental group were compared with the respective WT group by calculating a t statistic from the survival times and comparing it with an empirical null distribution obtained by permutation of the data. The 25th percentile, median, and 90th percentile survivals for each group were compared with the WT group using quantile regression (32). All comparisons were made individually between each experimental group and their WT littermates of the same sex. For the point estimate comparisons (mean, median, and 90th percentile), Holm’s method (33) was used to correct for multiple comparisons. If any animals died from accidental causes after being assigned to a survival study, the death was represented in the data as a right-censored event. The IRKO−/− study was post hoc, and the censoring method is described in the Results section. The R language was used for all statistical tests (34).

Body Weight and Food Consumption

Over the course of each experiment, body weight was measured for eight mice of each sex per genotype (except the PGC-1αTG experiment where 20 males were used per genotype and the IRKO experiment where there were seven males in the heterozygous group). For the IRKO, PTP-1B, and PGC-1αTG experiments, the weight measurements started at a median age of 9.5, 25.4, and 10.4 weeks, respectively. Weights were measured monthly for the IR experiment. For the PTP-1B experiment, weights were measured weekly for the first 4 weeks, biweekly for the next 26 weeks, and monthly afterward. For the PGC-1αTG experiment, weights were measured biweekly for the first 9 weeks and monthly afterward.

Food consumption was also measured for each experiment as follows: during weekly cage changes, chow remaining in cage hoppers, and food debris sifted from cage
IRKO+/− mice did not differ from that of WTs. Although mean

tional hazards test; Figure 1). The mean life span of female

p group. Although these mice are insulin insensitive (29, 30),

and 2). The survival study had 11 males and 8 females in the

18 months. There was no significant effect of IR hemizygo-

measurements were collected for the IR, PGC-1αTG, and

PTP-1B experiments, respectively.

For both body weight and food consumption, a lin-

earized mixed-effect model was fitted with genotype, sex

where applicable, time or age, and the square of time or

age as the fixed predictor variables. Animal identity or
cage identity was used as the random variables, to account
for within-individual variation in these repeated measures
data. Initially, all possible interaction terms with the tem-

poral variable and the square of the temporal variable were

included in the model. The lme function from the nlme
package for R was used to fit these models (35). Then, an

automated backward regression procedure (stepAIC func-
tion from the MASS package for R) was used to eliminate in a marginality-preserving manner terms not necessary
for model fit as determined from the Akaike Information
Criterion (36). The question of interest was whether geno-
type affected the response variable (food consumption or

body weight) in a given experiment, and if the coefficient
for a term in the model containing genotype was found to be significantly different from zero, it was interpreted as
evidence that genotype affected the response.

RESULTS

Life Span Is not Reduced in Insulin-Insensitive Insulin
Receptor Knockout Mice

From 6 to 18 months of age, both male and female IRKO+/−
mice were hyperinsulinemic, but they remained euglycemic
(Supplementary Figures 1A and B). Neither plasma concen-
trations of insulin nor glucose increased between 6 and 18 months. There was no significant effect of IR hemizygo-
sity on body weight or food intake (Supplementary Tables 1
and 2). The survival study had 11 males and 8 females in the
control group and 19 males and 27 females in the IRKO+/−
group. Although these mice are insulin insensitive (29,30),
there was no shortening of life span (p = .079, Cox propor-
tional hazards test; Figure 1). The mean life span of female
IRKO+/− mice did not differ from that of WTs. Although mean
life span of male IRKO+/− mice also did not differ from that
of WT littermates, the longest lived (90th percentile) male
IRKO+/− mice were 14% older than WTs (p < .001; Table 2).

Further analysis of the apparent sex difference in mortality
used sex as a predictor variable in the Cox proportional
hazards model. We found a significant effect of sex, that is,
females have one fourth the hazard of males (Table 3). There

was also a significant antagonistic interaction between sex
and genotype: that is, the effect of the IRKO+/− genotype is
opposite in males and females. To find the sex-specific effects of the IRKO+/− genotype, separate Cox models were fit to the
male and female data with genotype as the only predictor
variable. The coefficients fitted by the respective models
were opposite in males and females—that is, IRKO+/− males
had about 1.5 times the hazard of WT females but IRKO+/− females had about 1.5 times the hazard of WT females (Table 3).

For each of the sexes examined individually, the effect was
nonsignificant, but in males, it approached significance
(p = .06). The opposite coefficient values for the single-sex
comparisons in the context of the significant interaction in
the full comparison are evidence that the IRKO+/− genotype
lengthens the survival of males and either shortens or has no
effect on the survival of females.

In contrast with PGC-1αTG and PTP-1B−/− survival stud-
ies below, this survival study was post hoc; it consisted of
animals that died of natural causes that were part of a lar-
ger cohort from which some animals were terminated for
other experiments. To determine if the post hoc nature of
this study biased the analysis, we obtained the age at which
each of the littermates of the animals in the data set died or
was terminated or sacrificed and added them to the data set as right-censored events. The new sample sizes were 39
males and 47 females in the WT control group and 37 males
and 44 females in the IRKO+/− group. We fit the same mod-
els as earlier to this extended data set but stratified accord-
ing to whether the data came from the original animals
or the littermates of those animals. The coefficients were
found to have the same signs and similar values as in the
original data set, and the same terms were found to be sig-
nificant (Table 3). Survival studies where animals were not
observed from weaning should be interpreted with caution,
but the agreement between the two data sets suggests that
any distortion introduced by the post hoc nature of these
data was not sufficient to substantially affect the results.
Life Span Is not Extended in Insulin-Sensitive PGC-1α TG Mice

PGC-1α TG mice have previously been shown to exhibit increased glucose tolerance, increased whole-body sensitivity to insulin, and increased sensitivity of insulin-mediated glucose disposal by skeletal muscle (28). The mice also show enhanced sensitivity of skeletal muscle to insulin as measured by increased insulin-mediated AKT phosphorylation (28). However, the response to PGC-1α overexpression is tissue specific and dichotomous: PGC-1α TG mice showed hepatic insulin resistance as measured by increased basal hepatic glucose production and reduced suppression of hepatic glucose production in euglycemic-hyperinsulinemic clamp studies (31). There was no significant effect of the PGC-1α overexpression on body weight or food intake (Supplementary Tables 1 and 2). As shown in Figure 2, although mean longevity was unaffected in PGC-1α TG mice, their mortality was significantly increased during the first half of life. Specifically survival of the 20th, 25th, and 30th percentiles was 15%–20% lower in PGC-1α TG mice ($p < .05–.01$).

Life Span Is Shortened in Insulin-Sensitive PTP-1B Knockout Mice

The line used in this study (27), as well as an independently developed line (37), show enhanced insulin sensitivity and resistance to diabetes. Insulin levels are markedly reduced in PTP-1B−/− mice (27). Despite the increase in insulin sensitivity, mean life span was reduced by 20% in females and 12% in males carrying the PTP-1B null mutation (Figure 3, Table 1). Food consumption declined over time less rapidly in PTP-1B−/− mice of both sexes (Supplementary Table 2 and Supplementary Figure 2). Body weight change over time in male PTP-1B−/− mice was altered in a manner that resulted in lower values than for WT controls over the time period they were measured (Supplementary Table 1 and Supplementary Figure 1). In female PTP-1B−/−, body weight declined more abruptly than in WT controls after peaking around 400 days of age (Supplementary Table 1 and Supplementary Figure 2).

<table>
<thead>
<tr>
<th>Table 1. Effect of Modulating the Expression of Three Genes that Influence Insulin Sensitivity and Signaling on Mean Life Span in Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Insulin receptor WT</td>
</tr>
<tr>
<td>Insulin receptor KO</td>
</tr>
<tr>
<td>PTP-1B WT</td>
</tr>
<tr>
<td>PTP-1B KO</td>
</tr>
<tr>
<td>PGC-1α WT</td>
</tr>
<tr>
<td>PGC-1α TG</td>
</tr>
</tbody>
</table>

Notes: KO = knockout; PTP-1B = protein tyrosine phosphatase 1B; WT = wild type; Peroxisome proliferator-activated receptor-α coactivator.

**$p < .01$, ***$p < .001$.

<table>
<thead>
<tr>
<th>Table 2. Effect of Modulating the Expression of Three Genes That Influence Insulin Sensitivity and Signaling on Maximum Life Span, as Measured at the 90th Percentile, in Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Insulin receptor WT</td>
</tr>
<tr>
<td>Insulin receptor KO</td>
</tr>
<tr>
<td>PTP-1B WT</td>
</tr>
<tr>
<td>PTP-1B KO</td>
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<tr>
<td>PGC-1α WT</td>
</tr>
<tr>
<td>PGC-1α TG</td>
</tr>
</tbody>
</table>

Notes: KO = knockout; PTP-1B = protein tyrosine phosphatase 1B; WT = wild type.

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Life Span Is Not Extended in Insulin-Sensitive PGC-1α TG Mice

PGC-1α TG mice have previously been shown to exhibit increased glucose tolerance, increased whole-body sensitivity to insulin, and increased sensitivity of insulin-mediated glucose disposal by skeletal muscle (28). The mice also show enhanced sensitivity of skeletal muscle to insulin as measured by increased insulin-mediated AKT phosphorylation (28). However, the response to PGC-1α overexpression is tissue specific and dichotomous: PGC-1α TG mice showed hepatic insulin resistance as measured by increased basal hepatic glucose production and reduced suppression of hepatic glucose production in euglycemic-hyperinsulinemic clamp studies (31). There was no significant effect of the PGC-1α overexpression on body weight or food intake (Supplementary Tables 1 and 2). As shown in Figure 2, although mean longevity was unaffected in PGC-1α TG mice, their mortality was significantly increased during the first half of life. Specifically survival of the 20th, 25th, and 30th percentiles was 15%–20% lower in PGC-1α TG mice ($p < .05–.01$).

Table 3. Effect of Genotype and Sex on Mortality (Cox Proportional Hazards)

<table>
<thead>
<tr>
<th>Effect</th>
<th>N (Censored)</th>
<th>Coefficient</th>
<th>SE</th>
<th>Z</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC-1α TG</td>
<td>78 (5)</td>
<td>0.128</td>
<td>0.229</td>
<td>0.557</td>
<td>.58</td>
</tr>
<tr>
<td>PTP-1B−/−</td>
<td>97 (3)</td>
<td>1.347</td>
<td>0.256</td>
<td>5.263</td>
<td>.00000014***</td>
</tr>
<tr>
<td>Female</td>
<td>41 (1)</td>
<td>0.162</td>
<td>0.220</td>
<td>0.737</td>
<td>.46</td>
</tr>
<tr>
<td>Female PTP-1B−/−</td>
<td>0.000</td>
<td>0.435</td>
<td>0.001</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IRKO−</td>
<td>65</td>
<td>−0.699</td>
<td>0.398</td>
<td>−1.758</td>
<td>.07869</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>−1.355</td>
<td>0.502</td>
<td>−2.699</td>
<td>.00695***</td>
</tr>
<tr>
<td>Female IRKO−</td>
<td>1.160</td>
<td>0.581</td>
<td>1.997</td>
<td>.04587*</td>
<td></td>
</tr>
<tr>
<td>Split by sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRKO− (males only)</td>
<td>30</td>
<td>−0.826</td>
<td>0.439</td>
<td>−1.882</td>
<td>.0598</td>
</tr>
<tr>
<td>IRKO− (females only)</td>
<td>35</td>
<td>0.430</td>
<td>0.414</td>
<td>1.038</td>
<td>.299</td>
</tr>
<tr>
<td>IRKO−, including all censored littermates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRKO−</td>
<td>77 (90)</td>
<td>−0.553</td>
<td>0.392</td>
<td>−1.410</td>
<td>.16</td>
</tr>
<tr>
<td>Female</td>
<td>44 (47)</td>
<td>−1.362</td>
<td>0.478</td>
<td>−2.850</td>
<td>.0044***</td>
</tr>
<tr>
<td>Female IRKO−</td>
<td>1.162</td>
<td>0.544</td>
<td>2.130</td>
<td>.033*</td>
<td></td>
</tr>
<tr>
<td>Split by sex</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRKO− (males only)</td>
<td>33 (43)</td>
<td>−0.826</td>
<td>0.439</td>
<td>−1.882</td>
<td>.0598</td>
</tr>
<tr>
<td>IRKO− (females only)</td>
<td>44 (47)</td>
<td>0.642</td>
<td>0.377</td>
<td>1.703</td>
<td>.0885</td>
</tr>
</tbody>
</table>

Notes: IRKO = insulin receptor knockout mice; PTP-1B = protein tyrosine phosphatase 1B.

* $p < .05$, ** $p < .01$, *** $p < .001$. 

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littermates when subjected to paraquat and showed other evidence of increased resistance to oxidative stress, a result that parallels the increased resistance to oxidative stress of daf2 mutants (26). These mice were insulin insensitive as indicated by hyperinsulinemia and impaired glucose tolerance. In an earlier report (25), these mice outlived WT ob mice) are short lived (38,39). The IRKO+/− mice showed no evidence of type 2 diabetes or an age-related progression toward greater insulin resistance (Supplementary Figure 1).

They were euglycemic and showed no trend toward hyperglycemia or further elevations of circulating insulin as they aged, at least until 18 months. We selected the C57BL/6 background for this mutant because it had previously been shown to be more resistant up to 6 months of age to development of hyperinsulinemia and type 2 diabetes (40). Another issue concerns the evidence of gender differences in the longevity effect of IRKO+/− that was revealed in the Cox proportional hazards analysis, suggesting that mortality in males is diminished by reduced insulin signaling (although in females, mortality is either unchanged or increases slightly). This result underscores the importance of measuring mortality in both sexes and parallels, albeit in an opposite direction, gender differences in the life-span response to reduced IGF-1 receptor (14,15) and other insulin-like signaling intermediates (41). The results with the IRKO+/− mouse are consistent with the recent report that life span was not shortened in a mouse in which the insulin receptor gene was replaced with a gene containing a substitution that mimicked one of the daf2 mutants (26). These mice were insulin insensitive as indicated by hyperinsulinemia and impaired glucose tolerance. In an earlier report (25), these mice outlived WT littermates when subjected to paraquat and showed other evidence of increased resistance to oxidative stress, a result that parallels the increased resistance to oxidative stress of insulin/IGF1-like signaling mutants in invertebrates (1,4).

**Discussion**

This is the first a priori exploration of the role of insulin sensitivity in murine longevity. We used three strains genetically modified specifically to either increase or reduce insulin sensitivity. The results indicate that impairment of insulin signaling by direct genetic manipulation of the insulin receptor and consequent reduction in insulin sensitivity does not shorten life span and can even lengthen life. Conversely, increasing insulin sensitivity by knocking out PTP-1B and thereby attenuating dephosphorylation of key intermediates in the insulin signaling cascade (27) shortens life span. Similarly, enhancement of components of insulin sensitivity in a whole-animal PGC-1α overexpressing model led to increased mortality in the first half of the life span. Thus, there was an inverse relationship between insulin sensitivity and longevity in the three strains examined.

Whether the inverse relationship between insulin sensitivity and life span observed in this study is more broadly applicable to other strains and conditions remains to be determined. However, similar to the insulin-signaling-impaired IRKO+/− mice in this study, impairment of insulin signaling by substituting the normal insulin receptor allele with a mutant allele using homologous recombination also did not shorten life (26).

In addition to the question of ubiquity of the relationship between insulin sensitivity and murine longevity, there are other caveats to consider. First, these genetic manipulations undoubtedly have pleiotropic effects beyond their effects of insulin signaling that could also contribute to the life-span phenotype. The IRKO+/− model is probably least susceptible to this possibility because it involves direct interference with insulin action at the level of the cognate receptor (ie, reducing receptor expression). That these mice were insulin insensitive is evidenced by their persistent hyperinsulinemia—at least up to 18 months of age, the last age examined. This result counters the notion that insulin insensitivity, per se, is necessarily deleterious in mammals. Although increased insulin sensitivity is often found in calorie-restricted and mutant mice with exceptional longevity, these are correlations and their causal role in longevity has not been established. Even if moderate insulin insensitivity is life-extending, at some point, greater insensitivity will be deleterious. Mice homozygous for the null mutation of the IR are fetal lethal (29) and mice with spontaneous mutations leading to type 2 diabetes (eg, ob/ob mice) are short lived (38,39). The IRKO+/− mice showed no evidence of type 2 diabetes or an age-related progression toward greater insulin resistance (Supplementary Figure 1). They were euglycemic and showed no trend toward hyperglycemia or further elevations of circulating insulin as they aged, at least until 18 months. We selected the C57BL/6 background for this mutant because it had previously been shown to be more resistant up to 6 months of age to development of hyperinsulinemia and type 2 diabetes (40). Another issue concerns the evidence of gender differences in the longevity effect of IRKO+/− that was revealed in the Cox proportional hazards analysis, suggesting that mortality in males is diminished by reduced insulin signaling (although in females, mortality is either unchanged or increases slightly). This result underscores the importance of measuring mortality in both sexes and parallels, albeit in an opposite direction, gender differences in the life-span response to reduced IGF-1 receptor (14,15) and other insulin-like signaling intermediates (41). The results with the IRKO+/− mouse are consistent with the recent report that life span was not shortened in a mouse in which the insulin receptor gene was replaced with a gene containing a substitution that mimicked one of the daf2 mutants (26). These mice were insulin insensitive as indicated by hyperinsulinemia and impaired glucose tolerance. In an earlier report (25), these mice outlived WT littermates when subjected to paraquat and showed other evidence of increased resistance to oxidative stress, a result that parallels the increased resistance to oxidative stress of insulin/IGF1-like signaling mutants in invertebrates (1,4).
Taken together, these results indicate that moderate insulin insensitivity that falls short of inducing type 2 diabetes does not appear to be life shortening in mice and may even be life-extending depending on gender, the environment, or to tissue specificity.

Mortality patterns differed in the two strains that were hypersensitive to insulin. PTP-1B−/− mice had markedly shortened life span—reflected in increased mortality throughout adult life. By contrast, PGC-1αTG mice only showed increased mortality during early adulthood. Two possible reasons for the midlife normalization of mortality in PGC-1αTG mice are an attenuation of insulin hypersensitivity due to a loss of expression of the insulin-sensitizing transgene or neutralization, the hypersensitivity by an age-related decrease in sensitivity to insulin. The proteins encoded by PTP-1B and PGC-1α subserve multiple pathways in addition to cognate insulin signaling. Thus, their negative effects on longevity cannot solely be ascribed to their effects on insulin sensitivity. However, the available evidence indicates that negative effect on longevity of the PTP-1B deletion is not the result of concomitant hypersensitization of the IGF-1 signaling pathway because plasma IGF-1 levels were not altered (unpublished observations and [27]) and in vitro sensitivity to IGF-1 was not increased in PTP-1B−/− mice (27). Nevertheless, lack of PTP-1B could have a life-shortening effect through modulation of other growth receptors. Overexpression of PGC-1αTG also may have pleiotropic actions beyond insulin signaling that could contribute to the effect or lack thereof on longevity. In addition, PGC-1αTG mice exhibited opposite effects on insulin sensitivity in liver and muscle, although whole-animal insulin sensitivity was significantly enhanced (28). Although other models of exceptional longevity, such as mice under dietary restriction or dwarf mice, show evidence of enhanced insulin sensitivity, the multifactorial effects of these models on pathways and mechanisms that are plausible contributors to the prolongation of life reveal the difficulty of determining whether they are important or ancillary with respect to extended life span. Indeed, as exemplified by the two models developed for this study, devising models to specifically determine the effect of hypersensitivity to insulin on life span seems more difficult than testing the role of reduced insulin sensitivity. One of the most unambiguous tests of this question would be to construct a set of genetically modified insulin receptors that covered a broad range of insulin action from large reductions in sensitivity to large increases.

In studies measuring the effects of genetic, pharmacologic, or nutritional interventions on longevity, measurement of food intake and body weight are needed to determine whether any life-span extension might be secondary to dietary restriction resulting from the intervention. No effects on food intake or body weight were observed in the IRKO−/− or PGC-1αTG mice. The modest reduction of food intake and body weight observed in PTP-1B−/− mice was not associated with life-span lengthening. Whether the reduced food intake and body weight are related to the shortened life span of these mice remains to be determined.

These results that suggest that reduced insulin signaling and concomitantly increased insulin resistance can reduce mortality, whereas interventions that increase insulin signaling and reduce resistance can increase mortality, have potential implications for understanding type 2 diabetes as well as human aging. The role of insulin resistance, per se, compared with that of hyperglycemia in the pathological sequelae of type 2 diabetes remains unclear. The finding that specific interference with insulin signaling can increase resistance to oxidative stress and reduce mortality in some conditions indicates that insulin resistance may be a homeostatic mechanism not only to maintain euglycemia but also to protect against oxidative/inflammatory processes during metabolic stress and in obesity. In the Baltimore Longitudinal Study of aging, analysis of insulin and glucose in the oral glucose tolerance test as predictors of mortality found that hyperglycemia at 2 hours after onset of oral glucose tolerance test was the only predictor of increased mortality. Hyperinsulinemia had no explanatory value when glucose was accounted for (42).

In conclusion, these results, as well as earlier studies that bear on this question, reveal that increasing insulin sensitivity, albeit through means that may also target other pathways, can shorten life span. The evidence is stronger than reducing insulin sensitivity, provided that diabetes is not induced, either has little effect or under some circumstances can lengthen life. Thus, there are some parallels between the modulatory effects of insulin signaling on life span in mice and those of insulin-like signaling in nematodes and flies.

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Supplementary Material
Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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