Response to Comment on “Brain IRS2 Signaling Coordinates Life Span and Nutrient Homeostasis”

Akiko Taguchi and Morris F. White*

Differences in reported life span of mice heterozygous for a null allele of the insulin receptor substrate–2 (Irs2) might involve the effects of diet, breeding strategies, and genetic background on insulin-like signaling cascades. A better understanding will emerge from studies focusing on the coordination of nutrient homeostasis and life span by insulin-like signaling in specific peripheral tissues and the central nervous system.

Our experiments show that systemic Irs2–/+ mice live about 18% longer than wild-type (WT) mice (1). Selman et al. (2) question this finding because their systemic Irs2–/+ mice are indistinguishable from their control group (3). Indeed, we were surprised by our results because we thought the Irs2–/+ mice might develop diabetes and die sooner than expected. The different experimental designs used by our laboratory and by Selman et al.—including diet, breeding strategies, and housing conditions—might explain the different outcomes. Selman et al. maintained their mice on a 5% fat diet (Teklad Global 18% Protein Diet, #2018; metabolizable energy = 3.3 kcal/g), whereas all of our mice were maintained on a 9% fat diet (Picolab Diet 20, #5058; metabolizable energy = 3.5 kcal/g). This difference alone might explain why our Irs2–/+ mice have a longer life span. Indeed, on a caloric-dense diet, mutant Drosophila (Chico) lacking the Irs ortholog live longer than control flies, whereas Chico dies before control flies when caloric density of the food is reduced (4). It will be important to investigate the interaction between calorie intake and Irs2-regulated life span of mice.

We also used a different breeding strategy than Selman et al. (2, 3), which might influence the outcome. Selman et al. tested the life span of 51 WT and 87 Irs2–/+ mice generated from male and female Irs2–/+ mice that were backcrossed 10 times onto a C57BL/6J background (3). We intercrossed male and female Irs2–/+ mice—previously backcrossed 6 times onto the C57BL/6J background—to generate over a 15-month interval 27 litters composed of WT mice, 73 Irs2–/+ mice, and 23 Irs2–/+ mice (table S1). Thus, our experimental cohort was produced at different times of the year and from many different parents that were not yet on a pure C57BL/6J background. More genetic heterogeneity rather than less might be a better choice to assess the effects of genes upon life span (5).

Selman et al. separated their mice into groups of same-sex littersmates within individually ventilated pathogen-free cages containing three to eight mice that were monitored daily and weighed monthly but otherwise left undisturbed until they died naturally (3). Our same-sex littersmates were also housed in a pathogen-free environment, but with no more than five mice per cage. However, many of the mice were also used to measure glucose and insulin tolerance, food intake, and circulating insulin and glucose concentrations (table S1). In addition, 52% of the WT mice, 56% of the Irs2–/+ mice, and all the Irs2–/+ mice were removed from the longevity study at various times for use in other experiments (table S1, Censor = 0). We used this design to obtain metabolic assessments from the same cohort of mice used for the life-span determination.

In their comment, Selman et al. (2) conclude that the life-span extension of Irs2–/+ mice in our experiments arises from atypical survival profiles owing to experimental design, husbandry, or environmental factors rather than a genetic effect. Realizing the complexity of our experimental design, we used the Cox proportional regression to establish whether the Irs2–/+ mice had a longer life span than WT mice (1). However, in our original report, we only analyzed the subset of mice that were followed to a natural death, which included 30 WT mice and 31 Irs2–/+ mice (table S1, Censor = 1). To determine whether censoring biased our original analysis, we reanalyzed all the mice, including the mice that were removed for experimental purposes. In addition to covariates for sex, parental identities, and date of birth, we also included a quantitative covariate indicating the size of the litter into which each mouse was born and categorical covariates to indicate whether experimental tests were conducted on the mice (table S1). Consistent with our previous results, Cox regression revealed a risk of death lower by a factor of 38 (P < 0.0001) for Irs2–/+ mice compared with WT mice. Male mice had a greater risk of death than female mice (risk ratio = 5.6, P = 0.002), and whether or not experimental tests were conducted upon the mice had no significant effect (table S2). However, the litter size influenced life span; the risk of death was reduced 37% (risk ratio = 0.6, P = 0.032) for each additional mouse in the litter (table S2). Larger litters tend to reduce postnatal nutrition and growth, which correlates with an increased life span (6). Thus, in addition to the strong Irs2 gene effect, our results reveal the expected effect of sex and an effect of litter size. Even Selman et al. found a beneficial gene effect in female Irs2–/+ mice (risk ratio = 0.7, CI = 0.41 to 1.09, P = 0.108) at marginal significance (3).

Most investigators, including Selman et al., use Kaplan-Meier curves and a log-rank test to determine the effect of gene deletions on life span (2, 3). This analysis is ideal for small animals like C. elegans or Drosophila that produce many offspring with relatively short life spans where covariates can be tightly controlled; however, to use the Kaplan-Meier analysis we must ignore the confounding covariates that influence the result. Regardless, Kaplan-Meier analysis revealed a robust difference (log rank X2 = 39.8, P = 2.8 × 10–10) between WT mice and Irs2–/+ mice, confirming that the male and female Irs2–/+ mice survived 17% (mean) to 19% (median) longer than WT mice (Table 1). Moreover, the Kaplan-Meier analysis did not identify any confounding covariates that influence the result. Table 1. Kaplan-Meier analysis of life span in WT and Irs2–/+ mice. The data in table S1 were analyzed by Kaplan-Meier analysis in SPSS V16. The number of censored mice (N) and the percentage of the total (%) that were removed before reaching a natural death are indicated. The mean and median survival is reported with the standard error (SE) and the 95% confidence interval (95% CI).

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Table 1. Kaplan-Meier analysis of life span in WT and Irs2–/+ mice. The data in table S1 were analyzed by Kaplan-Meier analysis in SPSS V16. The number of censored mice (N) and the percentage of the total (%) that were removed before reaching a natural death are indicated. The mean and median survival is reported with the standard error (SE) and the 95% confidence interval (95% CI).

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<th>Median survival</th>
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<td>44</td>
<td>16</td>
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<td>790 ± 14</td>
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<tr>
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<tr>
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<td>28</td>
<td>51</td>
<td>934 ± 31</td>
<td>873–995</td>
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Howard Hughes Medical Institute, Division of Endocrinology, Children’s Hospital Boston, Harvard Medical School, Boston, MA 02215, USA.

*To whom correspondence should be addressed. E-mail: morris.white@childrens.harvard.edu
profile has a different shape than Selman et al. — owing to a later onset and narrower range of deaths in our cohort — it has a similar shape to the survival curves published recently by others (Fig. 1A) (7). Whether our survival curve is atypical is at best open for discussion. Many factors could increase the number of early deaths that affect the shape of a survival curve, including mice generated from small litters sizes (Fig. 1B). Thus, we disagree with Selman et al. that our survival curves led to erroneous conclusions.

It might be difficult to determine exactly why our systemic Irs2+/− mice live longer than those of Selman et al. The life span of a group of mice can vary between test sites even when many environmental variables are controlled (5). A better understanding of the variables will be important before life span collected at different times and in different places can be compared reliably between institutions. Our finding that the deletion of neuronal Irs2 also increases life span of overweight and glucose-intolerant mice by 18% supports the inference that the brain is the location where reduced insulin-like signaling can extend life span (1).

References
7. G. Laurent et al., Cell Metab. 7, 113 (2008).

Supporting Online Material
www.sciencemag.org/cgi/content/full/320/5879/1012c/DC1
Tables S1 and S2

References
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Fig. 1. (A) Kaplan-Meier curves (SPSS V16) for WT mice (blue line) and Irs2+/− mice (red line) and theoretical Cox regression curves (SAS V9.1) for WT mice (gray line) and Irs2+/− mice (black line). The survival curve (gray dashed line) for WT mice taken from (7) is shown for comparison. (B) Theoretical Cox regression survival curves (SAS V9.1) showing the effect of litter size on survival of WT mice (2 mice, light gray; 4 mice, gray; 10 mice, black) and Irs2+/− mice (2 mice, light red; 4 mice, red; 10 mice, dark red) determined at the mean value for the other covariates (see table S1 for details).
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