Comment on "Brain IRS2 Signaling Coordinates Life Span and Nutrient Homeostasis"

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Taguchi *et al.* (Reports, 20 July 2007, p. 369) reported that mice heterozygous for a null mutation in insulin receptor substrate–2 (Irs2) display a 17% increase in median life span. However, using the same mouse model, we find no evidence for life-span extension and suggest that the findings of Taguchi *et al.* were due to atypical life-span profiles in their study animals.

Taguchi *et al.* (*1*) demonstrated a 17% increase in median and maximum life span in $Irs2^{+/-}$ compared with wild-type (WT) mice on a C57BL/6J background. Although time-consuming and expensive, mouse longevity studies require replication to confirm that findings are robust and reproducible across laboratories and hence of broad applicability to mammalian aging. We made a parallel study of $Irs2^{+/-}$ mice (*2*) using the same model (*3*, *4*) but, in contrast to Taguchi *et al.*, we did not see life-span extension (*2*).

Using $Irs2^{+/-}$ parents, we bred WT and $Irs2^{+/-}$ mice and aged them in a specific-pathogen–free facility in individually ventilated cages under our standard husbandry conditions (*2*, *5*). We studied 51 WT and 87 $Irs2^{+/-}$ mice, which is close to the expected Mendelian ratio of progeny genotypes. Kaplan-Meier survival curves were indistinguishable between the two genotypes (Fig. 1A), with no significant differences in cumulative mortality rate (log-rank test: $X^2 = 1.79$, $P > 0.05$). Parental identity, sex, genotype, and birth date had no significant influence on life span (Cox regression analysis, table S1).

We sought explanations for the discrepancy by examining the respective study designs and directly comparing the published longevity data (*1*, *2*). Both studies used the same gene-targeted animal (*3*, *4*) on a C57BL/6J background and similar husbandry conditions, although Taguchi *et al.* used a relatively high-fat diet (9% fat) compared with our study (5% fat). Differences in dietary composition might influence longevity (*6*–*8*). Taguchi *et al.* reported data from 30 WT and 31 $Irs2^{+/-}$ mice, a 1:1 ratio, which implies that not all progeny from their $Irs2^{+/-} \times Irs2^{+/-}$ intercrosses used to generate the study groups (and which should give a 1:2 ratio of WT:$Irs2^{+/-}$ animals) were entered into the study. One possibility is that a potential failure to study all the available $Irs2^{+/-}$ progeny from these crosses in the life-span trials introduced an uncontrolled variable (e.g., parental identity or recruitment date), which increased the life span of the $Irs2^{+/-}$ population. Indeed, these factors appeared to influence the longevity of offspring independent of genotype in the Taguchi *et al.* study (*1*). A difference in the number of backcrosses onto the C57BL/6J background (6 for Taguchi *et al.* and 10 for our animals) and potential differences in colony health status and stocking density may also explain the observed differences.

Comparison of the longevity profiles of the WT control animals in the two studies (*1*, *2*) shows similar cumulative mortality risk profiles and median life span (Table 1; $X^2 = 1.62$, $P > 0.05$). However, Kaplan-Meier survival curves constructed using Taguchi *et al.*'s life-span data demonstrated a later onset of deaths and a more precipitous fall in survival in WT mice from their $Irs2^{+/-}$ study compared with those from our study, resulting in a truncated period over which all WT deaths occurred in their study (Fig. 1B). Importantly, the life-span profiles of the same-strain control mice for the brain-specific *Irs2* mutant described by Taguchi *et al.* did not resemble those from their $Irs2^{+/-}$ study and instead were similar to the WT data from our $Irs2^{+/-}$ study (Fig. 1 and Table 1). Furthermore, there were significant differences between mean age of death of the oldest 10% and youngest 10% WT animals from Taguchi *et al.*'s $Irs2^{+/-}$ study, compared with both our and Taguchi *et al.*'s brain-specific *Irs2* mutant study ($X^2 = 22.14$, $P < 0.001$ and $X^2 = 11.03$, $P < 0.01$ for the oldest and youngest 10% of each population, respectively), and the range of ages at time of death was also markedly different in this study (Table 1). Thus, the survival curves of the control mice from Taguchi *et al.*'s $Irs2^{+/-}$ study are very different from our study (Fig. 1 and Table 1) and their own brain-specific *Irs2* mutant study (Fig. 1B and Table 1) and do not appear to resemble those of others who have studied life span in C57BL/6 mice [e.g., (*9*–*14*)]. In addition, although Taguchi *et al.* reported a significant increase in median life span in $Irs2^{+/-}$ mice, the 10th decile of survivorship, which is generally accepted to be an indication that the aging process has been altered (*11*, *12*), was not significantly different ($X^2 = 0.290$, $P > 0.05$) from our $Irs2^{+/-}$ animals (Table 1). Therefore, the WT life-span data supporting Taguchi *et al.*'s conclusion of increased longevity in $Irs2^{+/-}$ mice is atypical for this mouse strain, and robust conclusions about the role of systemic IRS2 in longevity cannot be made.

In contrast, our studies, using large numbers of WT and $Irs2^{+/-}$ animals that showed typical mortality curves for C57BL/6J mice, did not

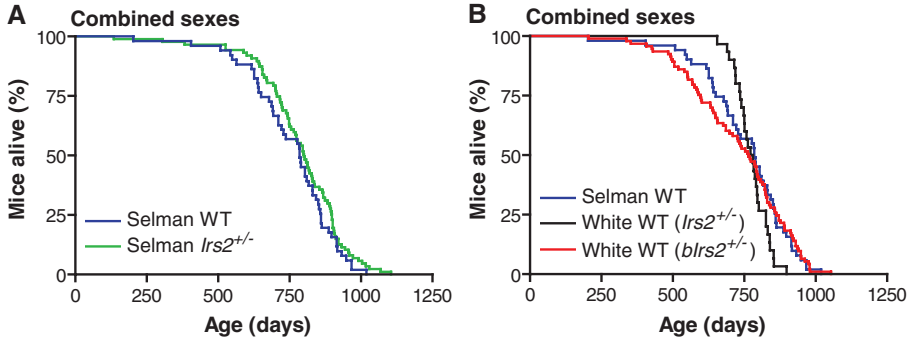

Fig. 1. Life span of systemic $Irs2^{+/-}$ mice and WT mice from (*2*) and a comparison of WT mice from the studies of Selman *et al.* (*2*) and Taguchi *et al.* (*1*). (**A**) Kaplan-Meier survival curves are shown for WT and $Irs2^{+/-}$ mice for combined sexes of the *Irs2* strain from (*2*). Cumulative mortality risk profiles, as determined by log-rank analysis, were not significantly different in $Irs2^{+/-}$ mice compared with WT mice ($X^2 = 1.79$, $P > 0.05$). Survival was assessed from 138 mice (87 $Irs2^{+/-}$; 51 $Irs2^{+/+}$). Green indicates $Irs2^{+/-}$ mice and blue indicates WT mice. (**B**) Kaplan-Meier survival curves are shown for WT mice for combined sexes of the *Irs2* strain from either Selman *et al.* or Taguchi *et al.* Cumulative mortality risk profiles, as determined by log-rank analysis, were not significantly different in WT mice compared across the three studies ($X^2 = 1.62$, $P > 0.05$). Survival was assessed from 174 mice. Blue indicates WT mice from the systemic *Irs2* of Selman *et al.* ($n = 51$), black indicates WT mice from the systemic *Irs2* comparison of Taguchi *et al.* ($n = 30$), and red indicates WT mice from the brain-specific *Irs2* comparison of Taguchi *et al.* ($n = 93$).

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Table 1. Comparative survival characteristics of systemic *Irs2*$^{+/-}$ and WT mice from Selman *et al*. (*2*) and Taguchi *et al*. (*1*). Life span is reported in days (±SEM, where appropriate) for WT and *Irs2*$^{+/-}$ mice. Oldest 10% and youngest 10% were calculated as the mean life span of the longest (or shortest) living 10% of animals within a genotype. *n* = sample size.

Genotype	Median	Mean ± SEM	Range	Oldest 10%	Youngest 10%	*n*
			Selman et al. (*2*)			
WT	785	755 ± 22	203–1019	967 ± 15	442 ± 65	51
Irs2$^{+/-}$	800	788 ± 17	135–1105	1011 ± 17	480 ± 57	87
			Taguchi et al. (*1*)			
WT*	776	775 ± 10	655–898	868 ± 15	681 ± 13	30
WT†	722	730 ± 18	205–1054	975 ± 11	403 ± 31	93
Irs2$^{+/-}$	924	905 ± 22	402–1042	1037 ± 4	634 ± 116	31

*WT mice are control mice from the systemic *Irs2* comparison of Taguchi *et al*. †WT mice are control mice from the brain-specific *Irs2* comparison of Taguchi *et al*.

implicate systemic IRS2 signaling in the regulation of longevity. Indeed, in separate studies, we found that double heterozygote *Irs1*$^{+/-}$:*Irs2*$^{+/-}$ mice had no increase in life span ($X^2 = 3.39$, $P > 0.05$) (figure S1 and table S2) and no alterations in the life span of the oldest 10% and youngest 10% of mice (oldest 10%: $X^2 = 0.511$, $P > 0.05$ and youngest 10%: $X^2 = 0.802$, $P > 0.05$), with no recruitment, sex, or parental effects (table S3). These findings strongly suggest that partial loss of function in this pathway does not increase life span in mammals. We recently presented data that provisionally suggests that global deletion of *Irs1* increases life span (*2*), indicating that appropriate manipulation of systemic IRS signaling has the potential to extend longevity. Therefore, we are able both to generate typical life-span profiles for C57BL/6J mice and to detect life-span extension under our husbandry conditions, which suggests that the absence of life-span extension in our *Irs2*$^{+/-}$ mice is unlikely to be attributable to our study conditions. Moreover, deletion of *Irs1* delayed a range of age-related changes (*2*), suggesting that aging was genuinely retarded in *Irs1*$^{-/-}$ mice. Such evidence is lacking for *Irs2*$^{+/-}$ mice. We therefore conclude that the atypical mouse survival profiles (which may have resulted from experimental design, husbandry, or environmental factors) probably account for the apparent life-span extension in *Irs2*$^{+/-}$ mice reported by Taguchi *et al*. The discrepancies in the findings underscore the value of replication of mouse life-span measurements in different laboratories before categorical statements about the role of specific factors in the regulation of mammalian life span can be made.

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Supporting Online Material

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Fig. S1
Tables S1 to S3

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