# Comment on "HST2 Mediates SIR2-Independent Life-Span Extension by Calorie Restriction"

Matt Kaeberlein, *et al.*

*Science* **312**, 1312b (2006);
DOI: 10.1126/science.1124608



Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 

# Comment on "*HST2* Mediates *SIR2*-Independent Life-Span Extension by Calorie Restriction"

Matt Kaeberlein,[1]* Kristan K. Steffen,[2] Di Hu,[2] Nick Dang,[2] Emily O. Kerr,[2] Mitsuhiro Tsuchiya,[2] Stanley Fields,[3] Brian K. Kennedy[2]*

Calorie restriction (CR) increases life span in yeast independently of Sir2. Lamming *et al*. (Reports, 16 September 2005, p. 1861) recently proposed that Sir2-independent life-span extension by CR is mediated by the Sir2 paralogs Hst1 and Hst2. Contradictory to this, we find that CR greatly increases life span in cells lacking Sir2, Hst1, and Hst2, which suggests that CR is not mediated by Sir2, Hst2, or Hst1.

Calorie restriction (CR), instituted by reducing the glucose concentration of the media from 2% to 0.5% or lower, increases yeast replicative life span and was proposed to work through a Sir2-dependent mechanism (*1*). We recently reported that CR increases life span in the long-lived BY4742 strain to a greater extent in cells lacking Sir2 than in wild-type cells, as long as extrachromosomal ribosomal DNA (rDNA) circles are kept at low levels by deletion of the gene coding for the replication fork block protein, Fob1 (*2*). This discovery has since been confirmed in a report by Lamming *et al*. (*3*), in which they extended our work and reported that CR fails to increase the life span of cells lacking Sir2, Hst2, and Hst1. Based on this finding, Lamming *et al*. (*3*) propose that Sir2-family proteins (sirtuins) are activated by CR, and a Sir2-redundant function of Hst2 (and to a lesser extent Hst1) as a repressor of rDNA recombination accounts for Sir2-independent life-span extension by CR.

To test the model of Lamming *et al*. (*3*), we generated yeast lacking Sir2, Hst1, Hst2, and Fob1 and determined the effect of CR on life span. Contradictory to the results of Lamming *et al*. (*3*), we observed a significant life span extension at 0.5% (23% increase in mean life span, $P = 0.003$), 0.05% (66% increase in mean life span, $P = 1.3 \times 10^{-8}$), and 0.005% (72% increase in mean life span, $P = 4.9 \times 10^{-9}$) glucose in BY4742 *sir2*Δ *hst1*Δ *hst2*Δ *fob1*Δ mother cells (Fig. 1A). Lamming *et al*. (*3*) also report that a genetic model of CR, deletion of the gene encoding hexokinase, *HXK2*, fails to increase the life span of BY4742 *sir2*Δ *fob1*Δ *hst1*Δ *hst2*Δ mother cells. In contrast, we found that *sir2*Δ *fob1*Δ *hst1*Δ *hst2*Δ *hxk2*Δ mother cells were significantly longer lived (62% increase in mean life span, $P = 6.3 \times 10^{-7}$) than *sir2*Δ *fob1*Δ *hst1*Δ *hst2*Δ mother cells (Fig. 1B). These data are consistent with the model that Sir2-independent life-span extension by CR is not mediated by Hst2 or Hst1 (or both).

In addition to BY4742, the W303AR5 strain was used by Lamming *et al*. (*3*) to examine Sir2-independent life-span extension by CR. In

[1]Department of Pathology, [2]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA. [3]Departments of Genome Sciences and Medicine, Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA.

*To whom correspondence should be addressed. E-mail: kaeber@u.washington.edu (M.K.); bkenn@u.washington.edu (B.K.K.)

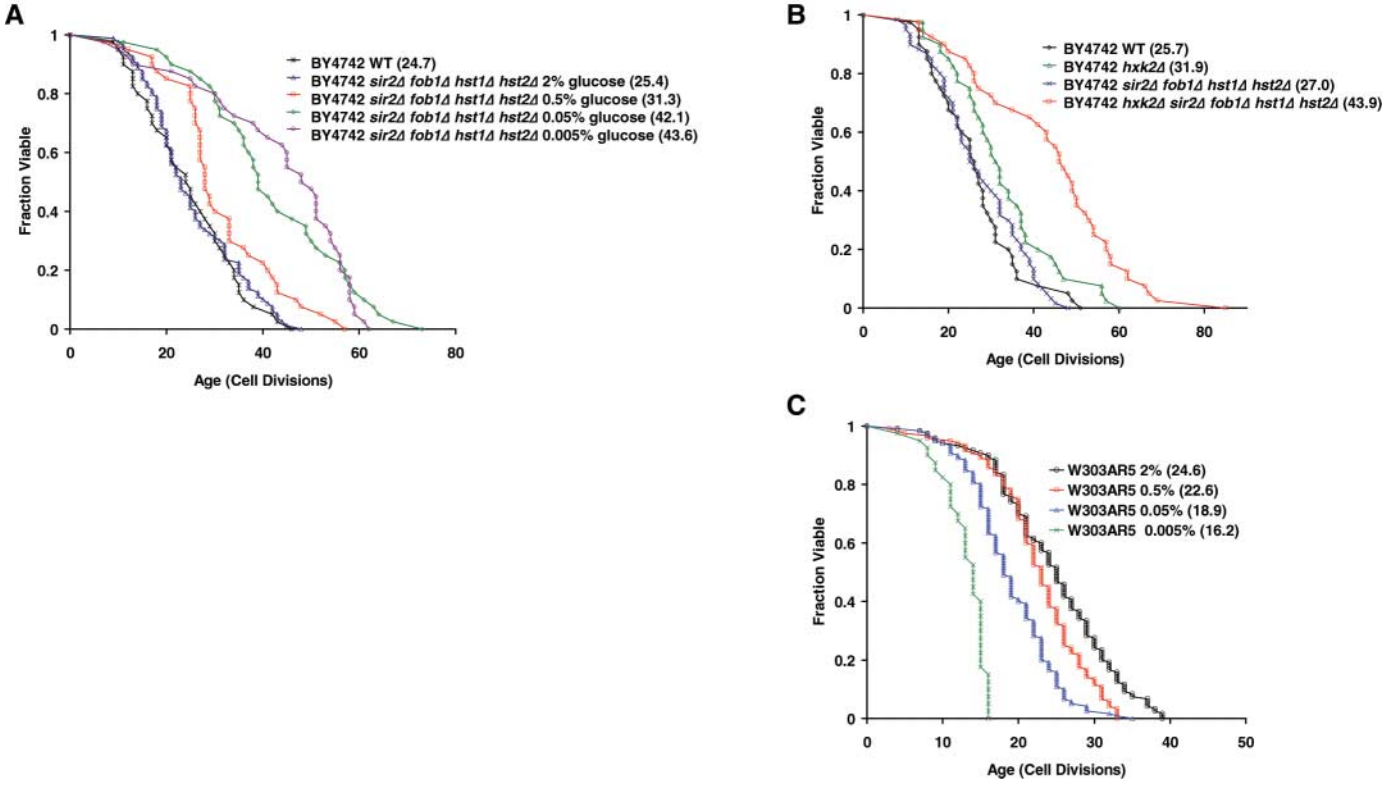


**Fig. 1.** Life-span extension by CR occurs independently of Sir2, Hst1, and Hst2. (**A**) CR significantly increases the life span of BY4742 *sir2*Δ *fob1*Δ *hst1*Δ *hst2*Δ mother cells at 0.5%, 0.05%, and 0.005% glucose. (**B**) Deletion of *HXK2* significantly increases the life span of BY4742 *sir2*Δ *fob1*Δ *hst1*Δ *hst2*Δ mother cells. (**C**) CR fails to increase the life span of W303AR5 mother cells. Mean life spans shown in parentheses.

previous work from the Sinclair and Guarente labs (*4*–*6*), experiments measuring the effect on life span of CR by growth on low glucose have been carried out solely in the PSY316 strain background, with W303AR5 used for rDNA recombination analysis and life span experiments involving *SIR2* or *FOB1*. To the best of our knowledge, the report by Lamming *et al*. (*3*) is the first to claim life-span extension from growth on reduced glucose in W303AR5. We therefore further examined the effect of CR in W303AR by determining the life span of W303AR5 cells at different glucose concentrations ranging from 2% to 0.05%. We found no significant life-span extension by CR at any reduced glucose level (Fig. 1C). It is unclear why the data of Lamming *et al*. (*3*) differ from ours. All of our experiments were carried out using the standard yeast replicative life-span methodology (*2*, *7*, *8*), with researchers performing the microdissection blind to the identity of individual strains within each experiment.

In addition to our findings reported here, the notion that Sir2 and other sirtuins redundantly mediate the CR response, as proposed by Lamming *et al*. (*3*), is difficult to reconcile with several observations. First, CR increases life span by a greater magnitude in *fob1*Δ cells relative to wild-type cells, irrespective of Sir2, Hst1, and/or Hst2 activity (*2*). Second, PSY316, a strain that shows robust life-span extension in response to CR, shows no life-span effect in response to increased expression of Sir2 (*9*). Third, the in vivo activity of Sir2, as measured by telomere silencing, is not enhanced by CR (*7*, *8*). Finally, CR does not extend life span in a *sir2*Δ *FOB1* strain (*1*), and Lamming *et al*. (*3*) offer no explanation as to why CR does not activate Hst2 and Hst1 to offset the loss of Sir2 under these conditions. Further, the report by Lamming *et al*. (*3*) that Hst2 inhibits rDNA recombination in a Sir2-independent fashion contradicts previous findings by Gasser's group (*10*).

It is important to resolve the controversy over whether sirtuins function to mediate life-span extension in response to CR. Parallel studies are ongoing in other model systems such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and mice. Our data here demonstrate that Sir2, Hst1, and Hst2 are not required either alone or in combination for life-span extension by CR in yeast, consistent with the model that CR is not mediated by sirtuins in this organism.

**References**


1. S. J. Lin, P. A. Defossez, L. Guarente, *Science* **289**, 2126 (2000).
2. M. Kaeberlein, K. T. Kirkland, S. Fields, B. K. Kennedy, *PLoS Biol.* **2**, E296 (2004).
3. D. W. Lamming *et al.*, *Science* **309**, 1861 (2005); published online 28 July 2005 (10.1126/science.1113611).
4. R. M. Anderson, K. J. Bitterman, J. G. Wood, O. Medvedik, D. A. Sinclair, *Nature* **423**, 181 (2003).
5. M. Kaeberlein, M. McVey, L. Guarente, *Genes Dev.* **13**, 2570 (1999).
6. S. J. Lin *et al.*, *Nature* **418**, 344 (2002).
7. M. Kaeberlein *et al.*, *Science* **310**, 1193 (2005).
8. M. Kaeberlein *et al.*, *PLoS Genet.* **1**, e69 (2005).
9. M. Kaeberlein *et al.*, *J. Biol. Chem.* **280**, 17038 (2005).
10. S. Perrod *et al.*, *EMBO J.* **20**, 197 (2001).


4 January 2006; accepted 4 April 2006
10.1126/science.1124608