farms alone. If this is the case, then subsequent dilution through migration and admixture, after the arrival of the first farmers, would need to be invoked, implying multiple episodes of population turnover, which are not necessarily observable in the archaeological record. This, in turn, would mean that the classic model of European ancestry components (contrasting hunter-gatherers with early Neolithic farming pioneers) requires revision.

The geographic origin of the demographic processes that brought the early farmer mtDNA types to central Europe now becomes a major question. On the one hand, all of the early farmer remains analyzed here are associated with the LBK culture of central Europe. Based on ceramic typology, the LBK culture is thought to have originated in present-day western Hungary and southwestern Slovakia, with a possible predecessor in the southeast European Starčevo-Kris culture (19, 20). These cultural source locations may provide the most plausible origins or routes for the geographic spread of the early farmers, considering that the LBK was the first major farming culture in central and northern Europe and is archaeologically attested to have disseminated over five centuries and covered nearly a million square kilometers. Alternatively, the farmers’ mtDNA types may have an origin closer to the Neolithic core zone in southwestern Asia. Further ancient DNA analysis of early farmer samples from southeastern Europe and Anatolia will be required to resolve this question.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1176689/DC1 Materials and Methods
Fig. S1 Tables S1 to S6 References
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Ribosomal Protein S6 Kinase 1 Signaling Regulates Mammalian Life Span

Colin Selman,1 Jennifer M. A. Tullet,2 Daniela Wieser,3 Elaine Irvine,4 Steven J. Lingard,5 Agharul I. Choudhury,1 Marc Claret,1 Hind Al-Qassab,1 Danielle Carmignac,4 Faruk Ramadani,5 Angela Woods,5 Iain C. A. Robinson,4 Eugene Schuster,3 Rachel L. Batterham,1 Sara C. Kozma,7 Jennifer M. A. Tullet,2 Daniela Wieser,3 Elaine Irvine,1 Steven J. Lingard,1 Agharul I. Choudhury,1 Marc Claret,1 Hind Al-Qassab,1 Danielle Carmignac,4 Faruk Ramadani,5 Angela Woods,5 Iain C. A. Robinson,4 Eugene Schuster,3 Rachel L. Batterham,1 Sara C. Kozma,7 George Thomas,7 David Carling,6 Klaus Okkenhaug,5 Janet M. Thornton,3 Linda Partridge,2 David Gems,2 Dominic J. Withers1,8†

Caloric restriction (CR) protects against aging and disease, but the mechanisms by which this affects mammalian life span are unclear. We show in mice that deletion of ribosomal S6 protein kinase 1 (S6K1), a component of the nutrient-responsive mTOR (mammalian target of rapamycin) signaling pathway, led to increased life span and resistance to age-related pathologies, such as bone, immune, and motor dysfunction and loss of insulin sensitivity. Deletion of S6K1 induced gene expression patterns similar to those seen in CR or with pharmacological activation of adenosine monophosphate (AMP)–activated protein kinase (AMPK), a conserved regulator of the metabolic response to CR. Our results demonstrate that S6K1 influences healthy mammalian life span and suggest that therapeutic manipulation of S6K1 and AMPK might mimic CR and could provide broad protection against diseases of aging.

Genetic studies in Saccharomyces cerevisiae, Caenorhabditis elegans, and Drosophila melanogaster implicate several mechanisms in the regulation of life span. These include the insulin and insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway and the mammalian target of rapamycin (mTOR) pathway, both of which activate the downstream effector ribosomal protein S6 kinase 1 (S6K1) (1, 2). Although the role of these pathways in mammalian aging is less clear, there is mounting evidence that IIS regulates life span in mice (1). Global deletion of one allele of the IGF-1 receptor (Igfr1), adipose-specific deletion of the insulin receptor (Insr), global deletion of insulin receptor substrate protein 1 (Irs1), or neuron-specific deletion of Irs2, all increase mouse life span (1). Life-span-extending mutations in the somatotrophic axis also appear to work through attenuated IIS (3). Igfr1 has also been implicated as a modulator of human longevity (4). However, the action of downstream effectors of IIS or mTOR signaling in mammalian longevity is not fully understood. S6K1 transduces anabolic signals that indicate nutritional status to regulate cell size and

1Institute of Healthy Ageing, Centre for Diabetes and Endocrinology, Department of Medicine, University College London, London WC1E 6JJ, UK. 2Institute of Healthy Ageing, Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK. 3European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK. 4Division of Molecular Neuroendocrinology, Medical Research Council National Institute for Medical Research, London NW7 1AA, UK. 5Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge CB22 3AT, UK. 6Cellular Stress Group, Medical Research Council Clinical Sciences Centre, Imperial College, London W12 0NN, UK. 7Department of Cancer and Crinology, Medical Research Council National Institute for Medical Research, London NW7 1AA, UK. 8Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge CB22 3AT, UK. *Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge CB22 3AT, UK. *Metabolic Signaling Group, Medical Research Council Clinical Sciences Centre, Imperial College, London W12 0NN, UK. *Present address: Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB2 2TZ, UK.
†To whom correspondence should be addressed. E-mail: d.withers@ucl.ac.uk or d.withers@imperial.ac.uk.
growth and metabolism through various mechanisms (5). These include effects on the translational machinery and on cellular energy levels through the activity of adenosine monophosphate (AMP)-activated protein kinase (AMPK) (6, 7). Furthermore, S6K1 serine phosphorylates IRS1 and IRS2, which decreases insulin signaling (5). Given the key role of S6K1 in IIS and mTOR signaling and the regulation of aging in lower organisms by mTOR, S6k, and their downstream effectors (2), we used log-rank testing to evaluate differences in life span of wild-type (WT) and S6K1−/− littermate mice on a C57BL/6 background (8, 9). Data for both sexes combined showed median life-span in S6K1−/− mice increased by 80 days (from 862 to 942 days) or 9% relative to that of WT mice (χ² = 10.52, P < 0.001) (Fig. 1A and Table 1). Maximum life span (mean life span of the oldest 10% within a cohort) was also increased (1077 ± 16 and 1175 ± 24 days, P < 0.01 for WT and S6K1−/− mice, respectively). Analysis of each sex separately showed that median life span in female S6K1−/− mice was increased by 153 days (from 829 to 982 days) or 19% relative to that of WT mice (χ² = 11.07, P < 0.001) (Fig. 1B and Table 1). Female maximum life span was also increased (Table 1). In contrast, deletion of S6K1 in male mice had no effect on median (χ² = 0.34, P > 0.05) (Fig. 1C and Table 1) or maximum life span (Table 1). Similar gender effects on life span have been reported in other long-lived IIS mouse mutants (8, 10).

regression analysis of pooled male and female life-span data revealed no effect of recruitment date, parental identity, or gender, but that of genotype was significant (table S1). Therefore, deletion of S6K1 increases longevity in female mice.

Female S6K1−/− mice also showed improvements in a number of age-sensitive biomarkers of aging. In forced motor activity on a rotating rod (rotarod) assays to assess motor and neurological function, 600-day-old female S6K1−/− mice performed better than WT littermates (Fig. 2A). Performance in open-field testing to analyze general activity and exploratory drive were also enhanced (Fig. 2, B and C). An increase in abundance of memory T cells and a reduced number of naïve T cells are seen in mice with age, and the extent of these changes may be correlated with longevity (11). Female S6K1−/− mice at 600 days of age had significantly fewer memory and more naïve T cells than did WT mice (Fig. 2D), although male mice also displayed this phenotype (fig. S1A). Micro-computed tomography scanning of tibia from 600-day-old female S6K1−/− mice revealed attenuation of the normal age-dependent loss of cancellous bone volume seen in C57BL/6 mice (12) (Fig. 2, E and F). However, there was no difference in the incidence of macroscopic tumors in S6K1−/− and WT animals [8% (4 out of 48) for S6K1−/− and 8% (4 out of 49) for WT mice, respectively].

Young, male S6K1−/− mice fed a high-fat diet (13) display increased insulin sensitivity and reduced adiposity relative to those of WT mice, phenotypes also seen in WT mice under caloric restriction (CR), an evolutionarily conserved environmental manipulation that extends life span (14). Insulin sensitivity (assessed by the updated homeostasis model, HOMA2) was significantly greater in 600-day-old female S6K1−/− mice than in WT animals (Fig. 2G), and glucose tolerance was improved (Fig. 2H), in contrast to the impaired glucose tolerance seen in young animals (fig S1B). Fat mass and plasma leptin levels were lower in old female S6K1−/− mice (Fig. 2, I and J), despite increased food intake (fig. S1C). Core temperature and resting metabolic rate (with general linear modeling to account for body-mass differences) were not significantly different (fig. S1, D and E). Although S6K1−/− mice were smaller than their littermates throughout their lives (Fig. 2K), endocrinologically they did not resemble long-lived pituitary dwarfs (15), because their total circulating IGF-1, pituitary growth hormone, thyroid-stimulating hormone, and prolactin concentrations were normal (Fig. 2, L and M, and fig. S1, F and G). Male S6K1−/− mice at 600 days of age had normal fasting and fed glucose levels (fig. S1, H and I).

We compared the effect of S6K1 deletion on genome-wide hepatic gene expression in 600-day-old female mice to transcriptional changes induced by long-term CR (16). The 500 gene categories most overrepresented among genes with altered expression in S6K1−/− mice showed...
changes associated with highly significant functional categories (\(P < 10^{-8}\), two-tailed) in both comparisons (fig. S2, A and B). This is consistent with the existence of common mechanisms underlying the effects of S6K1, CR, and IIS on aging.

We examined transcription of individual genes in liver, skeletal muscle, and white adipose tissue (WAT) in 600-day-old female \(S6K1^{-/-}\) and WT mice, looking for genes previously associated with longevity (tables S2, A and B, S3, A and B, and S4, A and B). Significant cross talk exists between peroxisome proliferator-activated receptor (PPAR)-\(\gamma\), coactivator 1\(\alpha\) (PGC-1\(\alpha\), AMPK, and nicotinamide adenine dinucleotide (oxidized form)-dependent deacetylase sirtuin-1 (SIRT1) signaling, which may be critical to cellular energy metabolism and perhaps aging (17). Increased expression of genes associated with these pathways was observed in liver (Parg1a, Parg1b, Foxo1, Foxo3a, Cpt1b, Pdk4, Glut1, and Cyp) and muscle (Parg1a, Ppara, Foxo1, Foxo3a, Pdk4, Glut1, Sirt1, and Ucp3) of \(S6K1^{-/-}\) mice. Adipose tissue is a key tissue in longevity assurance in \(C.\) elegans, \(D.\) melanogaster, and mice (18). In WAT of \(S6K1^{-/-}\) mice, fewer PGC-1\(\alpha\)-regulated genes (Foxo3a, Pdk4, Nampt, and Angptl4) showed increased expression compared with changes seen in liver and muscle, but there was also increased expression of the \(\alpha2\) catalytic and \(\beta1\) regulatory subunits of AMPK (log 2 fold change = 1.7, \(P = 2.88 \times 10^{-6}\) and 1.2, \(P = 4.95 \times 10^{-5}\), respectively, Cyber-T analysis). AMPK activity is increased in WAT, muscle, and liver of \(S6K1^{-/-}\) mice (7). Moreover, comparison of gene expression patterns in muscle of \(S6K1^{-/-}\) mice with those of mice treated with the AMPK activator aminimidazole carboxamide ribo-
null mutants with or without aak-2(ok524) mutation. In (D), rsks-1(ok1255) is significantly different (\( P < 0.001 \); one-way ANOVA) from all other groups from day 2 onward, but rsks-1(ok1255);aak-2 is not significantly different from WT or aak-2. (A) to (C) show data from one representative experiment, and (D to F) show combined data from three similar independent experiments. Values (A and D to F) are means ±SEM. In (A), \( n = 3 \), and in (D), \( n > 8 \) for each strain and time point. For (E) and (F), \( n > 20 \) for each group. Asterisks indicate statistical differences by using two-tailed \( t \) tests, \( * P < 0.05, \ *** P < 0.001 \).

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Genome-Wide RNAi Screen Identifies Letm1 as a Mitochondrial Ca\textsuperscript{2+}/H\textsuperscript{+} Antiporter

Dawei Jiang, Linlin Zhao, David E. Clapham*

Mitochondria are integral components of cellular calcium (Ca\textsuperscript{2+}) signaling. Calcium stimulates mitochondrial adenosine 5'-triphosphate production, but can also initiate apoptosis. In turn, cytoplasmic Ca\textsuperscript{2+} concentrations are regulated by mitochondria. Although several transporter and enzymes in the tricarboxylic acid cycle (7, 8), and [H+]mito (lower panel) measurements in digitonin-permeabilized S2-pericam cells treated with scrambled control (circles; n = 3, 81 cells). (C) Transmembrane voltage (V'mito) measured with 5 nM TMRM in control S2-pericam cells was reduced by the ETC inhibitors rotenone and antimycin (5 μM, n = 3, 81 cells) or by the protonophore trifluoromethoxy carbonyl cyanide phenylhydrazone (FCCP; 10 μM, n = 3, 102 cells). By contrast, V'mito increased in dLetm1 knockdown cells (n = 3, 113 cells) compared to cells treated with scrambled control dsRNA (n = 3, 168 cells). (D) Relative mRNA level of dLetm1 and actin in control and dLetm1 dsRNA-treated S2 cells by quantitative reverse transcription–polymerase chain reaction (RT-PCR) (n = 3). (E) Ca\textsuperscript{2+}- and pH gradient-driven [Ca\textsuperscript{2+}]mito and [H+]mito changes in permeabilized S2-pericam cells treated with control (circles; n = 4, 143 cells) or dLetm1 dsRNAs (triangles; n = 4, 113 cells). A representative trace shows the effect of applying the H'K' antiporter nigericin (1 μM, dashed colored line) on dLetm1 dsRNA-treated cells (n = 3, 87 cells). (F) pH-dependent [Ca\textsuperscript{2+}]mito and [H+]mito changes in permeabilized S2-pericam cells treated with scrambled control (circles; n = 6, 214 cells) or dLetm1 dsRNAs (triangles; n = 6, 187 cells). BAPTA-maintained test solution [Ca\textsuperscript{2+}] = 50 nM. A representative trace shows the effect of nigericin (1 μM, dotted line) on dLetm1 dsRNA-treated cells (n = 3, 104 cells). All data shown are the mean ± SEM (*P < 0.05, two-tailed Student’s t test).

Fig. 1. dLetm1 knockdown reduces Ca\textsuperscript{2+}/H\textsuperscript{+} antiport. (A) [Ca\textsuperscript{2+}]mito (upper panel) and [H+]mito (lower panel) measurements in digitonin-permeabilized S2-pericam cells treated with scrambled control (circles; n = 6, 194 cells) or dLetm1 dsRNAs (triangles; n = 6, 167 cells). (B) Experiment as in (A), but with 2 μM Ca\textsuperscript{2+} applied to cells treated with scrambled control (circles; n = 4, 93 cells) or dLetm1 dsRNAs (triangles; n = 4, 81 cells). (C) Transmembrane voltage (V'mito) measured with 5 nM TMRM in control S2-pericam cells was reduced by the ETC inhibitors rotenone and antimycin (5 μM, n = 3, 81 cells) or by the protonophore trifluoromethoxy carbonyl cyanide phenylhydrazone (FCCP; 10 μM, n = 3, 102 cells). By contrast, V'mito increased in dLetm1 knockdown cells (n = 3, 113 cells) compared to cells treated with scrambled control dsRNA (n = 3, 168 cells). (D) Relative mRNA level of dLetm1 and actin in control and dLetm1 dsRNA-treated S2 cells by quantitative reverse transcription–polymerase chain reaction (RT-PCR) (n = 3). (E) Ca\textsuperscript{2+}- and pH gradient-driven [Ca\textsuperscript{2+}]mito and [H+]mito changes in permeabilized S2-pericam cells treated with control (circles; n = 4, 143 cells) or dLetm1 dsRNAs (triangles; n = 4, 113 cells). A representative trace shows the effect of applying the H’K’ antiporter nigericin (1 μM, dashed colored line) on dLetm1 dsRNA-treated cells (n = 3, 87 cells). (F) pH-dependent [Ca\textsuperscript{2+}]mito and [H+]mito changes in permeabilized S2-pericam cells treated with scrambled control (circles; n = 6, 214 cells) or dLetm1 dsRNAs (triangles; n = 6, 187 cells). BAPTA-maintained test solution [Ca\textsuperscript{2+}] = 50 nM. A representative trace shows the effect of nigericin (1 μM, dotted line) on dLetm1 dsRNA-treated cells (n = 3, 104 cells). All data shown are the mean ± SEM (*P < 0.05, two-tailed Student’s t test).