The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet

Authors:

Sarah J. Mitchell[1,2,3,8], Alejandro Martin-Montalvo[1,8], Evi M. Mercken[1], Hector H. Palacios[1], Theresa M. Ward[1], Gelareh Abulwerdi[1], Robin K. Minor[1], George P. Vlasuk[4], James L. Ellis[4], David A. Sinclair[5], John Dawson[6], David B. Allison[6], Yongqing Zhang[7], Kevin G. Becker[7], Michel Bernier[1] and Rafael de Cabo[1].

[8] These authors contributed equally to this work

SUPPLEMENTAL EXPERIMENTAL PROCEDURES. Related to main experimental procedures.

Survival Study. Moribund animals were euthanized and every animal found dead or euthanized was necropsied. A mouse was considered moribund when was showing one or more of these clinical signs: incapable to eat or to drink, failure to move when prodded, drop in body weight of 7-10 grams in a week, severe bleeding, inability to walk. Criteria for euthanasia were based on an independent assessment made by a veterinarian, and only cases where the condition of the animal was considered incompatible with continued survival are represented as deaths in the curves. The age at which a moribund mouse was euthanized was taken as the best available estimate of its natural lifespan. Mice found dead were also noted at each daily inspection. Animals removed at sacrifice for experimental procedures were considered as censored deaths.

Determination of serum marker concentrations and HOMA calculation. Serum concentrations of aspartate aminotransferase (AST), total cholesterol (CHOL), creatinine kinase (CK), creatinine and low-density lipoprotein (LDL) were measured using the Cobas Integra 400 automated analyzer (Roche Diagnostics, Basel, Switzerland). Glucose was measured in whole blood using the Ascensia Elite glucose meter (Bayer, Mishawaka, IN). Insulin was measured using an enzyme-linked immunosorbent assay (Crystal Chem, Downers Grove, IL) according to the manufacturer's instructions. Insulin resistance was calculated from fasted glucose and insulin values using the HOMA2 Calculator software available from the Oxford Centre for Diabetes, Endocrinology and Metabolism, Diabetes Trials Unit website (www.dtu.ox.ac.uk). TNF-α was measured in a multiplex plate according to the manufacturer's instructions (Millipore, Billerica, MA).

Western blotting. Tissues were lysed in radioimmunoprecipitation buffer supplemented with ethylenediaminetetraacetic acid and ethylene glycol tetraacetic acid (Boston BioProducts, Ashland, MA), protease and phosphatase inhibitors (Sigma-Aldrich, St Louis, MO), and Trichostatin A (Sigma-Aldrich). Following centrifugation (14,000 rpm, 30 min at 4ºC) protein concentration was quantified using the Bradford assay method (Bio-Rad, Hercules, CA). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing

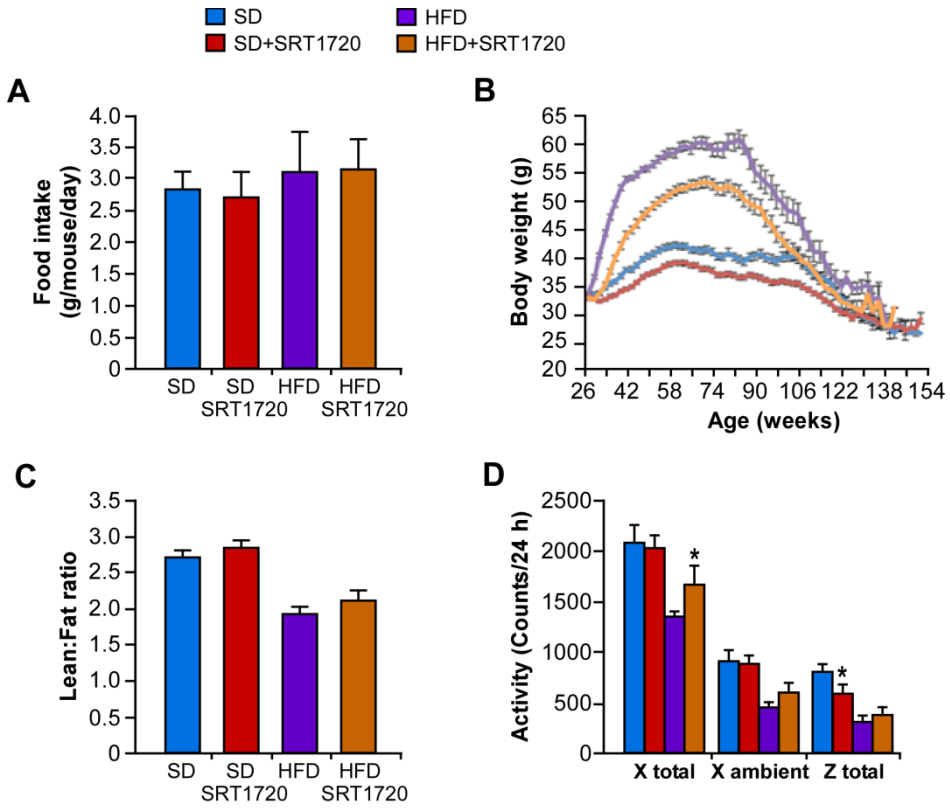
conditions and then transferred to nitrocellulose membranes. Western blots were performed according to standard methods. Membranes were blocked in either 5% bovine serum albumin or 5% milk, and then incubated (overnight at 4ºC or room temperature for 3-4 h) with the antibody of interest, followed by incubation with a horseradish peroxidase-conjugated secondary antibody. The visualization of immunoreactive bands was performed using the ECL Plus Western blotting detection system (GE Healthcare, Pascataway, NJ). The primary antibodies were directed against SIRT1 (cat#: S5447-200UL; Sigma-Aldrich); NF-κβ p65 (cat#: 1546-1; Epitomics, Burlingame, CA); COX-2 (cat#: ab52237; Abcam, Inc., Cambridge, MA); BAX (cat#: 2772s; Cell Signaling Technology, Danvers, MA); and GAPDH (cat#: sc-365062; Santa Cruz Biotechnology, Santa Cruz, CA). The quantification was performed by volume densitometry using ImageJ software (NIH, Bethesda, MD) and normalization to GAPDH. n=6 per group; age 40 weeks; diet 13 weeks.

Microarray. RNA from tissues was isolated using the RNeasy kit (Qiagen, Valencia, CA) and then hybridized to BD-202-0202 Illumina Beadchips. Raw data were subjected to Z-normalization, as described elsewhere (Cheadle et al., 2003; Lee et al., 2012). Principal component analysis (PCA) was performed on the normalized Z-scores of all of the detectable probes in the samples using the DIANE 6.0 software, available from: (http://www.grc.nia.nih.gov/branches/rrb/dna/diane_software.pdf). Significant genes were selected by the z-test < 0.05, false discovery rate < 0.30, as well as z-ratio > 1.5 in both directions and ANOVA p value < 0.05. Parametric analysis of gene set enrichment (PAGE) was analyzed as previously described (Kim and Volsky, 2005). All raw data are available in the Gene Expression Omnibus database (accession number GSE50987). n=4-5 animals per group, 40 weeks age, 13 weeks diet.

Quantitative Real-time RT-PCR. Total RNA was extracted from frozen tissue samples or cells using the RNeasy kit (Qiagen). Complementary DNA was synthesized from total RNA with the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) and random hexamer primers. The real-time polymerase chain reaction measurement was performed on individual cDNAs by using SYBR green dye to measure duplex DNA formation with the Roche Lightcycler system. The calculation of mRNA expression was performed by the $2^{-\Delta\Delta CT}$ method

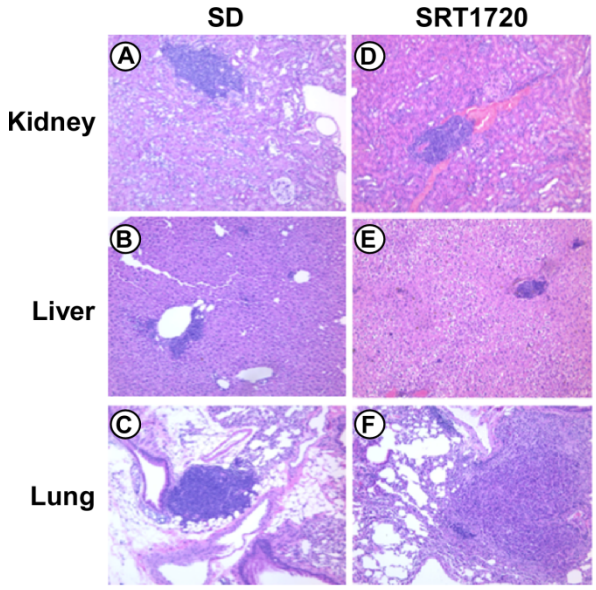
normalized to the expression of β-actin. Primers used in this study were CISH (forward: GCATAGCCAAGACGTTCTCC; reverse: AATGTACCCTCCGGCATCTT), SOCS2 (forward: TGTGCAAGGATAAACGGACA; reverse: GGTAAAGGGAGTCCCCAGA), IL10 (forward: GCAACTGTTCCTGAACTCAACT; reverse: ATCTTTTGGGGTCCGTCAACT), IL-1β (forward: GACAAAATACCTGTGGCCTTG; reverse: GATGTACCAGTTGGGGAACTCT), and β-actin (forward: ACCTTCTACAATGAGCTGCG; reverse: CTGGATGGCTACGTACATGG). n=4-5 animals per group, 40 weeks age, 13 weeks diet.

SUPPLEMENTAL FIGURES



Suppl. Figure 1

Figure S1, related to Figure 1. (a) Average daily food intake over the course of the study. (b) Body weight measurement over the duration of the study (152 weeks) for mice fed a standard diet (SD) without or with SRT1720 supplementation (SD SRT1720), and high fat diet (HFD)-fed mice without or with SRT1720 supplementation (HFD SRT720). (c) Lean-to-fat ratio measured using nuclear magnetic resonance spectroscopy. (d) Average activity counts over 24 hours for mice in the CLAMS system. Data are mean ± SD. * $p \leq 0.05$ compared to non-SRT1720 treated animals.



Suppl. Figure 2

Figure S2, related to Figure 2. Representative photographs from blinded histopathological analysis of kidney (panels A, D), liver (panels B, E) and lung (panels C, F) are shown for mice on standard diet (SD) and after SRT1720 supplementation (118 weeks age, 92 weeks diet). Magnification 10x.