Supporting Online Material for

Increase in Activity During Calorie Restriction Requires Sirt1

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Materials and Methods
Methods:

Mice:
Sirt1\(^{+/+}\) and Sirt1\(^{-/-}\) mice have been described previously (McBurney M. et. al Mol. Cell Biol. 23(1) 38-54 (2003)). All mice were housed on a 12:12-hr light:dark cycle at 25±1°C. 3-6 months old animals were either fed ad libitum (AL) or subjected to a 40% calorie restricted (CR) diet, which was provided daily for 9 months. Both wild type and KO mice experienced weight reduction (Wild type AL: 37.8±3.8g. Wild type CR: 19.9±1.9g. KO AL: 18.29±2.7g. KO CR: 15.4±1.4g). Only wild type and Sirt1 KO littermates were used. All animal procedures were in accordance with the MIT animal care committee.

Plasma chemical analysis:
Blood was collected from the tail veins of 8-10 pairs of littermates and kept on ice until centrifugation (5000rpm, 10min at 4°C). The plasma was either used immediately for assays or stored at -80°C until analysis. Glucose concentrations were determined using OneTouch Ultra glucose meter (LifeScan, Milpitas, CA). Triglyceride (Wako) and IGF-1 (Diagnostic Systems Laboratories, Inc.) were measured using ELISA kits.

Home cage behavior analysis:
Mice were singly housed in typical static microisolator cages and were video recorded over two 23-24 hour periods in the home cage. JVC digital handycams were mounted on tripods angled perpendicular to the cage, giving a side-view of the cage. The cameras input into a quad which was connected to a Dell computer with an ATI All-In-Wonder© video card. To allow viewing the mice during the dark cycle red light was used. For CR mice, recording began at the same time as feeding. The video data was analyzed using HomecageScan© software, which is described in Steele A.D., Jackson W.S., and S. L. Lindquist (manuscript in preparation) and at http://www.cleversysinc.com/products_hcs.html. For measures of activity, we analyzed 3-4 pairs of KO mice and their WT littermate controls on each of the two diets. We acquired home cage behavior records of individual mice for two periods (23 or 24 hr recordings). Bars in Figure 1A show the activity levels averaged over the sets of mice.

Rotarod Analysis
Pairs of wild type and KO littermates were placed on an Ugo Basile rotarod (myneurolab), which was accelerated at a continuously increasing speed 20 seconds after the mice were placed on the rod. We recorded the amount of time a mouse remained on the rod before falling. Mice were trained on the apparatus for three trials before the actual test, and the data of three tests was averaged for 4 pairs of littermates.

Treadmill analysis
Littermate pairs were placed on a Single Lane Gait Analysis Treadmill Model Exergait© (Colombus Instruments) and the speed of the treadmill was accelerated
1 cm/s every 5 seconds until the mouse could not maintain its gait or the treadmill reached the maximum speed of 50 cm/s. The maximum speed at which the mouse could maintain its stride was recorded. Mice were trained once, then data was recorded for the three trials and then averaged for 4 pairs of littermates.