

Supplementary data

Methods:

Glucose-tolerance tests (GTTs) and insulin-tolerance tests (ITTs)

Both the GTT and ITT assays were performed following 16 hours of food deprivation in 8-month-old mice. Food was removed from the AL and Rapa mice immediately after the CR mice had eaten their daily allotment. GTTs were performed with an intraperitoneal injection of glucose (1.5 g/kg body wt) solution in saline into the mice, and blood glucose was measured 0, 15, 30, 60, and 120 min after glucose injection. ITTs were performed with an intraperitoneal injection of human recombinant insulin in saline (Novalin; Novo Nordisk) at a dose of 0.75 U/kg body weight and blood glucose was measured 0, 15, 30, and 60 min after insulin injection. Blood glucose concentrations were measured using a One-Touch Ultra glucometer. The data were expressed as mean \pm standard error of the mean and analyzed using one-way analysis of variance with the Holm-Sidak post-hoc test.

Rapamycin levels

Rapamycin levels were measured in blood and liver tissue by a HPLC system coupled to an AB Sciex API 3200 tandem mass spectrometer with turbo ion spray.

Figure Legends:

Figure supplementary 1. Blood glucose levels and stomach weight at the time of tissue collection. The blood glucose concentrations were measured using a One-Touch Ultra glucometer at the time of tissue collection (Supp. Figure 1A). Stomach weight was measure at both, 1hr after feeding (4 PM afternoon), or at the time of tissue collection

(8:30-10:30AM) Supp. Figure 1B. The data were expressed as mean \pm standard error of the mean and analyzed using one-way

Figure supplementary 2. Effect of dietary restriction and rapamycin on intermediary metabolites of Tri Carboxylic Acid cycle (TCA).

Levels of glucose several intermediary metabolites of the TCA cycle were measured by metabolomics analysis, e.g., citrate, succinate, fumarate, malate were determined in AL (cyan), DR (pink), Rapa (blue), and DR+Rapa (red) in mice fed for 6 months of treatment (starting at 2 months of age). Each metabolite was measured by Metabolome, and the data were obtained from 8-11 mice per group and expressed as mean \pm SD using a box and whisker plot, where plus symbol (+) represents the average. Data were analyzed using unpaired t test with Welch's correction.

Figure Supplementary 3. Effect of dietary restriction and rapamycin on genes that controls lipolysis.

Lypolysis was assessed in liver samples by measuring the gene expression of the enzymes that participate in lipolysis: Adipose Triglyceride Lipase [(ATGL), solid bars], Monoacylglyceride Lipase [(MGL), gray bars], and Hormone-Sensitive Lipase [(HSL), open bars], using RT-PCR. The data were obtained from 6 mice per group: AL, DR, Rapa, and DR+Rapa. The data were expressed as mean \pm SE and analyzed using one-way ANOVA with the Tukey's post-hoc test; an asterisk denotes those values that are significantly different from AL mice at the $p \leq 0.05$ level.

Figure supplementary 4. Measurement of serum glycerol.

Glycerol levels were measured in serum from un-fasted mice treated for 6 months with rapamycin. The amount of glycerol in serum were determined by colorimetric assay using a kit purchased from Sigma (St. Louis, MO).

Figure supplementary 5. Effect of dietary restriction and rapamycin on glucose and insulin tolerance test. The GTT **(A)** and ITT **(B)** were performed as described in Methods. The data were obtained from 10 mice per group for AL (solid square), Rapa (open square), DR (open circle), and DR+Rapa (solid circle) mice. The data were expressed as mean \pm SEM and analyzed using one-way ANOVA with the Holm-Sidek post-hoc test; an asterisk denotes those values that are significantly different ($p \leq 0.05$) from AL mice.