Supporting Information

Metabonomic analysis of plasma

Plasma samples were randomly measured at 300K using three proton NMR (^{1}H-NMR) complementary pulse sequences, namely standard, Carr-Purcell-Meiboom-Gill (CPMG), and diffusion-edited as previously reported (Rezzi et al., 2007). All spectra were collected using 64 K data points and a spectral window of 12000 Hz. Before Fourier transformation, free induction decays were multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz for standard spectra and 1 Hz for CPMG and diffusion-edited spectra. The spectra were manually phased, baseline corrected and referenced to the chemical shift of the methyl resonance of lactate at δ 1.33 using the software package TOPSPIN (version 2.0, Bruker Biospin, Rheinstetten, Germany).

The spectra were converted into 22 K data points over the range of δ 0.2-10.0 using an in-house developed MATLAB (The MathWorks Inc., Natick, MA, USA) routine. The region containing the water resonance (δ 4.5-5.19) was removed. The spectra were normalized to a constant total sum of all intensities within the specified range prior to chemometric analyses. Multivariate pattern recognition techniques used in this study were based on principal component analysis (PCA), projection to latent structure (PLS), projection to latent structure discriminant analysis (PLS-DA), and the orthogonal-projection to latent structure discriminant analysis (O-PLS-DA) (Trygg and Wold, 2002) using the software package SIMCA-P+ (version 11.5, Umetrics AB, Umeå, Sweden) and in-house developed MATLAB (The MathWorks Inc., Natick, MA, USA) routines. PCA was first applied to NMR variables (subjected to Pareto scaling, by dividing each variable by the square root of its standard deviation) to detect the presence of inherent similarities between metabolic profiles. Variations between the different plasma metabolic phenotypes were analyzed using scores and

loadings plots. Biochemical components (NMR spectral variables) responsible for the differences between individual plasma samples detected in the scores plot can be extracted from the corresponding loadings plot. Additional detailed classification studies were performed using PLS and O-PLS-DA to exclusively focus on the effects of CR on aging (Trygg and Wold, 2002). All the models were calculated using the NMR data as the X matrix and class information (aging, CR) as a dummy Y matrix and using unit-variance scaled variables (each variable divided by its standard deviation). Details about the statistical parameters used for model building are given in the Figure captions. Here, the test for the significance of the Pearson product-moment correlation coefficient was used to calculate the cut-off value of the correlation coefficients of 0.46 at the level of $p < 0.05$. To test the validity of the model against over-fitting, the goodness of fit (R^2X) and predictability (Q^2Y) values were computed and the standard 7-fold cross validation method was used. The statistically discriminant NMR variables were vizualized according to the method developed by Cloarec et al. (Cloarec et al., 2005). This method provides coefficients plot with a shape similar to a NMR spectrum, offering a straightforward means to identify statistically significant variations in metabolite concentrations. The weight of a variable in the discrimination is given by the square of its correlation coefficient (r^2), which is color coded from zero in blue to high values in red. The interpretation of the resulting coefficients plot is performed considering the degree of correlation of each variable, e.g., color code, and its orientation, e.g., positive or negative, indicating relative changes in metabolite concentrations between classes of subjects.

Insulin sensitivity

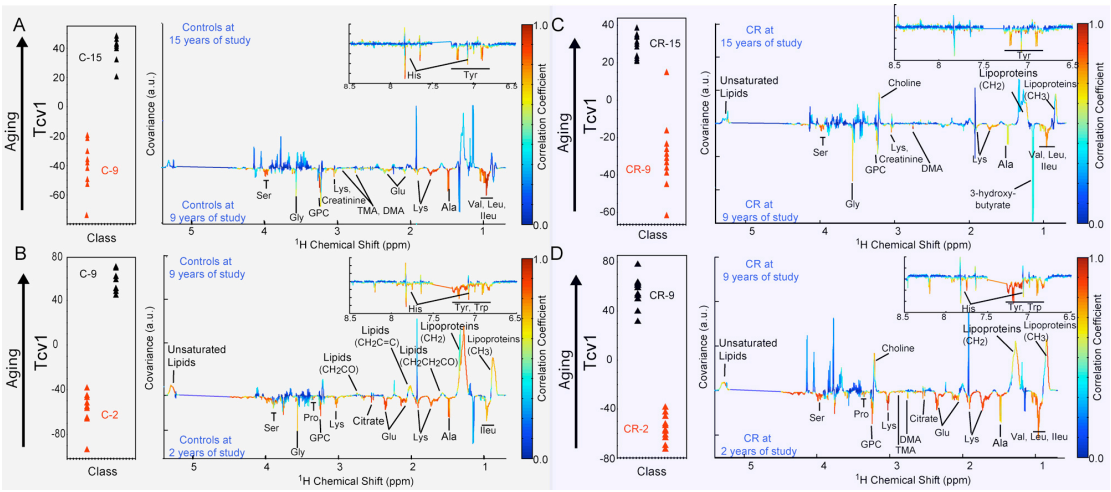
Following an overnight fast, monkeys were anesthetized with ketamine HCl (10 mg/kg, IM) and diazepam (1.25 mg/kg, IM). A catheter was inserted either through the saphenous or femoral vein and positioned in the inferior vena cava. Four pretreatment 2 ml blood samples were taken at -15, -10, -5, and -1 minutes, a 300

mg/kg glucose bolus administered over one minute, and 2 ml blood samples drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes post glucose administration. Tolbutamide (5 mg/kg, IV) was infused 20 minutes after glucose administration to induce an additional increment in circulating insulin levels (Gresl et al., 2003).

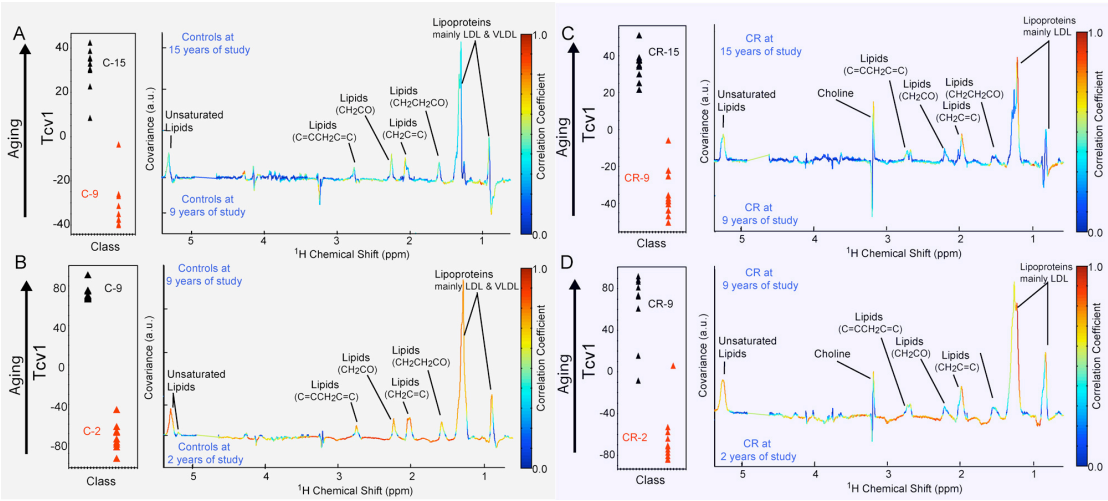
Body composition

Following an overnight fast, animals were sedated with ketamine HCl (10 mg/kg, IM) and weighed. Animals were further administered a mixture of ketamine HCl and xylazine (7 mg/kg, 0.6 mg/kg xylazine, respectively, IM) for additional muscle relaxation and anesthesia maintenance. Upon scan completion, yohimbine (0.06 mg/kg, IV) was given to reverse xylazine. Approximate scan time was 20 minutes per animal. Total body scans were acquired with the animal in the supine position and analyzed using Lunar pediatric software (version 1.5e for acquisition, version 4.0a for analysis, Lunar, Madison, WI). DXA coefficients of variation ([mean/standard deviation]/*100) for total body fat and lean masses were 1.8% and 0.8%, respectively (Colman et al., 1999).

SI Fig. 1 O-PLS-DA scores and coefficients plots derived from ^{1}H-NMR CPMG spectra of blood plasma describe metabolic changes associated with aging in control (C) and restricted (CR) animals from years 2 to 9 (B, D) and from years 9 to 15 (A, C). The cross-validated scores plot showed statistically significant separations between the plasma profiles with aging for controls and CR animals. The corresponding O-PLS-DA coefficients identified the specific metabolites contributing to the separation. The color code corresponds to the correlation coefficients of the NMR variables. 1 predictive and 1 orthogonal components were calculated, the respective (Q^2Y, R^2X) are: **A** (64.7%, 37.7%), **B** (70.8%, 40.7%), **C** (32.6%, 26.5%), **D** (70.0%, 35.5%). Aging-related metabolic changes were characterized by decreased levels of circulating plasma amino acids and increased concentrations of plasma lipoproteins.



SI Fig. 2 O-PLS-DA scores and coefficients plots derived from ^{1}H-NMR diffusion-edited spectra of blood plasma describe metabolic changes, mainly lipoproteins, associated with aging in control (C) and restricted (CR) animals from year 2 to 9 (B, D) and from year 9 to 15 (A, C). The cross-validated scores plot showed statistically significant separations between the plasma profiles with aging for controls and CR animals. The corresponding O-PLS-DA coefficients identified the specific metabolites contributing to the separation. The color code corresponds to the correlation coefficients of the NMR variables. 1 predictive and 1 orthogonal components were calculated, the respective (Q_Y^2, R_X^2) are: **A** (64.0%, 65.0%), **B** (64.0%, 62.0%), **C** (59.0%, 52.0%), **D** (64.0%, 60.0%). Aging-related metabolic changes were characterized by decreased levels of circulating plasma amino acids and increased concentrations of plasma lipoproteins.



SI Fig. 3 O-PLS-DA scores and coefficients plots derived from ^{1}H-NMR CPMG (left panel, **A** and **B**) and diffusion-edited (right panel, **C** and **D**) spectra of blood plasma describe the CR induced metabolic changes between control and CR animals at 9 and 15 years of study. The CPMG and diffusion-edited spectra favor the measurement of low molecular weight metabolites and large macromolecules (mainly lipoproteins), respectively. Signals that change in the negative and positive directions represent metabolites relatively increased and decreased in the CR group versus the control group. 1 predictive and 2 orthogonal components were calculated, the respective (Q^2Y, R^2X) are: (15.9%, 34.5%), (53.7%, 39.3%), (19.6%, 58.8%), (59.4%, 61.7%). CR subjects were characterized with relative lower concentrations of plasma VLDL associated with higher levels of HDL, creatinine, Glu, OAc and gluconate.