Supporting Online Material

Materials and methods

Animals and diets. All animals have lived their entire lives at the WNPRC, and have known birthdates. Complete clinical and experimental histories are maintained for each animal. Prior to the start of this experiment, no animals had any clinical or experimental history that would be expected to affect disease susceptibility or mortality. Animals in this study are fed a semipurified, nutritionally fortified, low fat diet containing 15% protein and 10% fat. As part of the study design, animals are treated for presenting conditions. For example, diabetics receive insulin sensitizers or insulin and animals with diverticulosis receive fiber supplements.

Analysis of age-associated diseases. Accurate determination of the effect of CR on longevity required that invasive manipulation of the animals be kept to a minimum. As such, we monitored age-associated diseases that are most prevalent in humans using non-invasive detection methods.

Cancer: Animals with intestinal adenocarcinoma often have reduced food intake and/or weight loss and, in some cases, a mass may be palpated. Radiology or ultrasonography, along with screening of feces for small amounts of blood (S*1, S2*), were used to detect the presence of tumors. Incidence and type of neoplasia were confirmed by surgical removal of the mass and subsequent histopathology.

Cardiovascular disease: Typically cardiovascular disease is not detected in caged animals(S*3*), though on physical exam, a heart murmur may be noted. We used thoracic radiology, echocardiography, and electrocardiography to evaluate the cause of murmurs and the severity of the underlying disease process.

MRI capture and analysis. Imaging studies of brain aging in nonhuman primates have shown an age effect on brain volume(S*4*) similar to normal human aging. All living animals (n=47) from this study underwent MRI at 3.0 Tesla field strength (GE Medical Systems, Milwaukee, WI). Using voxel-based morphometry(S*5*) we segmented out GM volume and applied a voxel-wise regression to determine the regional effects of age, diet, and age x diet interactions on GM volume. Animals were divided into two age-equivalent groups: CR (n=26, M/F 15/11, age range 18.8-31.2 yrs, mean age 24.5 yrs) and control (n=16, M/F 9/7, age range 18.8-28.9 yrs, mean age 23.7 yrs). Images were acquired using an 18 cm diameter quadrature transmit/receive volume coil. A three-dimensional coronal T1-weighted inversion recovery-prepped spoiled gradient echo (IR-prepped SPGR) was acquired with the following parameters: repetition time = 8.772 ms; echo time = 1.876 ms; inversion time = 600 ms; flip angle = 10°; number of excitations = 2; acquisition matrix = 256x256; field of view = 160mm. 124 coronal slices with a thickness of 0.7 mm were acquired resulting in 0.6 x 0.6 x 0.7 mm voxels. The brain images were segmented into tissue

probability maps using locally developed priors(*5*), then spatially transformed to the standard space defined by Saleem and Logothetis(S*6*) using a GM template and smoothed with a Gaussian kernel to 4 mm full width at half maximum. GM probability maps were analyzed further on a voxel-wise basis.

Statistical methods. Analyses of age-associated diseases and mortality include all animals with known diagnoses or cause of death prior to 2/22/2008. To determine the effect of CR on mortality, a Cox proportional hazards (PH) regression with sex and group (CR or control) as predictors was used to estimate the survival and hazard functions. For those whose deaths were not age-associated, animals were censored and their age at death was used as the time variable in the Cox regressions. The PH assumption was tested by fitting a non-PH Cox regression with a CR by time interaction. For both the mortality and age-associated disease analyses the interaction was not significant, and thus, PH models were considered valid.

To determine the effect of CR on the onset of age-associated diseases, a Cox PH regression was used with sex and group (CR or control) as predictors to estimate the hazard function. In this analysis, the age at which the animal experienced its first age-associated diagnosis was used as the time variable. Animals that received a *non-age-associated diagnosis* as cause of death were divided into two groups. Animals that died of *non-age-associated causes* (7 control, 9 CR) were censored and their age of death used as the time variable in

the regression. Animals that died of an *age-associated cause without ever receiving an age-associated diagnosis* (4 Control and 2 CR) were not censored, death was considered as their age-associated diagnosis, and their age at death was used as the time variable.

Analysis to determine the effect of CR on regional GM volume involved voxel-based morphometry methods that were adapted for tissue segmentation using priors, spatial transformation to a template, and spatial smoothing. Regression was used within the framework of the general linear model to test the effect of CR, age and interactions with age. Other covariates in the model included sex and global brain volume. All effects were tested with t contrasts. Interactions were excluded from the tests of main effects. The voxel level p-value was set at $p<0.005$ and resultant clusters contained at least 75 contiguous voxels (though all but one cluster contained more than 230 contiguous voxels).

References

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