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Effects of aging and calorie restriction on white matter in rhesus macaques

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Abstract

Rhesus macaques on a calorie restricted diet (CR) develop less age-related disease, have virtually no indication of diabetes, are protected against sarcopenia, and potentially live longer. Beneficial effects of caloric restriction likely include reductions in age-related inflammation and oxidative damage. Oligodendrocytes are particularly susceptible to inflammation and oxidative stress, therefore, we hypothesized that CR would have a beneficial effect on brain white matter and would attenuate age-related decline in this tissue. CR monkeys and controls underwent diffusion tensor imaging (DTI). A beneficial effect of CR indexed by DTI was observed in superior longitudinal fasciculus, fronto-occipital fasciculus, external capsule, and brainstem. Aging effects were observed in several regions, although CR appeared to attenuate age-related alterations in superior longitudinal fasciculus, frontal white matter, external capsule, right parahippocampal white matter, and dorsal occipital bundle. The results, however, were regionally specific and also suggested that CR is not salutary across all white matter. Further evaluation of this unique cohort of elderly primates to mortality will shed light on the ultimate benefits of an adult-onset, moderate CR diet for deferring brain aging.
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Caloric restriction (CR)—reduced calorie intake without essential nutrient deficiency—has been associated with improved cognition, a slowing of brain aging, and potential protection against neurodegenerative diseases in animal models (Gillette-Guyonnet and Vellas, 2008). Although the exact mechanisms underlying beneficial effects of CR are not known, mechanisms may include reductions in inflammatory and oxidative processes (Anson et al., 2003; Forster et al., 2000; Prolla and Mattson, 2001). Oligodendrocytes, the brain's myelin forming cells, are particularly susceptible to inflammation (Felts et al., 2005; Lehnardt et al., 2002). In addition—as has been pointed out by previous investigators (Bartzokis, 2004) —the high lipid and iron content and high metabolic activity of olidodendroglia make them especially vulnerable to oxidative damage (Juurlink et al., 1998; Richter-Landsberg and Vollgraf, 1998; Smith et al., 1999). Although the effect of CR has been measured in neurons (Eckles-Smith et al., 2000; Okada et al., 2003; Shi et al.,

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2007; Stranahan et al., 2009) and gray matter (Colman et al., 2009), little is known about the effects of CR on brain white matter.

One method of studying white matter *in vivo* is to use diffusion tensor imaging (DTI), an MR technique that is sensitive to the random thermally driven motion of water molecules and as a consequence is sensitive to the structural organization of white matter (Basser and Pierpaoli, 1996; Basser, 1995). DTI has been widely used to study disease and early brain development, in addition to normal and pathological aging. Two commonly reported DTI measures are fractional anisotropy (FA), a measure of the directionality of water molecule motion, and mean diffusivity (MD), an indicator of isotropic water molecule motion. In human aging, brain white matter exhibits both a decrease in white matter FA and an increase in MD (Abe et al., 2002; Engelter et al., 2000; Pfefferbaum et al., 2000; Salat et al., 2005), with several studies supporting an anterior to posterior gradient of change with age (Bendlin et al., 2009; Head et al., 2004; Pfefferbaum et al., 2005; Yoon et al., 2007). In rhesus monkeys, structural white matter alterations with age appear to be similar to humans and occur in prominently frontal regions, including the superior longitudinal fasciculus, the cingulum bundle, and anterior corpus callosum (Makris et al., 2007). With the exception of a few studies (Colman et al., 2009; Matochik et al., 2004), little is known about the effect of CR on primate brains in aging.

We sought to investigate the effect of CR on white matter microstructure in primates. Rhesus monkeys (*Macaca mulatta*) from the Wisconsin National Primate Research Center, in an ongoing study of CR (Colman et al., 2009; Ramsey et al., 2000), underwent MRI that included DTI. Animals were in one of two groups, a group of control animals who were fed *ad libitum*, or a group of monkeys that were on a moderate CR diet (approximately 30% reduced) for approximately 12 or 17 years. Voxel-wise analyses were performed on DTI data acquired on CR and control animals. This method of analysis can be largely automated, has high reliability, allows for the evaluation of brain regions that may not have been considered *a priori*, and produces results that largely concur with region of interest (ROI) based methods (Snook et al., 2007; Zhang et al., 2009). In the case of CR (of which the effects are unknown) it was desirable to consider all white matter tracts as opposed to limiting the analysis to select regions. We hypothesized that CR would exert a beneficial effect on white matter, indexed by higher FA in the CR animals compared with controls. To date, there are few *in vivo* studies of the effects of aging on the rhesus monkey brain; therefore, we also performed a cross-sectional analysis of age in a combined group of CR and control animals. Finally, because CR is known to inhibit mechanisms associated with aging, we hypothesized that CR would interact with age, resulting in the CR animals showing less age-related white matter decline compared with controls. FA was the primary outcome measure to assess benefits of a CR diet.

1. Methods

1.1. Subjects

Forty-eight rhesus monkeys (21 controls; 27 CR) between 18 and 31 years of age were studied from a longitudinal assessment of CR at the Wisconsin National Primate Research Center located in Madison, WI, USA, a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Details of the CR manipulation have been published previously (Kemnitz et al., 1993; Ramsey et al., 2000). Animals were maintained at 21 °C; humidity 50–65%; 12h/12h light/dark cycle. Water was available *ad libitum*. Food was present for 6–8 hours per day. Animals in the CR group were fed approximately 70% of their *ad libitum* diet (Ramsey et al., 2000). They were monitored at least twice daily by Animal Care and research staff. The study was approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. Of the original 48 animals that underwent scanning, 33 were included in the final DTI analysis.

2.2. Imaging

Images were acquired on a General Electric 3.0 T Signa MR Unit (GE Medical Systems, Milwaukee, WI, USA) using a quadrature transmit/receive volume coil with an 18 cm diameter at the Waisman Center for Brain Imaging and Behavior. Prior to the MRI scan, monkeys were administered ketamine (up to 15 mg/kg intramuscular) or alternative anesthesia in consultation with a Wisconsin National Primate Research Center veterinarian and xylazine (up to 0.6 mg/kg, IM). Occasionally, animals were administered a booster injection of ketamine (7–15 mg/kg, IM or intravascular, IV) with or without xylazine (0.2–0.6 mg/kg, IM or IV) during the scan to maintain the plane of anesthesia. DTI was performed in the axial plane using a single-shot, spin-echo, diffusion-weighted echo-planar imaging sequence with diffusion gradients in 12 optimal directions. A diffusion-weighting of b = 816 second/mm^2 was used. Other imaging parameters for this sequence were tr = 10000, TE = 77.2 ms, NEX = 6, FOV = 160 mm, matrix = 120 × 120, section thickness = 2.5 mm, no gap. Image distortion was minimized by a higher-order shimming protocol that was run before the DTI scan. Animals received a 2D T2-weighted scan with parameters: tr = 4500, TE = 88.51 ms, NEX = 2, FOV = 160 mm, matrix = 256 × 192, flip = 90°, section thickness = 1.7 mm, gap = 0.3 mm that was used to assist in spatial normalization of the DTI scan as detailed below. Other scans collected included T1-weighted, magnetization transfer, and T2-relaxation scans, but these were not analyzed as part of the present study.

Images were inspected for abnormalities or artifacts that could potentially impact analyses of microstructure. Six

animals were excluded for lesions visible on T1- and T2-weighted images. Six animals were excluded due to breathing motion, phase artifact, or scanner drift that produced images with poor quality. Additionally, despite sedation, three animals did not complete the DTI scan due to excessive involuntary movement during MRI. Of the total 15 excluded animals, nine excluded animals were from the CR group and six were controls.

2.3. Image processing

DTI processing was conducted by personnel blind to diet status of the animals. Image distortions in the DTI data caused by eddy currents were corrected using tools available in the FMRIB Software Library (FSL) (Smith et al., 2004) Diffusion Toolbox (FDT). Three-dimensional maps of the diffusion tensor and derived measures, MD and FA, were calculated using DTIFIT in FSL. DTI maps were further corrected for spatial distortion and brought into a common space for voxel-wise analysis through a combination of two transforms. First, to ameliorate warping artifact, we estimated nonlinear transforms of each subject's nondiffusion weighted map (b = 0 image) to their own coplanar T2-weighted image using Statistical Parametric Mapping software (University College London, London, UK, SPM5). Second, parameters were estimated for a normalization of the subject's corrected T2-weighted images to a rhesus macaque T2-weighted template (McLaren et al., 2009) via 12-parameter affine transformation and nonlinear deformations. The two estimated parameters for each subject were combined and then applied to the subjects' nondiffusion-weighted image (b = 0), FA, and MD maps. Combining the transformations avoided resampling the data twice. Normalized maps maintained the intensities of the original images. DTI maps that were normalized to the T2-weighted template were visually inspected using the "check registration" function in SPM5 to ensure accurate normalization. To optimize signal-to-noise and facilitate comparison across participants, the normalized images were smoothed using a 4 mm full-width-at-half maximum Gaussian kernel.

2.4. Statistics and analysis

First, we assessed the effect of age by performing a cross-sectional linear regression analysis where age was the predictor variable and FA was the dependent variable. To test the hypothesis that CR exerts a beneficial effect on white matter, FA was compared between groups using a two-sample *t*-test. To investigate the hypothesis that CR has an age-inhibiting effect on white matter, we tested the interaction between group (CR vs. control) and age using ANOVA. We hypothesized that CR would interact with age; with CR animals showing less age-related decline in white matter integrity compared with control animals. The same analyses were also performed with MD as the dependent variable. Due to the unknown effects of CR on brain white matter, we adopted an exploratory approach and the t-statistic threshold was set at $p < 0.005$ (uncorrected). To reduce the number of multiple comparisons, we employed a binary white matter mask. The mask was generated by thresholding a white matter prior probability mask (McLaren et al., 2009) at 0.4. Results were thresholded at a minimum cluster size of five contiguous voxels. Gender differences were controlled by covarying for female or male gender in all analyses.

Results are displayed on a standardized rhesus monkey atlas, the 112RM-SL atlas described by McLaren et al. (2009), an MRI based atlas comprising 112 rhesus monkeys, which is defined by the brain coordinate space of the Saleem-Logothetis atlas (Saleem et al., 2002). The locations of significant results on statistical parametric maps were identified by overlaying the results on either an average FA image or on the T1-weighted 112RM-SL underlay and referring to the results of autoradiography conducted in rhesus monkeys (Schmahmann and Pandya, 2006).

3. Results

There was no significant difference in years of age at the start of the CR study between the CR group (M = 9.5; SD = 2.6) and control (M = 9.47; SD = 2.4); no difference in age at the time of scanning between the CR group (M = 23.74, SD = 2.66) and control (M = 23.62, SD = 2.98); and no significant difference in length of time (yrs) in the study between the CR group (M = 13.33, SD = 2.23) and control (M = 13.00, SD = 2.07). The CR monkeys weighed (lbs) significantly less (M = 9.04, SD = 1.74) than control (M = 11.70, SD = 3.35), $t = 2.9$, d.f. = 31, $p < 0.05$. The CR group had a slightly larger proportion of males (67%) than the control group (53%). Table 1 provides descriptive information for individual animals.

3.1. Fractional anisotropy

3.1.1. Effect of age

A robust negative relationship between age and FA was evident when the monkeys in both the CR and control conditions were considered together. Brain regions where older age was associated with lower FA included white matter tracts in the thalamus, the body and tapetum of the corpus callosum, anterior internal capsule, superior longitudinal fasciculus, inferior longitudinal fasciculus, medial longitudinal fasciculus, and a small cluster in the inferior cerebellar peduncle. This association between FA and age is shown on a T1-weighted template brain in Figure 1. Table 1 indicates individual FA values for a subset of larger clusters. Table 2 indicates the specific regions where older age was associated with lower FA by 112RM-SL coordinates.

3.1.2. Effect of group

Compared with control, the CR group showed higher FA in several regions including: bilateral superior longitudinal fasciculus, with the most prominent difference apparent on the right, fronto-occipital fasciculus, a small cluster in ex-

Table 1
Individual FA and MD findings

Animal	Group	Sex	Age (years)	Thalamic bundle (−2, 14, 18) FA	Corpus callosum (8, 11, 24) FA	Superior longitudinal fasciculus (−6, 1, 26) FA	Superior colliculus (6, 4, 14) MD	Thalamic bundle (0, 12, 16) FA	Superior longitudinal fasciculus (−7, 12, 30) MD
1	Control	male	28.84	0.2742	0.3108	0.3692	0.00082	0.00082	0.00083
2	Control	female	26.84	0.247	0.2585	0.2954	0.00092	0.00094	0.00072
3	Control	male	26.64	0.2479	0.3263	0.3479	0.00096	0.00100	0.00065
4	Control	male	25.63	0.3024	0.3441	0.3663	0.00093	0.00087	0.00067
5	Control	female	26.00	0.321	0.3254	0.3669	0.00085	0.00083	0.00073
6	Control	male	25.59	0.2998	0.3258	0.4025	0.00080	0.00080	0.00070
7	Control	female	24.24	0.2659	0.2815	0.3433	0.00084	0.00087	0.00074
8	Control	male	23.61	0.2889	0.3436	0.3757	0.00086	0.00086	0.00069
9	Control	female	23.04	0.3102	0.3723	0.3739	0.00086	0.00078	0.00070
10	Control	female	22.09	0.2806	0.3562	0.4205	0.00079	0.00081	0.00069
11	Control	female	21.85	0.3196	0.331	0.3766	0.00086	0.00088	0.00070
12	Control	male	21.18	0.3039	0.3545	0.4066	0.00081	0.00076	0.00066
13	Control	female	21.06	0.3163	0.3346	0.4212	0.00083	0.00080	0.00066
14	Control	male	18.79	0.3299	0.3628	0.3991	0.00085	0.00084	0.00068
15	Control	male	18.93	0.3389	0.3752	0.4166	0.00088	0.00079	0.00063
16	CR	male	26.39	0.3229	0.3262	0.3564	0.00097	0.00092	0.00074
17	CR	male	26.42	0.2379	0.3106	0.3447	0.00091	0.00095	0.00072
18	CR	male	25.97	0.2873	0.3526	0.2768	0.00096	0.00089	0.00068
19	CR	male	25.73	0.2442	0.3246	0.3499	0.00078	0.00085	0.00066
20	CR	female	26.20	0.2614	0.3382	0.3903	0.00095	0.00092	0.00072
21	CR	female	25.80	0.2655	0.3254	0.3806	0.00087	0.00087	0.00068
22	CR	male	25.50	0.2552	0.3261	0.3305	0.00088	0.00094	0.00072
23	CR	female	26.04	0.2576	0.3484	0.3774	0.00086	0.00087	0.00070
24	CR	male	25.27	0.2725	0.3432	0.3416	0.00090	0.00089	0.00069
25	CR	male	24.06	0.315	0.3875	0.3714	0.00085	0.00082	0.00067
26	CR	male	23.81	0.3006	0.3341	0.3606	0.00082	0.00078	0.00064
27	CR	female	23.65	0.2855	0.3207	0.3726	0.00077	0.00087	0.00069
28	CR	female	22.07	0.3032	0.3492	0.405	0.00076	0.00079	0.00066
29	CR	female	22.22	0.2704	0.293	0.3424	0.00085	0.00088	0.00073
30	CR	male	20.04	0.3001	0.3593	0.3862	0.00087	0.00079	0.00063
31	CR	male	20.25	0.339	0.3626	0.4438	0.00082	0.00077	0.00061
32	CR	male	19.10	0.3204	0.3617	0.4102	0.00080	0.00079	0.00067
33	CR	male	18.83	0.3196	0.3459	0.4043	0.00076	0.00078	0.00067

ternal capsule and small portions of the brainstem, and a small cluster in right parahippocampal white matter. Regions where CR animals showed higher FA compared with controls are shown in Figure 2 and listed by 112RM-SL coordinates in Table 3. As shown in Figure 3, control monkeys had higher FA compared with CR in external capsule, internal capsule, a small cluster of short association fibers in the parietal lobe, superior longitudinal fasciculus and a small cluster in uncinate fasciculus (Table 4).

3.1.3. Group by age interaction

An interactive effect on FA was found in several small clusters including fibers in the mediodorsal thalamus, inferior longitudinal fasciculus, short association fibers in the frontal and ventral prefrontal cortex, frontal superior longitudinal fasciculus, external capsule, internal posterior capsule, cingulum, and dorsal occipital bundle. With the exception of small clusters in thalamus, and left inferior longitudinal fasciculus, most of the clusters indicated a larger slope in controls. Shown in red in Figure 4 are regions where controls showed a greater negative slope with age compared with CR, that is, where higher age was associated with lower FA in controls. In blue, are those regions where the opposite was true, CR showed a greater negative slope with age compared with controls. Table 5 lists 112RM-SL coordinates for the locations where age interacted with group.

3.2. Mean diffusivity

3.2.1. Effect of age

Higher MD with higher age was observed in superior colliculi fibers, lateral geniculate nucleus, thalamus, a small cluster in posterior internal capsule, inferior cerebellar peduncle, superior longitudinal fasciculus, and medial longitudinal fasciculus. The associations between MD and age are shown on a T1-weighted template brain in Figure 1. Table 1 indicates individual MD values for a subset of larger clusters. Table 6 lists coordinates for the regions where higher age was associated with higher MD.

3.2.2. Effect of group

When testing for a beneficial effect of CR, we found that the CR group showed lower MD in the tapetum of the

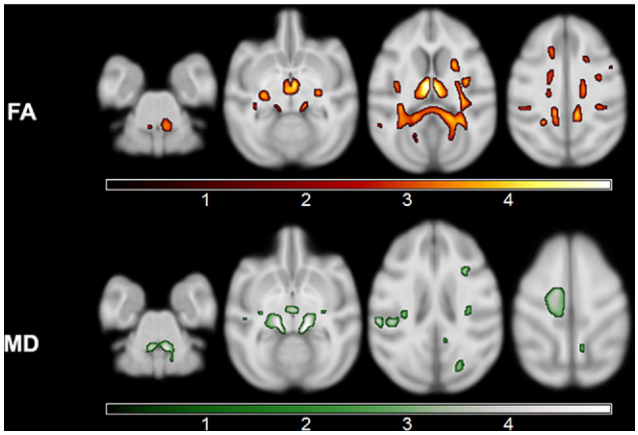

Fig. 1. White matter microstructure and age: calorie restricted and control monkeys combined. There was a negative correlation between FA and age. There was a positive correlation between MD and age. FA is shown on the top, and MD is shown below. The color in the images represents the magnitude of the *t* statistic.

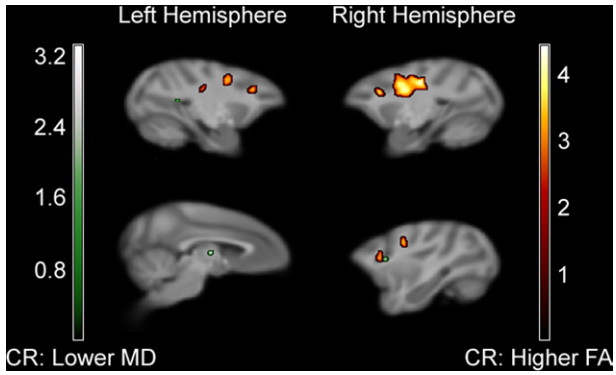

Fig. 2. The CR animals showed several regions of greater FA and lower MD compared with controls. The cooler colors (green) indicate regions where the CR animals showed lower MD compared with controls. Hot colors (orange/red) indicate regions where the CR animals showed higher FA compared with Controls. The color in the images represents the magnitude of the *t* statistic.

corpus callosum, thalamus, and a small portion of superior longitudinal fasciculus (Figure 2). These regions are listed by 112RM-SL coordinates in Table 3. When testing the extent to which the control monkeys showed lower MD compared with CR monkeys, regions that reached significance included the superior colliculi, superior longitudinal fasciculus, short association fibers in the parietal lobe, medial longitudinal fasciculus, dorsal occipital bundle, internal capsule, and external capsule. These regions are shown in Figure 3, and coordinates can be found in Table 4.

Table 2
Negative correlation between FA and age

Region	Location	Peak t-value	Cluster size
ThB	−2, 14, 18	5.03	5358
Body of CC	8, 11, 24	3.34	
Body of CC	−8, 18, 26	3.04	
ICa and TSB	10, 24, 20	4.67	6740
Tapetum of CC	8, −2, 17	4.24	
StB	14, 7, 17	4.09	
SLF	−6, 1, 26	4.57	742
SLF	4, 2, 27	4.22	666
ILF	−10, −4, 26	4.19	264
Corticocortical fibers (parietal)	16, 10, 34	3.89	94
ThB	−12, 9, 12	3.78	671
MLF	−22, 5, 22	3.54	225
SLF; EC	−16, 14, 20	3.50	311
CC; CB	−6, 30, 23	3.45	343
Inferior Cerebellar Peduncle	4, −4, 1	3.39	242
SLF	21, 22, 23	3.15	48
ILF	−24, −5, 18	3.01	28
ILF	20, −12, 16	2.99	40
SLF	−9, 13, 30	2.97	12
Occipito-Splenial fibers	−8, −12, 18	2.85	5
Inferior Cerebellar Peduncle	−4, −5, 1	2.84	9
ILF	18, 12, 10	2.82	9
SS	−16, −10, 22	2.79	8

CC, Corpus Callosum; CB, Cingulum bundle; dOB, dorsal occipital bundle; EC, external capsule; Ica, anterior internal capsule; ILF, inferior longitudinal fasciculus; MLF, medial longitudinal fasciculus; SS, sagittal stratum; StB, striatal bundle; SLF, superior longitudinal fasciculus; ThB, Thalamic Bundles; TSB, temporal subcortical bundle.

3.2.3. Group by age interaction

A significant interaction between group and age was found in short association fibers of the occipital lobe. Plotting MD against age in the two groups revealed a significantly greater positive slope in the controls compared with CR animals, where older age was also associated with higher MD. No regions showed the opposite pattern (a greater positive slope in CR). Table 5 lists the 112RM-SL coordinates for regions where age interacted with group.

Table 3
Greater FA and lower MD in CR

Region	Location	Peak t-value	Cluster size
FA: Control < Diet			
SLF; StB; TSB; FOF	11, 11, 28	4.43	2350
SLF	12, 30, 23	3.80	360
SLF	−12, 30, 24	3.35	118
FOF	−11, 20, 28	3.30	222
SLF	4, 2, 28	3.10	64
FOF	−10, 8, 24	2.97	34
EC	13, 10, 19	2.91	15
Brainstem	4, −8, −8	2.83	11
MD: Control > Diet			
Tapetum of CC	−8, −2, 18	3.32	155
Thalamic fibers	0, 14, 16	2.96	65
SLF	14, 27, 18	2.84	11

CC, Corpus Callosum; EC, external capsule; FOF, frontal occipital fasciculus; StB, striatal bundle; SLF, superior longitudinal fasciculus; TSB, temporal subcortical bundle.

Table 4
Greater FA and lower MD in control

Region	Location	Peak t-value	Cluster size
FA: Control > Diet			
EC	−18, 34, 16	3.39	53
ICa and ICp; TSB	−7, 16, 16	3.37	1012
Corticocortical fibers (parietal)	14, 18, 33	3.10	5
SLF	8, 2, 30	2.96	29
UF	−4, 37, 13	2.86	8
SLF	6, −8, 24	2.77	5
MD: Control < Diet			
Superior Colliculus fibers	−6, 4, 13	3.90	237
SLF	−17, 4, 31	3.53	138
SLF	5, −3, 32	3.52	349
Parietal corticocortical fibers	10, 4, 30	3.10	
SLF	−4, −2, 34	3.50	134
MLF	−20, 4, 22	3.48	77
SLF	−14, −1, 26	3.30	108
SLF	3, −10, 27	3.09	149
dOB	10, −19, 17	2.93	97
Ica	8, 18, 16	2.90	39
Superior Colliculus fibers	6, 6, 14	2.81	5

dOB, dorsal occipital bundle; EC, external capsule; Ica, anterior internal capsule; Icp, posterior internal capsule; MLF, medial longitudinal fasciculus; SLF, superior longitudinal fasciculus; TSB, temporal subcortical bundle; UF, uncinate fasciculus.

5. Discussion

A calorically restricted diet is known to impart beneficial effects on health and extend lifespan in a variety of species. Several mechanisms underlying this effect have been proposed (Anderson et al., 2009; Masoro, 2005; Mattson, 2008; Ramsey et al., 2000). It is acknowledged that the mechanisms that underlie CRs many effects are not likely to be mutually exclusive, but strong evidence suggests that a reduction in inflammatory processes underlies at least part of CRs beneficial effects (Bhattacharya et al., 2006; Jung et al., 2009; Kim et al., 2006) along with decreases in oxidative stress (Barja, 2004; Sohal and Weindruch, 1996). With regard to the rhesus monkeys in this cohort, CR is associated with lower levels of proinflammatory cytokines including IL-6 (Willette et al., 2010). Furthermore, Zainal et al. have shown in this same cohort that CR decreases oxidative stress, which in turn confers a beneficial effect on skeletal muscle tissue (Zainal et al., 2000). Accordingly, decreased inflammation and oxidation are considered prime candidates for the beneficial effect of CR on lifespan, and protection against age-related degeneration.

5.1. Age-related microstructural alterations

In human aging, brain white matter undergoes several alterations including a well-substantiated loss of volume (Courchesne et al., 2000; Jernigan et al., 2001; Raz et al., 2005), and microstructural alterations (Abe et al., 2002; Bendlin et al., 2009; Engelter et al., 2000; Pfefferbaum et al., 2000; Salat et al., 2005; Sullivan et al., 2006). Postmortem study of the brain in rhesus monkeys indicates that white matter is significantly compromised with age in the anterior commissure (Sandell and Peters, 2003), frontal lobe white matter, corpus callosum (Peters and Sethares, 2002), and primary visual cortex (Peters et al., 2008) due to the inclusion of dense cytoplasm, ballooning of myelin sheaths, formation of redundant myelin, and the circumferential splitting of thick sheaths (Feldman and Peters, 1998; Peters and Sethares, 2002; Peters et al., 2000). Brain imaging studies of volume indicate that the rhesus monkey undergoes a decline in forebrain white matter with age (Wisco et al., 2008). Age-associated white matter microstructural alterations have also been measured with DTI in superior and inferior longitudinal fascicles, cingulum bundle, and corpus callosum (Makris et al., 2007; Makris et al., 2009).

In the present study, age range was somewhat restricted compared with previous studies of brain aging, with animals falling along the spectrum of middle-aged adult to old/old (18–31 years of age). The median survival age of laboratory fed rhesus monkeys is ~ 25 years of age (Bodkin et al., 2003), with a maximum life span of ~ 35 years (Tigges et al., 1988). Similar to previous studies (Makris et al., 2007; Makris et al., 2009), we nevertheless found a relationship between age and white matter microstructure (as indexed by FA) in the corpus callosum (body and tapetum) and superior longitudinal fasciculus. Additional tracts identified using voxel-wise analysis in the present study included inferior longitudinal fasciculus, medial longitudinal fasciculus, inferior cerebellar peduncles, thalamic fibers, superior colliculi fibers, and tracts in the vicinity of lateral geniculate nucleus.

Although several of the affected regions were frontal, most of the age associations were clustered in midbrain. Moderate results in frontal brain regions are plausible given

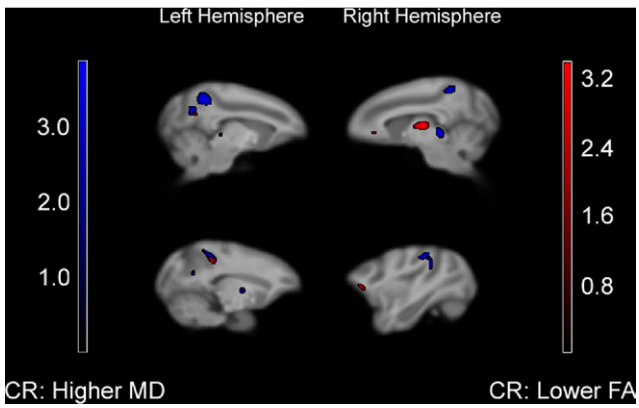

Fig. 3. In some regions, the control group showed greater FA and lower MD compared with the CR animals. Regions indicated by blue scale color are regions where the control group showed less MD compared with controls. Regions indicated by red scale colors are those where the control group showed higher FA compared with controls. The color in the images represents the magnitude of the *t* statistic.

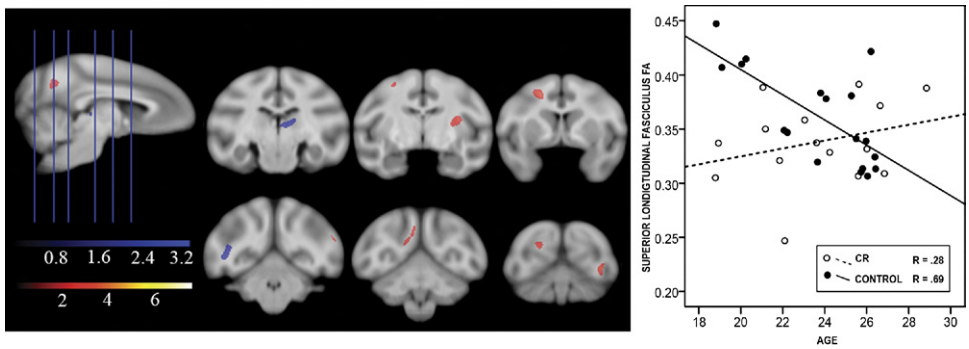

Fig. 4. Interaction with age. Both CR and control animals showed evidence for greater declines with age in relation to the other group, dependent on the region examined. CR animals showed attenuated age-related decline in several brain regions, including left superior longitudinal fasciculus, right inferior longitudinal fasciculus, right external capsule, left frontal short association fibers and left dorsal occipital bundle (shown in red below). Shown in blue are brain regions where the opposite was true, that is, CR showed a greater negative slope with age. These regions included portions of the thalamus and left inferior longitudinal fasciculus. The scatter plot on the right shows individual FA values from the left superior longitudinal fasciculus plotted against age. The control group (filled circles) shows a steeper slope with age compared with CR.

the age of the animals studied. Frontal white matter decline typically commences around the fourth decade of life in humans. In rhesus monkeys, myelin alterations such as circumferential splitting of the myelin sheath are present at 12–15 years of age (Sandell and Peters, 2003). It is possible that in later years, alterations in frontal white matter drop off from previous decline, and hence were not detected using correlation analysis in this older cohort.

One of the largest areas where an effect of age was detected was in a significant cluster situated over lateral geniculate nucleus. The lateral geniculate is the main structure through which visual information passes on the way to cortex, and studies indicate that neuronal counts in this region do not differ between young and old monkeys (Ahmad and Spear, 1993; Spear et al., 1994). However older animals do show differences in neuronal responses in this region compared with young animals. A lack of neuronal difference coupled with a behavioral difference may suggest alterations to myelin; post mortem examination of white matter in elderly rhesus may provide further information on age-related changes in this region.

5.2. Age and caloric restriction

Experiments in this cohort of animals have shown an interaction between age and diet that clearly favors CR animals. Monkeys undergoing CR develop less age-related disease, have virtually no indication of diabetes, are protected against sarcopenia, and tend to have an overall lower

Table 5
Age by group interaction

Region	Location	Peak t-value	Cluster size
FA			
Mediodorsal thalamic fibers	4, 8, 16	3.25	105
ILF	−20, −2, 16	2.85	21
ILF	26, −2, 22	3.51	20
SLF	−9, 23, 28	3.30	135
ILF	17, −17, 14	3.19	159
EC	15, 15, 18	3.07	82
CB (parahippocampal)	16, 1, 8	2.93	5
Frontal corticocortical fibers	−12, 16, 32	2.93	12
ICp	−11, 12, 14	2.90	11
SLF	−2, −8, 28	2.88	14
dOB	−10, −17, 24	2.83	10
MD			
Occipital corticocortical fibers	−7, −14, 30	2.95	22

CB, cingulum bundle; dOB, dorsal occipital bundle; EC, external capsule; ICp, posterior internal capsule; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus.

Table 6
Positive correlation between MD and age

Region	Location	Peak t-value	Cluster size
Superior colliculus fibers	6, 4, 14	5.03	2416
LGN fibers	9, 10, 8	3.56	
Superior colliculus and LGN fibers	−6, 6, 12	4.96	2641
Thalamic fibers	0, 12, 16	4.43	499
Inferior cerebellar peduncle	4, −6, 2	4.39	857
SLF	−7, 12, 30	3.93	2210
MLF	−24, 5, 22	3.92	145
MLF	−18, 5, 22	3.78	134
Occipito-splenial fibers	10, −12, 20	3.74	316
SLF	12, 27, 23	3.48	112
MLF	18, 5, 18	3.36	158
SLF	4, −6, 31	3.12	49
Occipito-splenial fibers	−6, −4, 18	3.07	45
SLF	14, 10, 20	3.04	208
MLF	16, 0, 24	2.98	42
SLF	−13, 8, 22	2.98	55
SLF	4, −4, 22	2.86	14
SLF	−3, −4, 25	2.81	7
ICp	−14, 10, 14	2.78	8

ICp, posterior internal capsule; LGN, lateral geniculate nucleus; MLF, medial longitudinal fasciculus; SLF, superior longitudinal fasciculus.

rate of mortality (Colman et al., 2009). In the present analysis of white matter, we hypothesized that the interaction between age and CR would show a similar pattern, where CR animals would exhibit less age-related declines when compared with controls. The analyses revealed several regions where this pattern was present, in particular, frontal superior longitudinal fasciculus and external capsule, small portions of cingulum, and dorsal occipital bundle. These results do suggest a protective effect of CR; however, the detected clusters were small and widespread compared with the main effect of age on white matter, and did not encompass large areas of the affected tract. Although we speculate that a reduction in inflammation or oxidative stress underlies the attenuation in age-related decline observed in this study, we cannot rule out that other health differences between the CR animals and control may have exerted independent effects on the brain diffusion measures.

Contrary to our hypothesis, we also found brain regions where the opposite pattern was true. Clusters where the age slope was steeper in CR animals were found in thalamus, inferior longitudinal fasciculus, and prefrontal cortex. These clusters were also small, but indicate that CR does not exert a global protective effect against FA decline with age. At least one other study has failed to establish a significant interaction between age and CR (Matochik et al., 2004), though striatum was the only area considered in that study.

5.3. Caloric restriction

Although we did not find strong evidence for widespread attenuation of age effects, an examination of CR, while controlling for age, revealed several regions where CR animals had higher FA compared with controls. Higher FA was present in bilateral superior longitudinal fasciculus, a set of long fiber bundles that form arcs through the brain sending branches into frontal, parietal, occipital, and temporal lobes; with CR primarily affecting frontal portions of this tract. Differences were also found in the anterior portions of the fronto-occipital fasciculus, external capsule, portions of the brainstem, dorsal occipital bundle, and a small cluster in right parahippocampal white matter. When MD was taken into account, the beneficial effects of CR were measurable in the thalamus, the tapetum of the corpus callosum (a small bundle of white matter that extends from the corpus callosum into temporal white matter), and superior longitudinal fasciculus.

We did not find a general beneficial effect across the brain; rather the CR animals exhibited localized regions of putative benefit. Bartzokis and others have hypothesized that certain brain regions may be more susceptible to the negative effects of inflammation and oxidation (Bartzokis et al., 2007; Bartzokis, 2004). In particular, brain regions that show late myelination in the course of development are hypothesized to show a greater susceptibility to brain insult compared with early myelinating regions. Our present results reflect this possibility, with beneficial effects of CR clustering to the anterior of the brain.

Interestingly, CR also showed effects opposite to our hypothesized direction of action. That is, we found brain regions where CR was associated with lower FA compared with control. Few studies have examined the effect of dieting on the brain, a handful of studies have examined extreme nutritional deficiency. In contrast to CR (which is a state of undernutrition without malnutrition), low body weight associated with starvation in both intentional (e.g. anorexia nervosa, bulimia, and hunger strike) and unintentional cases (war) is linked with smaller brain volume (Drevelengas et al., 2001; Krieg et al., 1989). Dieting in obesity indicates that loss of body weight is linked to a corresponding loss of brain volume, specifically white matter volume (Haltia et al., 2007). Additionally, obesity is associated with larger white matter volumes in superior, middle, and inferior temporal gyri, fusiform gyrus; parahippocampal gyrus, brain stem, and cerebellum (Haltia et al., 2007), and greater volume in the putamen (Pannacciulli et al., 2006). Similarly, Matochik et al. have found larger putamen volumes in rhesus monkeys that were fed *ad libitum* compared with CR animals (Matochik et al., 2004).

Although our study differs in species and comparative age range from the majority of existing studies of CR and body weight, the results of several studies suggest that either CR or body weight is related to brain volume, in particular, white matter volume. As is reported elsewhere (Colman et al., 2009), despite comparable nutrient intake, the CR animals weighed significantly less than control animals. It is not unreasonable to postulate that weight differences between groups could be accompanied by alterations in the diffusion properties of white matter, particularly if a CR diet is associated with a decrease in myelin lipids. Regions where we found higher FA and lower MD in the controls compared with CR monkeys included tracts in the vicinity of regions identified in previous human studies of body weight. These include the uncinate fasciculus, which connects the anterior temporal lobe to the orbital cortex, the internal and external capsules which carry axons from putamen (Ai et al., 2003), and occipital white matter fibers. Furthermore, in contrast to the primarily anterior pattern that was found in favor of CR, brain regions where controls showed higher FA and MD compared with CR animals were located primarily in mid and posterior brain regions.

5.4. Limitations

A limitation of this study is the small sample size. Larger sample sizes would provide more power to examine interactions and would likely obviate the need for the liberal threshold used in this study. It is important to note that the results are presented at an uncorrected (for multiple comparisons) threshold of $p < 0.005$. However, the effects of a CR diet on brain white matter are unknown, and consequently, we adopted an exploratory approach to the data.

The number of multiple comparisons was somewhat limited by employing a conservative white matter mask, and by the inherently smaller brain size of rhesus compared with humans. Nevertheless, the chance of Type 1 error was increased compared with analyses performed using more stringent criteria and the results should be considered with that caution in mind.

Most of the animals in this study are still alive. It will be invaluable to have postmortem information gleaned from the necropsied brain; in particular so the imaging results can be compared with tissue pathology and cell counts. Furthermore, ultimate lifespan itself will serve as a critical variable in the future analysis of these imaging data. Finally, the results reported here are cross-sectional. Longitudinal analyses of these animals over time will more systematically inform us about which aspects of the inevitable decline in brain integrity can be forestalled at the end of the life span.

Disclosure statement

The authors declare no actual or potential conflicts of interest.

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