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ORIGINAL ARTICLE

Caloric Restriction in Older Adults—Differential Effects of Weight Loss and Reduced Weight on Brain Structure and Function

Kristin Prehn[1], Reiner Jumpertz von Schwartzenberg[2], Knut Mai[2], Ulrike Zeitz[2], A. Veronica Witte[1,5], Dierk Hampel[2], Anna-Maria Szela[1], Sonja Fabian[1], Ulrike Grittner[3,4], Joachim Spranger[2] and Agnes Flöel[1]

[1]Department of Neurology and NeuroCure Clinical Research Center, Charité Universitätsmedizin Berlin, Berlin, Germany, [2]Department of Endocrinology, Diabetes and Nutrition, Experimental and Clinical Research Center and Center for Cardiovascular Research, Charité Universitätsmedizin Berlin, Berlin, Germany, [3]Department of Biostatistics and Clinical Epidemiology, Charité Universitätsmedizin Berlin, Berlin, Germany, [4]Center for Stroke Research, Charité Universitätsmedizin Berlin, Berlin, Germany and [5]Department of Neurology, Aging and Obesity Group, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

Address correspondence to Kristin Prehn, Department of Neurology and NeuroCure Clinical Research Center, Charité Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany. Email: kristin.prehn@charite.de; Agnes Flöel, Department of Neurology and NeuroCure Clinical Research Center, Charité Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany. Email: agnes.floeel@charite.de.

Abstract

Dietary modifications such as caloric restriction (CR) have been suggested as a means to improve memory and prevent age-related decline. However, it is unclear whether those effects remain stable over time or are related specifically to negative energy balance during the weight loss phase of CR. Using a randomized interventional design, we investigated changes in recognition memory and neural correlates in postmenopausal obese women ($n = 19$): 1) after intense weight loss in the course of a 12-week low-caloric diet (reduced body weight and negative energy balance) and 2) after having sustained the reduced weight over 4 more weeks (reduced body weight, but energy balance equilibrium). Participants were contrasted to a control group ($n = 18$) instructed not to change dietary habits. In the CR group, we found improved recognition memory, paralleled by increased gray matter volume in inferior frontal gyrus and hippocampus, and augmented hippocampal resting-state functional connectivity to parietal areas. Moreover, effects were specific for transient negative energy balance and could not be detected after subsequent weight maintenance. Our data demonstrate for the first time in humans that beneficial effects of CR on brain structure and function are due to weight loss rather than an overall reduced weight.

Key words: aging, hippocampus, nutritional intervention, resting-state fMRI, VBM

Introduction

Obesity and overweight have been increasing dramatically in many countries of the world (Finucane et al. 2011) and represent a major health problem. Besides increasing the risk for diseases such as type 2 diabetes, hypertension, coronary heart disease, and stroke (Guh et al. 2009), obesity is associated with accelerated age-related cognitive decline (Elias et al. 2005; Cournot et al. 2006) and even with dementia in later life (Fitzpatrick et al. 2009).

The previous work on obesity and brain structure has provided evidence that brain areas most vulnerable to the aging

process (i.e., frontal and medial temporal brain regions) are also negatively affected by obesity (for a review, see Bischof and Park 2015). Brooks et al. (2013), for instance, found that older obese subjects have smaller prefrontal gray matter (GM) volume than individuals of normal weight. Although it is not entirely clear how obesity exactly compromises the brain's ability to reorganize in response to age-related structural deterioration (Bruce-Keller et al. 2009), global and regional disruptions in energy metabolism seem to play an important role. Specifically, it has been found that excessive energy intake, accompanied by chronically elevated levels of blood glucose, triglycerides, and free fatty acids, seems to impair the ability of neurons to adapt to oxidative and metabolic stress and chronic inflammation (Kapogiannis and Mattson 2011; Miller and Spencer 2014). Higher HbA1c and fasting glucose concentrations, in particular, have been found to be related to lower memory performance and hippocampal atrophy in healthy older individuals (Kerti et al. 2013).

Conversely, epidemiologic observations in humans and animal studies supported the notion that moderate life-long caloric restriction (CR; i.e., below the usual ad libitum levels yet without malnutrition) contributes to healthy aging and longevity. For instance, the long average life expectancy in residents of Okinawa is thought to be related to a special diet which is low in calories (Willcox et al. 2007). Two parallel 20-year longitudinal studies in rhesus monkeys also confirmed that CR increases lifespan and delays the onset of age-related pathology (Colman et al. 2009, 2014), or at least improves metabolic and vascular parameters (Mattison et al. 2012).

With regard to mechanisms, current models suggest that excessive energy intake can impair, whereas energy restriction may engage adaptive cellular stress response pathways. These pathways involve the upregulation of genes that encode cytoprotective proteins, enzymes, and neurotrophic factors which strengthen neural networks and enhance neural plasticity. Thus, by imposing mild stress on neurons, CR protects neurons against aging, injury, and disease (for reviews, see Mattson et al. 2004; Martin et al. 2006; Mattson 2010; Stranahan and Mattson 2012). Studies in mice, for example, found that CR-induced improvements in learning and memory were associated with neurogenesis in the hippocampus (Lee et al. 2002). Age-related impairments in cellular energy metabolism could also be reversed by CR as could pathology in Alzheimer's disease model mice (Halagappa et al. 2007).

Evidence from prospective interventional studies in humans, however, is scarce. In a previous study of our group, Witte et al. (2009) investigated the effect of CR on memory performance in healthy older normal to overweight subjects. Subjects were instructed to reduce calorie intake by 30% relative to previous habits over a period of 3 months. After CR and moderate weight loss, we found an improved verbal recognition memory performance. Memory improvement was related to improved glucose metabolism, indicated by lower fasting plasma insulin concentration. Although these results are very encouraging, it is unclear so far whether cognitive improvements induced by CR remain stable over time or are related specifically to negative energy balance during the weight loss phase. The question of sustainability is of great importance as long-term and intense CR has not always been beneficial for cognitive function (e.g., Bellush et al. 1996; Yanai et al. 2004). Moreover, in human participants, long-term and intense CR interventions show poor adherence (Appelhans et al. 2015) and may even convey negative health effects in individuals with incipient dementia (Wysokinski et al. 2015). Moreover, the neural correlates of CR in humans have not been investigated so far.

To answer the question of sustainability and to investigate the neural correlates of CR, we conducted a randomized and controlled proof-of-concept study. On the basis of our previous work, we compared verbal recognition memory performance (Floel et al. 2008; Witte et al. 2009), GM volume and functional connectivity in fronto-temporal brain areas, as well as insulin–glucose metabolism directly after the weight loss of CR and after a period of maintaining the reduced weight. According to previous experimental animal studies, neural plasticity is specifically promoted by energy restriction (e.g., Yu and Mattson 1999; Maswood et al. 2004; Arumugam et al. 2010). Therefore, we expected that recognition memory improvement would be larger directly after the weight loss than after the weight maintenance phase of CR. In addition, we hypothesized that memory improvement would be accompanied by increased GM volume in fronto-temporal brain regions (e.g., the prefrontal cortex and the hippocampus) and changes in hippocampal resting-state functional connectivity (RSFC).

Materials and Methods

Study Overview

Subjects were tested in recognition memory performance before (t1), directly after having lost more than 10% of their body weight in the course of a 12-week low-caloric diet (t2), and after having sustained the reduced weight over an additional period of 4 weeks in which they followed an isocaloric diet (t3). Subjects also underwent structural and functional neuroimaging at all 3 time points. In addition, blood samples were taken and anthropometric data as well as blood pressure was measured. Results were contrasted to a control group instructed not to change dietary habits. For an overview of the study, see Figure 1A.

Participants

Fifty-three older obese women were recruited for the study. Inclusion criteria were age between 40 and 80 years, a body mass index (BMI) >27 kg/m^2, and postmenopausal status. Exclusion criteria comprised history of severe untreated medical, neurological, and psychiatric diseases which may interfere with the planned interventions, such as instable coronary heart disease, kidney and liver disease, systemic infections, endocrinological disorders, and hypertension (systolic blood pressure >180 mmHg, diastolic blood pressure >110 mmHg). We also excluded subjects who changed dieting or smoking habits significantly in the last 3 months including a weight loss of 5 kg or more. Inclusion and exclusion criteria were first checked during a telephone screening. During the baseline visit, subjects underwent a standardized medical interview as well as a neurological examination, and were screened for cognitive impairment using the Mini-Mental State Examination (Folstein et al. 1975). Psychiatric comorbidity was monitored using the Beck's Depression Inventory (BDI; Kuhner et al. 2007).

Subjects were randomly assigned to the 2 groups: CR group and control group. From the 53 subjects who were initially included in the study, 10 were not available for follow-up testing due to personal reasons ($n = 5$ from the CR group). Given the limited sample size, we decided to exclude from analysis subjects in the CR group who did not lose at least 10% of body weight (CR group, $n = 4$) and subjects in the control group who lost more than 5% of body weight (control group, $n = 2$). Therefore, 37 subjects were available for the per-protocol analysis (CR group: $n = 19$; mean age = 61 years; control group: $n = 18$, mean age = 61 years).

Subjects in CR and control group were comparable with regard to age, years of education, handedness measured using the Edinburgh Handedness Inventory (Oldfield 1971), weight, BMI, waist

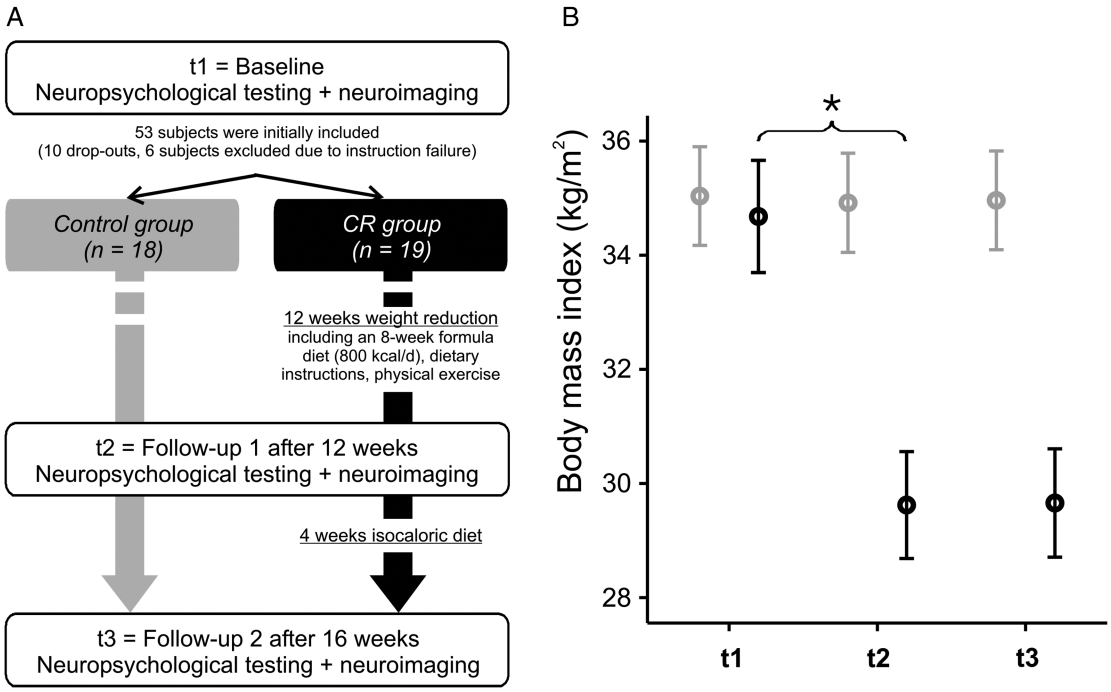

Figure 1. (A) Study flow and (B) changes in body mass index (BMI; mean and standard error of the mean in kg/m^2) in the CR (black color) and control group (gray color) at baseline (t1), follow-up 1 (t2), and follow-up 2 (t3). *$P < 0.05$ in a paired t-test indicating significant weight loss in the CR group. Healthy obese postmenopausal women randomized into 2 groups (CR group and control group) underwent baseline measurements and follow-up measurements including medical examination, neuropsychological testing and neuroimaging. Between t1 and t2 (after 12 weeks), participants in the CR group lost more than 10% of their body weight following CR. Weight loss was supported by a low-caloric formula diet (800 kcal/day) in the first 8 weeks. Between t2 and t3 (after additional 4 weeks), participants in the CR group followed an isocaloric diet without having a negative energy balance. Participants in the control group were instructed not to change their dietary habits. Between t1 and t2, subjects in the CR group showed significant weight loss ($t_{(18)} = 20.3$, $P < 0.001$) and were able to maintain reduced weight unto t3 (no significant difference in BMI between t2 and t3: $t_{(18)} = -0.4$, $P = 0.73$).

Table 1 Baseline characteristics for control and CR group (mean [SD])

	Control group	CR group
n	18	19
Age (years)	61 (4)	61 (6)
Education (years)	15 (3)	16 (3)
Right-handedness (%)	83.6 (22.0)	74.7 (35.6)
Weight (kg)	94.6 (9.5)	93.2 (11.0)
BMI (kg/m^2)	35.0 (3.7)	34.7 (4.3)
Waist circumference (cm)	113.7 (7.8)[a]	114.6 (10.5)
Systolic blood pressure (mmHg)	131.8 (16.9)	135.5 (16.3)
Diastolic blood pressure (mmHg)	86.8 (12.4)	89.0 (7.5)
HbA1c (%)	5.7 (0.5)	5.6 (0.9)[a]
Fasting glucose (mg/dL)	104.8 (14)	101.1 (19.9)
Mini-mental state examination (score)	29.2 (0.9)	29.3 (0.9)
Verbal intelligence (vocabulary test score)	31.7 (2.2)	32.5 (2.1)
Beck's Depression Index (score)	7.2 (4.7)[a]	6.0 (3.6)

BMI, body mass index; HbA1c, glycated hemoglobin A.
[a]Data were not available for one subject.

circumference, systolic and diastolic blood pressure, HbA1c, and fasting glucose blood concentration (all P's > 0.05; see Table 1 for demographic and baseline characteristics). There was also no difference in general verbal intelligence assessed using a vocabulary test between the groups (MWT-B; Lehrl 2005).

The study was conducted as part of a larger study focusing on muscle mass regulation "Effects of negative energy balance on muscle mass regulation" (registered at https://clinicaltrials.gov, NCT01105143) at the Department of Endocrinology at Charité University Medicine Berlin. The research protocol of the add-on study on memory and related neural correlates at the Department of Neurology was approved by the local Ethics Committee (EA2/050/10). The study was carried out in accordance with the principles of the Declaration of Helsinki, and all subjects provided written informed consent before investigation and received reimbursement for participation.

Caloric Restriction Intervention

In the CR group, weight loss was induced by lifestyle changes initiated via weekly counseling by clinical dieticians and using a structured weight reduction program. In the first 8 weeks of the 12 weeks weight loss phase, weight loss was supported by a low-caloric formula diet (800 kcal/day) in which all meals were replaced by a very low energy drink (Optifast 2®, Nestlé HealthCare Nutrition GmbH, Frankfurt/Main, Germany). Participants were advised to consume the formula diet exclusively (daily consumption of 5 packets with 160 kcal each: 20 g carbohydrates, 14 g proteins, and 3 g fat dissolved in 300 mL water) and not to consume any additional food. Therefore, participants received 35 portions of the formula diet for each week at the weekly counseling meetings. The 8 weeks of formula diet were followed by 4 weeks in which the formula diet was substituted by an energy-reduced diet to facilitate further weight loss. Meals in this diet comprised a balanced mix of macronutrients in accordance with the guidelines of the German Society for Nutrition (50–55% carbohydrates, 15–20% proteins, and 30% fat). Additionally,

participants were advised to increase physical activity to reach a goal of 150 min of activity per week. In sum, the CR intervention led to an average weight loss of 12.3 kg (i.e., 15% of initial body weight on average; minimum = 6.4 kg, maximum = 20.2 kg).

After the 12-week weight loss phase, subjects were instructed to maintain reduced weight over additional 4 weeks (until t3). That is, in the weight maintenance phase, subjects were instructed to follow an isocaloric diet without having a negative energy balance. The recommended daily calorie intake during the weight maintenance phase was individually specified based on the measured energy expenditure via calorimetry using a ventilation hood (Quark RMR, COSMED Deutschland GmbH, Germany) after a 20-min resting period, and adapted with regard to subsequent weight changes. The isocaloric diet again comprised a balanced mix of foods with 50–55% carbohydrates, 30% fat, and 15–20% proteins. Figure 1*B* illustrates the significant reduction in BMI in the CR group and shows that subjects were able to maintain reduced weight. Subjects in the control group were instructed not to change their dietary habits during the course of the study.

Assessment of Recognition Memory Performance, Other Cognitive Scores, Mood, Affect, and Physical Activity

Subjects were tested in verbal recognition memory performance using the German version of the Auditory Verbal Learning Test (VLMT; Helmstaedter et al. 2001). To avoid test–retest effects, 3 parallel versions were used. During the VLMT, subjects had to learn as many words as possible from a spoken list of 15 words presented in 5 consecutive trials. Subjects had to recall the words immediately after each trial and after a 30-min delay. Finally, subjects had to identify the previously learned words from a list which also includes 20 new words and 15 words from an interference list learned during the delay.

The VLMT recognition score (primary outcome) comprises the number of correctly recognized words minus false-positive identifications (maximum: 15 words). In addition to the recognition score, the VLMT allows for calculation of 3 more memory scores. The "learning" score is defined as the sum of correctly recalled words during the 5 immediate learning trials (maximum: 75 words). The "delayed recall" score is defined as the number of correctly recalled words after the 30-min delay (maximum: 15 words). The "consolidation" score is defined as the number of words recalled correctly after the fifth learning trial minus the number of words recalled correctly after the delay. Since the consolidation score indicates the number of forgotten items, lower values signify better performance.

Further neuropsychological testing assessed sensorimotor speed, attention, and executive functions and included digit span, trail making test (TMT) part A and B, Stroop color-word test and verbal fluency (Lezak 2004). Changes in affect and mood were measured using the BDI, the Positive and Negative Affect Schedule (PANAS; Watson et al. 1988), and the State-Trait Anxiety Inventory (Spielberger et al. 1970). All neuropsychological testing was performed by a trained clinical neuropsychologist blinded to the participants' group assignment.

To monitor whether participants increased or decreased physical activity over time, we used the standardized and validated Freiburger Questionnaire on Physical Activity (Frey et al. 1999) which has been developed to quantify regular physical activities in daily life (e.g., walking wherever possible, gardening, swimming, cycling, exercising in the gym, etc.). Participants are requested to indicate how many hours on average they spent on each activity in the last weeks. Time spent on each activity is multiplied by a metabolic equivalence score assigned to that specific activity (Ainsworth et al. 1993) to calculate energy expenditure in kcal per kg body weight.

Blood Sampling, Anthropometric Data, and Blood Pressure

As indicators of glucose metabolism, we determined fasting glucose, HbA1c, and maximum glucose concentration in the Oral Glucose Tolerance Test (OGTT). For blood sampling, subjects arrived at the laboratory at 8 AM in the morning after an overnight fast. First, fasting blood samples (fasting glucose and HbA1c) were taken. Then, the OGTT was conducted. Subjects were orally given a solution containing 75 g of glucose, and capillary blood samples were taken after 30, 60, 90, 120, and 180 min. Furthermore, we measured weight, height, waist circumference, as well as systolic and diastolic blood pressure at all 3 time points.

Statistical Analysis of Neuropsychological and Physical Parameters

Statistical analysis of neuropsychological data and physical parameters was performed using SPSS 18.0 (PASW, SPSS, IBM, Armonk, NY, USA). All parameters were measured at baseline (t1) and follow-up sessions (t2, t3). In the case of skewed distributions ($|\text{skewness}| > 1$), variables were transformed using a rank transformation. To assess changes over time in recognition memory performance in subjects of the CR in comparison with the control group, we computed a linear mixed model (random intercept model) fitting all data points (Verbeke and Molenberghs 2000). Time points (t1, t2, t3) were level-one units nested in the different individuals who were level-two units (111 test values, 37 subjects). Dummy coding was used for the time points with baseline as reference. The mixed model tested for group × time interactions at t2 and t3 by using these dummy variables for time point t2 and t3 (e.g., an increase in the CR compared with control group at t2 compared with the other time points).

Other memory and cognitive scores, scores for mood, affect, physical activity, indicators of glucose metabolism, anthropometric data, and blood pressure were also analyzed with a linear mixed model for exploratory purposes. No adjustments were made to control for multiple comparisons.

For illustration purposes and to test for changes within each group, additional post hoc paired *t*-tests were calculated between the time points (t1 vs. t2 and t1 vs. t3; Supplementary Table 1 and Figs 1*B* and 2). To explore the relationship between changes in glucose metabolism and changes in other variables, bivariate correlation analyses were run. The level of significance for all analyses was set at $\alpha = 0.05$ (two-tailed).

Magnetic Resonance Imaging

Data Acquisition

Magnetic resonance imaging was performed on a Siemens Trio system operating at 3T and using a 12-channel head coil. Imaging at all 3 time points (t1, t2, t3) comprised a high-resolution anatomical scan as well as a functional scan measuring blood oxygen level–dependent (BOLD) signal at rest. The anatomical scan consisted of 192 slices and was acquired in sagittal plane using a high-resolution T_1-weighted magnetization-prepared rapid acquisition with gradient echo sequence (repetition time = 1900 ms, echo time = 2.52 ms, flip angle = 9°, voxel size = 1 × 1 × 1 mm). Functional images were obtained using a T_2*-weighted EPI sequence (repetition time = 2300 ms, echo time = 30 ms, flip angle = 90°,

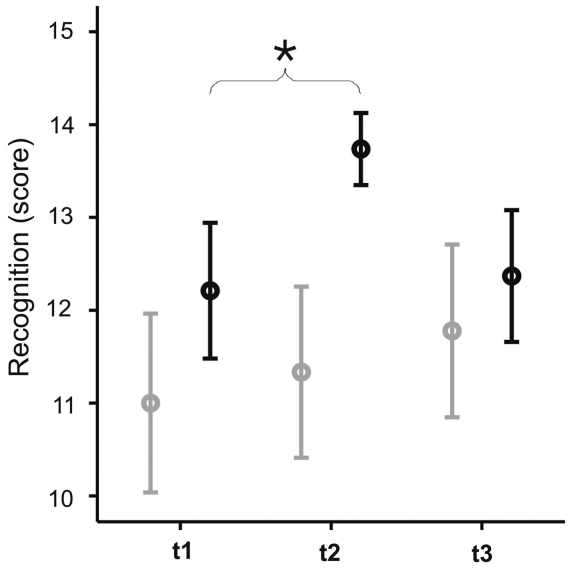

Figure 2. Recognition memory performance in the CR group (black color) and in the control group (gray color) at baseline (t1), follow-up 1 (t2), and follow-up 2 (t3). The recognition score (mean and standard error of the mean) represents the number of correctly recognized words after the 30-min delay adjusted for false-positive responses (min: −1, max: 15). *$P < 0.05$ in a paired *t*-test indicating significant improvement in the CR group.

voxel size = 3.0 × 3.0 × 4.0 mm). A total of 34 slices sampled for whole-brain coverage and across 150 time points (volumes) were collected. During the 6-min functional scan, subjects were instructed to keep their eyes closed and not to think of anything in particular.

Voxel-Based Morphometry

Analysis of high-resolution anatomical images was conducted using the SPM8 (Statistical parametric mapping software; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm) voxel-based morphometry (VBM) toolbox (VBM8; http://dbm.neuro.uni-jena.de/vbm) implemented in MATLAB 7.9.0 (Mathworks, Inc., Sherborn, MA, USA). Since the analysis of longitudinal anatomical data requires a customized processing that considers differences within each individual separately, data preprocessing was done using a specific batch. This batch registers baseline and follow-up images of each subject to the mean of both images and calculates structural differences (i.e., intraindividual changes) by applying spatial normalization parameters, which were estimated during segmentation of the mean image, to both images. In detail, data preprocessing for VBM comprised the following steps for each subject. In a first step, baseline and follow-up images (t1, t2, t3) were initially realigned to a T1 template in MNI (Montreal Neurological Institute) space. Second, a mean image (from t1, t2, and t3) was calculated and raw data were realigned using the mean image as the reference image. Then, images were bias-corrected to account for signal inhomogeneities and, in the next step, segmented into the different tissue classes (GM, white matter, cerebrospinal fluid). This segmentation procedure was further refined 1) by accounting for partial volume effects (Tohka et al. 2004), 2) by using adaptive maximum a posteriori estimations (Rajapakse et al. 1997), and 3) by applying a hidden Markov random field model (Cuadra et al. 2005). The resulting tissue maps were spatially normalized using a specific MNI template derived from 550 healthy control subjects of the IXI database (http://www.brain-development.org) and linear (12-parameter affine) transformations together with a nonlinear diffeomorphic image registration algorithm (DARTEL; Ashburner 2007). Spatial normalization parameters obtained from the segmented mean image were finally applied to the segmentations of the bias-corrected baseline and follow-up images, which were realigned again. Data were not modulated (i.e., scaled by the amount of contraction or expansion during normalization), because scaling is not necessary in longitudinal designs in which the focus is on relative differences between 2 images of the same participant (Freund et al. 2014). Finally, GM segments (wp1mr*) representing GM density were smoothed with a 10-mm full-width-at-half-maximum (FWHM; Silver et al. 2011) Gaussian kernel suitable for small sample sizes (Shen and Sterr 2013).

Processing of Hippocampal RSFC Data

Functional imaging data were preprocessed using the Data Processing Assistant for Resting State fMRI (DPARSF; http://www.restfmri.net/forum/DPARSF; Chao-Gan and Yu-Feng 2010), an automated pipeline for resting-state fMRI data analysis based upon SPM8 and implemented in MATLAB 7.9.0. During preprocessing, images were first corrected for acquisition time difference between the slices, and then realigned to the first volume to correct for head motion between volumes. Physiological noise was reduced 1) by regressing out signals from white matter, cerebrospinal fluid and the 6 head movement parameters, 2) by removing a linear trend and band-pass filtering the data (0.01–0.08 Hz) to reduce the effects of very low- and high-frequency physiological noise, and 3) by global signal removal.

Since we found significant differences in GM density in the right hippocampus (but not in the left), we used an anatomical region of interest (ROI) of the right hippocampus from the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002) as seed region to investigate hippocampal RSFC in line with our previous studies. The ROI was transferred into each subject's native space. Then, for each participant and time point, the time course of the preprocessed BOLD signal averaged within both seed regions was correlated with the BOLD signal in each voxel of the whole brain. The Pearson's *r* correlation coefficient 3D maps were z-transformed (Fisher's z) resulting in spatial maps representing the voxel-wise strength of functional connectivity to the seed region (zFC maps). For further statistics at the group level, functional images were spatially normalized using an MNI EPI template re-sampled into 3 × 3 × 3 mm isotropic voxels (see also Witte et al. 2014). Finally, the normalized functional data were smoothed with a 4-mm FWHM Gaussian kernel that is recommended for RSFC data (Chao-Gan and Yu-Feng 2010).

Statistical Analysis and Thresholding of Imaging Data

For the statistical analysis of VBM and RSFC data (i.e., individual wp1mr* GM segments and zFC maps), we used repeated-measures ANOVAs with a flexible factorial design comprising the factors "subject," "group," and "time" testing for group × time interactions as implemented in SPM8. To infer significant changes in GM density and hippocampal RSFC over time (t1–t2 and t1–t3) and between groups, 4 *t*-contrasts were formulated: 1) CR group > control group$^{t2>t1}$, 2) Control group > CR group$^{t2>t1}$, 3) CR group > control group$^{t3>t1}$, and 4) Control group > CR group$^{t3>t1}$.

In the analysis of GM changes, absolute GM thresholds of 0.2 were used to prevent edge effects located at the border regions of the tissue maps. To avoid false-positive results in the VBM analysis (Scarpazza et al. 2015), reported changes had to survive a voxel-wise family-wise error (FWE) correction for multiple comparisons at $P < 0.05$. In addition, clusters had to have a cluster size

Table 2 Results from the linear mixed model testing for group × time interactions at t2 and t3 (111 measures; CR group: $n = 19$, control group: $n = 18$; F-value [$df1$ for numerator, $df2$ for denominator], P-value)

	Group × time interaction at t2		Group × time interaction at t3	
	F ($df1$, $df2$)	P	F ($df1$, $df2$)	P
Weight	610.1 (1, 73)	**<0.001**	608.2 (1, 73)	**<0.001**
BMI	697.1 (1, 73)	**<0.001**	687.4 (1, 73)	**<0.001**
Waist circumference	120.6 (1, 77)	**<0.001**	140.6 (1, 77)	**<0.001**
Systolic blood pressure	1.6 (1, 94)	0.21	0.2 (1, 94)	0.68
Diastolic blood pressure	1.3 (1, 98)	0.27	3.4 (1, 98)	0.07
HbA1c	0.0 (1, 76)	0.86	10.7 (1, 75)	**0.002**
Fasting glucose	0.0 (1, 79)	0.96	1.6 (1, 79)	0.21
Maximum glucose in OGTT	5.8 (1, 98)	**0.02**	1.9 (1, 98)	0.17
Primary memory score				
VLMT: recognition	6.8 (1, 94)	**0.01**	0.2 (1, 94)	0.70
Further memory scores				
VLMT: learning	14.1 (1, 89)	**<0.001**	12.2 (1, 89)	**0.001**
VLMT: delayed recall	8.6 (1, 91)	**0.004**	5.4 (1, 91)	**0.02**
VLMT: consolidation	2.6 (1, 102)	0.11	1.64 (1, 102)	0.20
Further cognitive, affective, and activity scores				
Digit span	0.2 (1, 82)	0.68	1.6 (1, 82)	0.11
TMT part A	5.2 (1, 84)	**0.03**	15.8 (1, 84)	**<0.001**
TMT part B	1.9 (1, 82)	0.17	9.1 (1, 82)	**0.003**
Stroop color-word interference	7.3 (1, 77)	**0.008**	19.4 (1, 77)	**<0.001**
Verbal fluency, phon.	4.0 (1, 97)	0.05	6.3 (1, 97)	**0.01**
Verbal fluency, sem.	3.1 (1, 98)	0.08	8.7 (1, 98)	**0.004**
Beck's Depression Index	9.6 (1, 87)	**0.003**	19.7 (1, 87)	**<0.001**
Positive PANAS score	1.0 (1, 87)	0.31	1.7 (1, 87)	0.19
Negative PANAS score	1.2 (1, 100)	0.28	0.0 (1, 100)	0.90
Anxiety score	0.3 (1, 94)	0.57	2.4 (1, 94)	0.12
Energy expenditure	0.0 (1, 102)	0.88	0.3 (1, 102)	0.57

Note: Significant interactions (i.e., changes over time in the CR group compared with the control group) are indicated by bolding the number.

larger than 50 voxels (Holmes et al. 2013). Since we were specifically interested in structural changes in the hippocampus, we also applied a voxel-wise small-volume FWE correction (SVC) using an a priori defined bilateral hippocampus ROI obtained from the AAL atlas (Tzourio-Mazoyer et al. 2002). For explorative follow-up analyses and illustration purposes, we extracted GM values within the ROIs identified in the whole-brain analysis (left and right inferior frontal gyrus [IFG], right hippocampus) using the MarsBaR toolbox (marsbar.sourceforge.net) based upon SPM8.

Reported differences in hippocampal RSFC had to survive a more lenient cluster-wise FWE threshold of $P < 0.05$. Cluster-wise FWE correction here was done using an uncorrected cluster-defining threshold of $P < 0.001$. For explorative follow-up analyses, correlation coefficients in the functional ROIs were also extracted using the MarsBaR toolbox.

Results

Changes in Recognition Memory, Other Cognitive Scores, Mood, and Affect

The linear mixed model testing for group × time interactions for recognition memory performance (primary outcome) revealed a significant group × time interaction at t2, indicating better memory performance after the weight loss phase in the CR group in comparison with the control group and all other time points (for F- and P-values see Table 2, and for scores in each group at each time point see Supplementary Table 1 and Fig. 2). This result of the per-protocol analysis was reproduced in an intention-to-treat analysis (also including the 4 subjects of the CR group who failed to lose at least 10% of body weight and the 2 subjects of the control group who lost more than 5% of body weight while instructed not to change dietary habits; CR group: $n = 23$; control group: $n = 20$; $F_{1,111} = 5.6$, $P = 0.02$; mean difference between the groups at t2: 2.4 words, standard error: 0.9). With regard to the 3 other memory scores of potential interest (learning, delayed recall, and consolidation score), there was also a significant group × time interaction at t2 for learning score, delayed recall, but not for consolidation score.

However, no significant group × time interaction in recognition memory performance could be found at t3 (for F- and P-values, see Table 2), indicating no significant difference in memory performance between the groups after the weight maintenance phase in the CR group in comparison with the control group and all other time points. This result was reproduced in the intention-to-treat analysis ($F_{1,111} = 0.8$, $P = 0.38$). A significant group × time interaction at t3 was found for learning and delayed recall, but again not for the consolidation score. That is, positive effects of CR on recognition memory were specific for the weight loss phase and could not be detected after a period of maintaining the reduced weight, while improvements in learning performance and delayed recall remained stable over time.

With regard to further cognitive and affective domains, we found improvements in the CR group after the weight loss phase (at t2) in processing speed (TMT part A), Stroop color-word interference, and a reduction in Beck's depression score (see Table 2). All effects remained stable over time (until t3) and subjects in the CR group, in addition, improved at t3 in task switching (TMT part B) and verbal fluency (see Table 2).

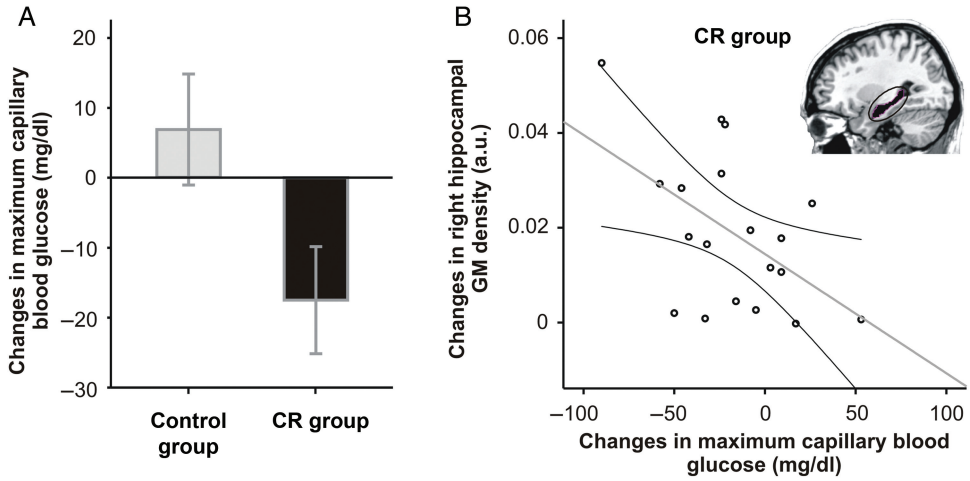

Figure 3. (A) Changes in maximum capillary blood glucose concentration (mean and standard error of the mean in mg/L) measured with the OGTT in subjects in the CR group (black color) and in the control group (gray color) between t1 and t2. (B) Changes in maximum glucose were negatively correlated with changes in right hippocampal GM density in the CR group, indicating that improved glycemic control was associated with increased GM density ($r = -0.52$; $P = 0.02$; changes in GM density in an anatomical ROI of the right hippocampus plotted against changes of maximal glucose concentration with regression line and 95% confidence limits).

The within-group comparisons (see Supplementary Table 1) revealed that some improvements over time were found in both the CR and the control group (in VLMT learning score, TMT part A and B, Stroop color-word interference; see Supplementary Table 1). These tests are assessing the number of words that participants are able to recall immediately, processing speed, and executive functions (task switching and resistance to interference). Therefore, the scores of these tests might have been influenced by learning effects regarding test taking ability and familiarity with the testing situation (thus, learning effects that occur despite using parallel test versions). Interestingly, we also found a decrease in positive affect in the control group between t1 and t2, which might indicate control subjects' dissatisfaction with participating in a study on the positive effects of weight loss and not being part of the intervention group.

Changes in Glucose Metabolism

The linear mixed model testing for group × time interactions revealed a significant group × time interaction for maximum glucose concentration in the OGTT at t2 (see Table 2), indicating that glycemic control was improved after the weight loss phase of CR. This effect diminished and could not be detected at t3. However, we found a significant group × time interaction for HbA1c at t3, which is regarded as a slower changing long-term marker of glucose metabolism (Kerti et al. 2013). Changes in maximum glucose concentration measured with the OGTT in CR and control group between t1 and t2 are displayed in Figure 3A. For within-group comparisons see Supplementary Table 1.

Changes in Gray Matter Volume

We found a significant increase in GM density in bilateral IFG between t1 and t2 in the CR compared with the control group (for coordinates, see Table 3 and Fig. 4; contrast: CR group > control group$^{t2>t1}$). Using voxel-wise SVC, we also found increased GM density in the right hippocampus. GM volume reductions in the CR group were found in the right olfactory cortex, the left postcentral gyrus, and the right cerebellum (vermis; contrast: control group > CR group$^{t2>t1}$).

Contrasting GM density between t1 and t3 in the CR compared with the control group (contrast: CR group > control group$^{t3>t1}$), we found no significant increase, indicating that effects reported for the comparison between t1 and t2 were specific for the weight loss phase of CR and diminished during a period of subsequent weight maintenance. However, we still found a reduction in GM density in the CR group in the right cerebellum after the weight maintenance phase (contrast: control group > CR group$^{t3>t1}$).

Results were replicated in an intention-to-treat analysis (i.e., by including also those subjects excluded from the per-protocol analysis; CR group: $n = 23$; control group: $n = 20$; Supplementary Material and Table 2).

Changes in Hippocampal RSFC

We found a significant increase in RSFC between hippocampus and left precuneus and left angular gyrus between t1 and t2 in the CR compared with the control group (for coordinates, see Table 4 and Fig. 5; contrast: CR group > control group$^{t2>t1}$). A decrease in RSFC was found between hippocampus and right IFG extending to the insular cortex (contrast: control group > CR group$^{t2>t1}$). We detected no difference, however, contrasting hippocampal RSFC between t1 and t3 between the groups (contrasts: CR group > control group$^{t3>t1}$, control group > CR group$^{t3>t1}$). Results in RSFC were replicated in an intention-to-treat analysis (CR group: n = 23; control group: n = 20; Supplementary Material and Table 3).

Exploratory Correlation Analyses

To investigate the question whether changes in brain structure and function (between t1 and t2) were related to changes in insulin–glucose metabolism, exploratory correlation analyses in subjects of the CR group were computed. Changes in GM density between t1 and t2 in right hippocampus were negatively correlated with changes in glycemic control (maximum blood glucose concentration measured with the OGTT; $r = -0.52$; $P = 0.02$; see Fig. 3B), indicating that increased GM volume was associated with metabolic changes induced by CR (i.e., improved glycemic control). In addition, we found a trend for a correlation of GM density changes in right hippocampus and changes in HbA1c ($r = -0.43$, $P = 0.08$), but no significant relation with changes in fasting glucose concentration ($r = 0.21$, $P = 0.38$). There were no significant correlations of GM changes in right or left IFG with any of the blood parameters. The decrease in HbA1c was also correlated

Table 3 Results of the whole-brain voxel-based analysis comparing GM density between groups (CR group: $n = 19$ vs. control group: $n = 18$) and time points (t1 vs. t2 and t1 vs. t3)

Anatomical region	L/R	Number of voxels in cluster	Z score of local maximum	MNI peak voxel coordinates		
				x	y	z
CR group > control group $^{t2>t1}$ (indicating an increase in the CR group compared with the control group)						
Inferior frontal gyrus (IFG)	L	770	5.59	−30	41	−11
Inferior frontal gyrus (IFG)	R	90	5.26	34	32	−3
Hippocampus[a]	R	107	3.98	36	−13	−12
Control group > CR group $^{t2>t1}$ (indicating a decrease in the CR group compared with the control group)						
Cerebellum/vermis	R	353	5.58	6	−60	−41
Postcentral gyrus	L	300	5.39	−57	−12	31
Olfactory cortex	R	138	4.98	6	22	−24
Olfactory cortex	L	134	4.87	−6	21	−21
CR group > control group $^{t3>t1}$ (indicating an increase in the CR group compared with the control group)						
No suprathreshold clusters						
Control group > CR group $^{t3>t1}$ (indicating a decrease in the CR group compared with the control group)						
Cerebellum/vermis	R	762	6.17	6	−60	−41

Reported clusters survived a voxel-wise family-wise error (FWE) correction at $P < 0.05$ and were larger than 50 voxels.
L, left hemisphere; R, right hemisphere.
[a]Voxel-wise small-volume corrected (SVC).

with increased RSFC of the right hippocampus with the DMN (i.e., precuneus and angular gyrus $r = -0.50$, $P = 0.04$). We also checked for correlations between neuroimaging (changes in GM density and hippocampal RSFC) and cognition in the CR group, however, no significant relations could be found.

Discussion

In this study, we investigated immediate and subsequent effects of CR on recognition memory performance, insulin–glucose metabolism, brain volume, and RSFC in healthy obese postmenopausal women. In particular, we differentiated between CR-induced weight loss (including negative energy balance) and reduced weight (including energy balance equilibrium). Thereby, we addressed the question whether beneficial effects of CR on brain structure and function remain stable over time or diminish during a subsequent period of maintaining a reduced weight.

Directly after the weight loss phase of CR, we found improvements in a number of cognitive functions including recognition memory performance as well as decreased OGTT maximum glucose concentrations. Improvements in recognition memory performance were specific for negative energy balance and could not be detected after the weight maintenance phase. Other cognitive changes (an increase in processing speed, learning ability, delayed recall, executive functions, as well as a reduction in depression) remained stable over time. Cognitive changes were paralleled by increased GM volume in IFG and hippocampus, and augmented hippocampal RSFC to precuneus and angular gyrus. Brain structural and functional changes were also specific for negative energy balance and diminished during a period of maintaining the reduced weight (i.e., a period of energy balance equilibrium).

Immediate and Subsequent Effects of CR with regard to Cognition and Affect

The result of improved cognitive functions and better memory performance directly after CR (in the domains: learning ability, recognition memory, and delayed recall) is in line with experimental animal studies. CR, for example, has been found to enhance learning and spatial memory performance in rats and mice (Bellush et al. 1996; Ingram et al. 1987; Stewart et al. 1989). We also replicated our previous finding (Witte et al. 2009) that CR improves recognition memory in normal to overweight and obese older subjects. Similar to our previous study (Witte et al. 2009), we demonstrated improved glucose metabolism as potential underlying mechanism, indicated by an immediate reduction in OGTT maximum glucose concentrations in response to the intervention. This result is consistent with previous studies showing that chronically elevated glucose levels are a risk factor for cognitive decline and dementia (Crane et al. 2013; Kerti et al. 2013).

With regard to the question of sustainability, we found that some improvements (e.g., recognition memory performance) were specific for negative energy balance and diminished during a period of balanced energy homeostasis in which subjects maintained reduced weight. Hence, improvements seem to be due to metabolic and neural modulations induced by negative energy balance and are probably not a consequence of reduced weight. This result further supports recent models suggesting the role of adaptive cellular stress response pathways which are stimulated by mild CR-induced stress (e.g., Mattson et al. 2004).

In addition to improvements in memory performance, we also found improvements in processing speed and executive functions as well as a reduction in depression scores. Executive functions improved with regard to cognitive control in terms of a decreased susceptibility to interference in the Stroop test. This result is consistent with a number of studies demonstrating that successful weight management and regulation of eating behavior is associated with improved cognitive control (Keranen et al. 2011; Neve et al. 2012; Nurkkala et al. 2015). The reduction in depression scores is also a typical side effect of weight loss (Herpertz et al. 2015) and might be related to improved cognitive control, the experience of self-efficiency and success in losing weight, or a result of improved quality of life. Changes in processing speed, executive functions, and depression scores remained stable over time.

Neural Correlates of Immediate and Subsequent Effects of CR

In the CR group, we found an increase in GM volume in bilateral IFG and right hippocampus confirming the hypothesis that CR

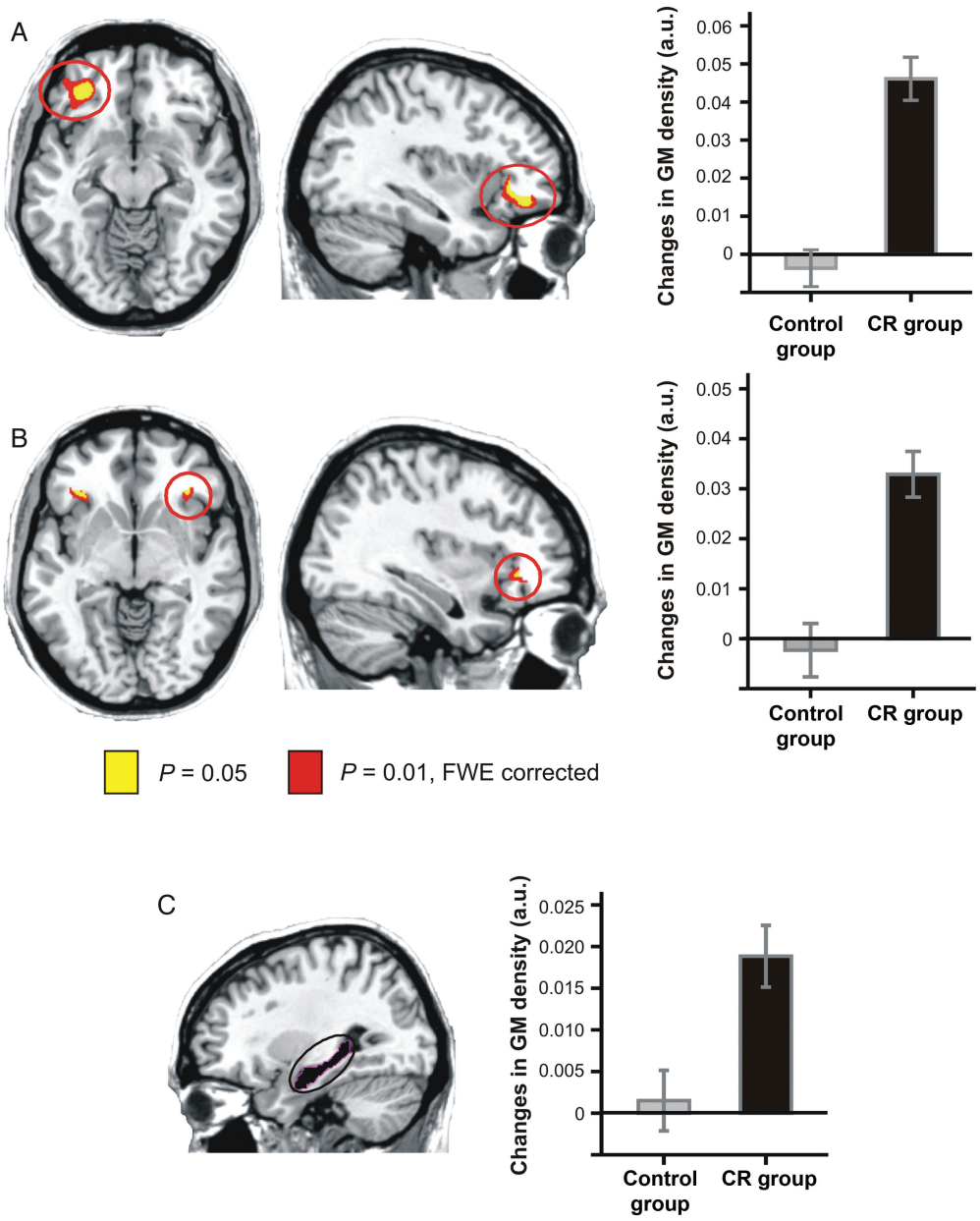

Figure 4. Changes in GM density after 12 weeks of CR (t1–t2) in the CR group compared with the control group. Left panel: A significant GM density increase was found in the CR group ($n = 19$) compared with the control group ($n = 18$) in left inferior frontal gyrus (IFG; (*A*)), right IFG (*B*), and in an anatomical ROI of the right hippocampus (*C*). Right panel: For illustration purposes, we plotted the change in GM density (mean and standard error of the mean in arbitrary units, a.u.) between t1 and t2 extracted from the ROIs identified in the whole-brain analysis (and the right hippocampus, respectively).

leads to enhanced neural plasticity in fronto-temporal brain regions. In line with this notion, an exploratory correlation analysis revealed that the GM volume increase in the right hippocampus was associated with improved glycemic control. Immediate effects of a low-caloric diet on brain structure have also been described by Haltia et al. (2007). The authors found white matter volume extensions in basal brain structures in young obese compared with lean subjects, which could be reversed partially by a low-caloric diet in a subgroup of the sample leading to a weight loss of 11 kg within 6 weeks. In this study, white matter changes were related to an abnormal lipid metabolism and accumulation of free fatty acids in the brain.

In addition to expected changes in IFG and hippocampus, we also found GM volume reductions in olfactory cortex, left postcentral gyrus, and cerebellum/vermis after CR. These 3 structures are part of the bottom-up appetitive network and play a role in interoceptive awareness, eating behavior, the determination of energy needs, and the drive to approach appetizing stimuli (Mahler et al. 1993; Zhu and Wang 2008; Weise et al. 2015). The vermis might play a particular role in this network. Brooks et al. (2012), for example, found reduced activation in the vermis in women with anorexia nervosa. Interestingly, volume reduction in the vermis persisted during the weight maintenance phase, indicating the impact of this brain region in the permanent regulation of eating behavior when adhering to an isocaloric diet. Volume changes in the other brain regions (i.e., the increase in fronto-temporal GM density), in contrast, were not stable during weight maintenance, and thus may rather be related to

Table 4 Results of the whole-brain analysis comparing RSFC of the right hippocampus between groups (CR group: $n = 19$ vs. control group: $n = 18$) and time points (t1 vs. t2 and t1 vs. t3)

Anatomical region	L/R	Number of voxels in cluster	Z score of local maximum	MNI peak voxel coordinates		
				x	y	z
CR group > control group $^{t2>t1}$ (indicating an increase in the CR group compared with the control group)						
Precuneus	L	156	4.79	−3	−66	39
Angular gyrus	L	80	4.76	−42	−72	39
Control group > CR group $^{t2>t1}$ (indicating a decrease in the CR group compared with the control group)						
Inferior frontal gyrus/insula	R	86	5.19	48	21	12
CR group > control group $^{t3>t1}$ (indicating an increase in the CR group compared with the control group)						
No suprathreshold clusters						
Control group > CR group $^{t3>t1}$ (indicating a decrease in the CR group compared with the control group)						
No suprathreshold clusters						

Reported clusters survived a cluster-wise FWE correction using an uncorrected cluster-defining threshold of $P < 0.001$.
L, left hemisphere; R, right hemisphere.

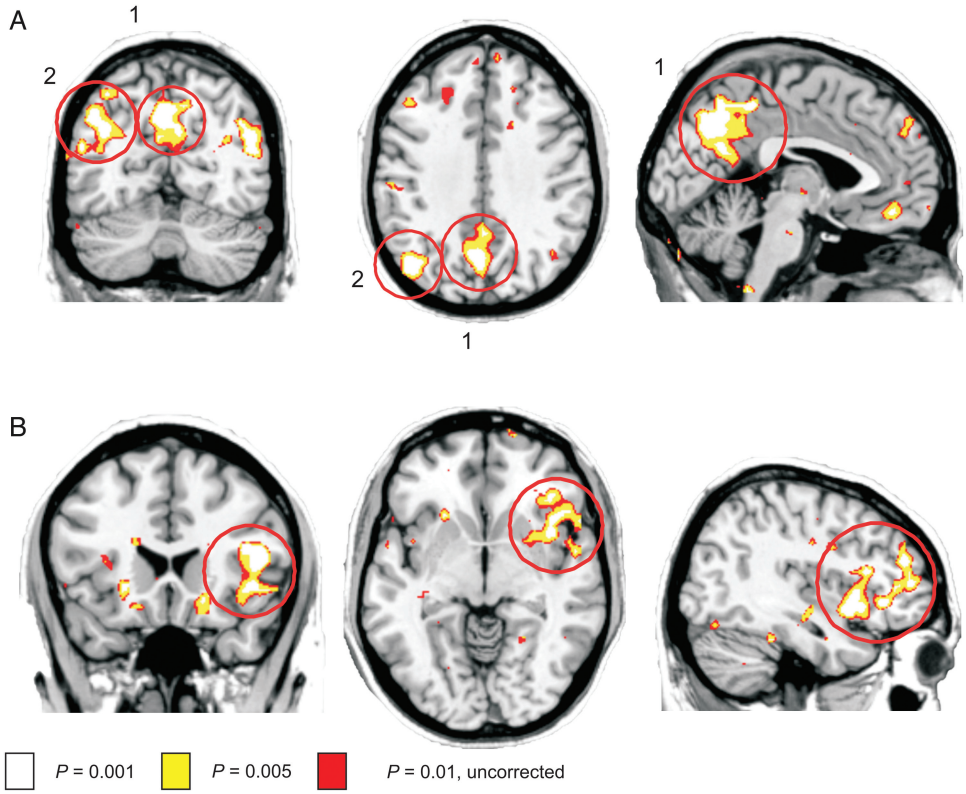

Figure 5. Changes in hippocampal RSFC after 12 weeks of CR (t1–t2) in the CR group compared with the control group. (A) Increased RSFC was found in the precuneus (1) and left angular gyrus (2). (B) Decreased RSFC was found in right inferior frontal gyrus extending to the insular cortex.

neuronal and metabolic changes induced by the negative energy balance.

A study by Mueller et al. (2015) recently demonstrated structural changes in similar brain regions (hippocampus, prefrontal cortex, cerebellum) following weight loss that was induced by intense physical training. Although no intervention regarding physical exercise was performed in our study, participants were advised to increase physical activity in the framework of the lifestyle intervention. However, a large number of studies have shown that exercise compliance levels are almost universally low and simply instructing participants to exercise more does usually not result in a significant increase in physical activity (e.g., Conraads et al. 2012; Eckard et al. 2015). To monitor whether participants increased physical activity, we used a standardized and validated self-report questionnaire which assessed time regularly spent with different activities (e.g., walking, gardening, swimming, cycling, exercising in the gym, etc.) and calculates energy expenditure in kcal/week. Energy expenditure did not differ between groups at each time point and did not change over time (see Table 2 and Supplementary Table 1 for within-group comparisons). Therefore, we consider it unlikely that structural changes observed in our study were induced by changes in physical activity.

Structural changes, such as a reduction in GM volume in the fronto-temporal cortex during aging, are usually accompanied by functional reorganizations. The loss of neurons in the hippocampus, for instance, is compensated by frontal hyperactivity (e.g., Gutchess et al. 2005) resulting in reduced specificity of functional networks. Changes in functional networks can be investigated by analyzing RSFC, which is defined as temporal correlations of neural activity between remote brain regions when no cognitive task is present (Friston 1994). Using RSFC, it has been demonstrated that older subjects display diminished coherence in the activity among brain regions included in the "default mode" network (DMN, e.g., precuneus, anterior and posterior cingulate, medial prefrontal, lateral temporal, parietal cortex, and hippocampus) which is essential for memory formation (Grady et al. 2006, 2012). Specifically, altered RSFC in the DMN has been frequently linked with decline in memory formation and with neurodegeneration (e.g., Greicius et al. 2004; Rombouts et al. 2005; Buckner et al. 2008). Thus, RSFC is a promising and reliable biomarker for the early detection of compensatory alterations during the process of aging (Biswal et al. 2010) as well as for intervention-induced normalization of abnormal network configurations (Chhatwal and Sperling 2012; Witte et al. 2014; Meinzer et al. 2015).

In the present study, we found an upregulation of RSFC between right hippocampus, right precuneus and angular gyrus directly after the weight loss phase of CR and a downregulation of hippocampal connectivity to the right IFG/insular cortex. Alterations in hippocampal RSFC did not persist through the weight maintenance phase and were thus transiently mediated by negative energy balance. The fronto-insular cortex is a central hub in cognitive control and salience networks (Seeley et al. 2007; Sridharan et al. 2008). That is, we found augmented connectivity within brain regions of the DMN (i.e., between hippocampus and parietal cortex), whereas internetwork connectivity (i.e., between hippocampus and fronto-insular cortex) was reduced. We speculate that reduced internetwork connectivity after CR reflects functionally more balanced and independently operating networks. This result fits well with the results of a study by Jacobs et al. (2014) who investigated age-related changes in neural connectivity during episodic memory consolidation. The authors found that increased negative coupling between default and executive control network is associated with better recognition performance in older adults, indicating that memory formation depends on suppressing interference from competing networks (see also Kelly et al. 2008; Wermke et al. 2008). These results are also in line with resting-state fMRI studies in obese participants showing altered connectivity in networks supporting the processing of salient reward and food stimuli (Kullmann et al. 2012; Musen et al. 2012; Fang et al. 2015).

In sum, our results indicate that various traits of brain structure and function (i.e., volume increase in IFG and hippocampus as well as increased DMN connectivity) constitute a neural correlate of transient negative energy balance during the weight loss phase of CR leading to immediate improvements in memory performance and cognitive control.

Limitations

Some limitations should be considered when interpreting our findings. First, it must be noted that we investigated a very specific sample (obese postmenopausal women). Although this relative homogeneity of study participants can be considered a strength of the present study (small range in BMI and age, healthy individuals free of signs for beginning cognitive impairment and brain pathologies), future studies are needed to replicate the findings in broader and more heterogeneous samples.

Second, the sample size was rather small (though comparable with sample sizes in other studies investigating the effect of dietary modifications on cognition and neural correlates; see Haltia et al. 2007; Witte et al. 2009). Therefore, we cannot fully exclude that a lack of group differences (e.g., comparing difference scores between t1 and t3) is due to the small number of participants. Moreover, the small sample size did not allow for additional comparisons among specific subgroups (e.g., subgroups with more or less intense weight loss). In the context of the small sample size, it should be noted that 10 subjects were not available for follow-up testing and 6 further subjects ($n = 4$ in the CR group, $n = 2$ in the control group) had to be excluded from analysis because they failed to follow dietary instructions or did not respond to the intervention. However, an intention-to-treat analysis, including the 4 subjects of the CR group who failed to lose at least 10% of body weight and the 2 subjects of the control group who lost more than 5% of body weight, confirmed the results of the per-protocol analysis with regard to both the primary outcome parameter (recognition score) and neuroimaging data.

Third, we did not measure inflammatory markers or activation of kinases and transcription factors. To further support the hypothesis that CR activates neuroprotective stress response pathways, these markers should be included (e.g., brain-derived and glia-derived neurotrophic factors, nerve growth factors, interleukin 6, TNF-α, and high-sensitive C-reactive protein).

Fourth, due to the fact that individuals in the control group did not experience weight loss, they also did not experience increased self-efficiency, success, or well-being which was reflected by a reduction of self-reported positive affect. Therefore, we cannot rule out that group-specific differences in memory performance after intervention might be in part due to lower motivation during test performance in the control group. However, given that improvements were also evident in dependent measures *t*-tests within the CR group itself and, moreover, paralleled by changes in brain structure and function, we consider this explanation as unlikely.

Conclusion and Outlook

This randomized controlled proof-of-concept study demonstrates for the first time in humans a beneficial effect of CR on brain structure and function. Moreover, results showed that most effects were specific for transient negative energy balance and diminished during a period of balanced energy homeostasis. Thus, population-based efforts to improve brain health via dietary interventions may have to aim for repeatedly induced CR, or substances mimicking the effects of short-term negative energy balance (Witte et al. 2014), rather than aiming for an overall reduced weight. This hypothesis has to be evaluated in larger interventional trials in the future. Our results in a sample of obese older subjects are particularly relevant as there is an urgent need in our aging society to identify approaches to prevent age-related cognitive decline and neurodegeneration (Grady 2012). Overweight and obesity have also been increasing dramatically in the past years (Finucane et al. 2011). Since excessive energy intake seems to amplify the aging process, lifestyle and nutritional modifications such as CR may be a cost-effective way of addressing both issues in the population at large. The findings of the present study will help to develop new therapeutic interventions to slow down cognitive decline in older people.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

Conflict of Interest: None declared.

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