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Caloric restriction increases ketone bodies metabolism and preserves blood flow in aging brain

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ABSTRACT

Caloric restriction (CR) has been shown to increase the life span and health span of a broad range of species. However, CR effects on in vivo brain functions are far from explored. In this study, we used multimetric neuroimaging methods to characterize the CR-induced changes of brain metabolic and vascular functions in aging rats. We found that old rats (24 months of age) with CR diet had reduced glucose uptake and lactate concentration, but increased ketone bodies level, compared with the age-matched and young (5 months of age) controls. The shifted metabolism was associated with preserved vascular function: old CR rats also had maintained cerebral blood flow relative to the age-matched controls. When investigating the metabolites in mitochondrial tricarboxylic acid cycle, we found that citrate and α-ketoglutarate were preserved in the old CR rats. We suggest that CR is neuroprotective; ketone bodies, cerebral blood flow, and α-ketoglutarate may play important roles in preserving brain physiology in aging.

1. Introduction

Brain energy demands are among the highest of all organs. As a result, the cerebral metabolic rates of glucose (CMR_{Glc}) and cerebral blood flow (CBF) are quite high at baseline. A widely accepted cause of the functional losses that accompany aging is decreased brain metabolic and vascular functions (Bentourkia et al., 2000; Wallace, 2005). In support of this viewpoint, a host of neuroimaging studies show that CMR_{Glc} and CBF decline with age (Bentourkia et al., 2000; Lin and Rothman, 2014; Wallace, 2005) and decline still more rapidly and profoundly in Alzheimer's disease (AD) (Cunnane et al., 2011; Hoyer, 1991; Nagata et al., 1997). The metabolic and hemodynamic reductions precede brain structural alteration (gray matter and white matter atrophy) and cognitive impairment (Bookheimer et al., 2000; Cunnane et al., 2011; Reiman et al., 2001). Therefore, preserving brain metabolism (i.e., glucose oxidative capacity) and hemodynamics are critical for optimizing health span (Stranahan and Mattson, 2012).

Caloric restriction (CR) is the most studied antiaging manipulation and has been shown to increase the life span of a broad range of species (Choi et al., 2011; Colman et al., 2009; Rahat et al., 2011). In the nervous system, CR has been shown to reduce oxidative stress, enhance neurotrophin levels, restore neuronal structure (Stranahan et al., 2009), and enhance cognitive function (Fontan-Lozano et al., 2008; Mattson, 2010; Valdez et al., 2010; Witte et al., 2009). In a recent study, we found that Fischer 344 Brown-Norway F1 (F344BNF1) rats under chronic CR had preserved mitochondrial function and neuronal activity (Lin et al., 2014). Specifically, compared with young rats, old rats with CR diet had similar fluxes of neuronal tricarboxylic acid (TCA) cycle and glutamate (Glu)-glutamine (Gln) neurotransmitter cycling (Lin et al., 2014). As TCA cycle flux is associated with metabolism (e.g., CMR_{Glc}) and neuronal activity is associated with vascular integrity (e.g., CBF) (Fox and Raichle, 1986; Lin et al., 2010), this indicates that CR may also be able to impede the age-related decline of brain metabolic and vascular functions.

In this study, our goal was to identify CR effects on cerebral metabolism and blood flow in the same animal model. Specifically, we used in vivo neuroimaging to measure CMR_{Glc} and CBF. We also used mass spectroscopy to determine the brain metabolites and identify the metabolic pathway associated with the changes under

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CR. We hypothesized that CR can preserve metabolic and vascular physiology in aging brain.

2. Material and methods

2.1. Animal

Experiments were conducted using male F344BNF1 rats because this particular strain has demonstrated extended longevity under CR (Turturro et al., 1999). Young control (5 months, N = 6), old control, and old calorie-restricted rats (24 months, N = 6 for each group) were obtained from the National Institute on Aging Caloric Restricted Colony. The sample size was determined with power analysis to perform the comparison at a 0.05 level of significance, with a 90% chance of detecting a true difference of all the measurements between the 3 groups.

At National Institute on Aging, all rats were fed ad libitum (National Institutes of Health [NIH]-31 diet) until 14 weeks of age. The CR regimen was initiated by incremental caloric reduction of 10% per week over 4 weeks, reaching full 40% CR by week 16. The vitamin-fortified NIH-31 (NIH-31 fortified) diet fed to CR rats provided 60% of the calories and additional vitamins supplement consumed by ad libitum rats. After arriving at our facilities, rats were housed individually (1 rat per cage) in a specific pathogen-free facility and were fed the same diet 1 hour before the onset of the dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio according to NIH guidelines.

2.2. Animal preparation for functional neuroimaging

Rats were anesthetized with 4.0% isoflurane for induction and then maintained in a 1.2% isoflurane and air mixture using a face mask. Heart rate (90–130 bpm.), respiration rate, and rectal temperature (37 ± 0.5 °C) were continuously monitored. A water bath with circulating water at 45–50 °C was used to maintain the body temperature. Heart rate and blood oxygen saturation level were recorded using a MouseOx system (STARR Life Science, Oakmont, PA, USA) and maintained within normal physiological ranges.

2.3. Cerebral metabolic rate of glucose (CMR_{Glc}) measurements

CMR_{Glc} was measured using fluorodeoxyglucose (^{18}FDG) positron emission tomography (PET) methods (Focus 220 microPET, Siemens, Nashville, TN, USA). A quantity of 0.5 mCi of ^{18}FDG dissolved in 1 mL of physiologic saline solution was injected through the tail vein. Forty minutes were allowed for ^{18}FDG uptake before scanning. Animals were then moved to the scanner bed and placed in the prone position. Emission data were acquired for 20 minutes in a 3-dimensional (3D) list mode with intrinsic resolution of 1.5 mm. For image reconstruction, 3D PET data was rebinned into multiple frames of 1-second duration using a Fourier algorithm. After rebinning the data, a 3D image was reconstructed for each frame using a 2D-filtered back projection algorithm.

Decay and dead time corrections were applied to the reconstruction process. Cerebral metabolic rate of glucose was determined using the mean standardized uptake value (SUV) equation: SUV (A × W)/Ainj, where A is the activity of the region of interest (i.e., brain region in the study), W is the body weight of the rat, and Ainj is the injection dose of the ^{18}FDG, as described in a previous study (Lin et al., 2012b).

2.4. Cerebral blood flow (CBF) measurements

We used magnetic resonance imaging (MRI) to measure CBF. Quantitative CBF (with units of mL/g per minute) was obtained with MRI-based continuous arterial spin labeling (CASL) techniques on a horizontal 7 T/30 cm magnet (Bruker, Billerica, MA, USA), as described previously (Lin et al., 2012b). A circular surface coil was placed on top of the head and a circular labeling coil was placed at the heart position for CASL. The 2 coils were positioned parallel to each other and were actively decoupled. Paired images were acquired in an interleaved fashion with field of view = 12.8 × 12.8 mm^2, matrix = 128 × 128, slice thickness = 1 mm, 10 slices, labeling duration = 2100 ms, repetition time = 3000 ms, and echo time = 20 ms. CASL image analysis employed codes written in MATLAB (Natick, MA, USA) and STIMULATE software (University of Minnesota, Minneapolis, MN, USA) to obtain CBF.

2.5. Brain metabolites measurements

We used high-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS) to measure brain metabolites. Rats were sacrificed at the end of the MRI study. Brain tissues from the cortex and hippocampus were separated and frozen with liquid N_2. Those brain tissues were then homogenized and extracted with icy cold 80% aqueous methanol and maintained on ice for 1 hour. The cell extracts were centrifuged at 13,800*g* for 10 minutes and the supernatants were transferred to glass autosampler vials for HPLC-ESI-MS analysis.

HPLC-ESI-MS analyses were conducted on a Thermo Fisher Q Exactive mass spectrometer with on-line separation by a Thermo Fisher/Dionex UltiMate 3000 HPLC. HPLC conditions were: column, Luna NH_2, 3 μm, 2 × 150 mm (Phenomenex; Torrance, CA, USA); mobile phase A, 5% acetonitrile in water containing 20-mM ammonium acetate, and 20-mM ammonium hydroxide, pH 9.45; mobile phase B, acetonitrile; flow rate, 300 μL/min; gradient, 85% B to 1% B over 10 minutes and held at 1% B for 10 minutes. Progenesis CoMet Software (Nonlinear Dynamics) was used to process the raw data files. We quantified the concentration of metabolites by measuring the area under the curve and detected the metabolites that exhibited significant differences in abundance. Metabolite identification was performed through METLIN database searching using a 5-ppm mass tolerance, manual interpretation of the MS/MS fragment patterns, and agreement with the HPLC retention time of authentic standards.

2.6. Blood ketone bodies measurements

When the rats were sacrificed, blood sample was collected in a 2-mL BD tube coated with of Lithium Heparin (Vacutainer K2 EDTA) to avoid blood coagulation. Twenty five microliter of blood sample was used to measure blood ketone bodies level of each rat using an STAT-Site Analyzer-Ketone Photometer and STAT-Site β-hydroxybutyrate (BHB) test cards. (Standbio Laboratory, Boerne, TX, USA).

2.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad, San Diego, CA, USA). Significance of differences among means of the 3 groups was evaluated using 1-way analysis of variance followed by Tukey's post hoc test. Evaluation of differences between 2 groups was done using Student *t* test. Values of $p < 0.05$ were considered significant.