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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.

Keywords

Aging; Brain metabolism; Cerebral blood flow; Neuroimaging; Ketone bodies; α-ketoglutarate; Mammalian target of rapamycin

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Disclosure statement

The authors declare no conflict of interest.

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 neuro-imaging 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 gluta-mate (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 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 13,800g for 10 minutes and the supernatants were transferred to glass auto-sampler 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. Pro-genesis 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.

3. Results

3.1. CR-reduced brain glucose metabolism

Fig. 1A shows a representative single slice of the CMR_{Glc} maps overlaid on the corresponding anatomical image. Color bar shows the minimal and maximal CMR_{Glc} in SUV in the linear scale. The maps show that old control rats overall had significantly lower CMR_{Glc} compared with the young controls; the old CR had significantly lower global

CMR_{Glc} relative to both control groups. The quantitative CMR_{Glc} values are shown in Fig. 1B. We further did regional analyses on the entire cortex, hippocampus, and hypothalamus. We chose cortex and hippo-campus because they are related to cognitive functions and the hypothalamus is related to glucose sensing. Similar to the pattern in global CMR_{Glc}, old control rats showed lower regional CMR_{Glc} than those in the young ones (though not significant in the cortex); old CR rats had significantly lower CMR_{Glc} compared with the 2 control groups in cortex (Fig. 1C), hippo-campus (Fig. 1D), and hypothalamus (Fig. 1E). The results suggest that CMR_{Glc} declines with age, and CR further reduces glucose utilization in aging.

3.2. CR preserved CBF

Fig. 2A shows a representative single slice of CBF maps overlaid on the corresponding anatomical image. Color bar shows the minimal and maximal CBF (in mL/g/min) in the linear scale. The maps show that old control rats overall had significantly lower CBF than that of the young controls. However, old CR rats had indistinguishable CBF compared with the young controls. Quantitation of global CBF can be seen in Fig. 2B. We also did regional analyses on cortex, hippocampus, and hypothalamus. Similar to global CBF, we found preserved CBF in old CR rats in cortex (Fig. 2C), hippocampus (Fig. 2D), and hypothalamus (Fig. 2E). The results indicate that CBF reduces with age, but CR is able to impede the decline.

3.3. CR reduced lactate level and increased utilization of ketone bodies

Using HPLC-ESI-MS methods, we observed significantly reduced glucose 1-phosphate/D-fructose 6-phosphate (Fig. 3A; $p < 0.01$) and lactate concentrations (Fig. 3B; $p < 0.01$) in cortex and hippocampus of the old CR rats compared with the age-matched controls. Glucose 1-phosphate is an intermediate metabolite between the glucose-glycogen recycling pathway and D-fructose 6-phosphate is an intermediate in the glycolytic pathway (from glucose to pyruvate); lactate is the end product of glycolysis. These results were consistent with our CMR_{Glc} imaging findings that glucose utilization is lower in the old CR rats.

As ketone bodies are the alternative fuel substrate in the brain when glucose utilization goes down (Akram, 2013), we examined concentration of ketone bodies, BHB. We found that BHB was significantly elevated in the CR group (Fig. 3C; 45% increase; $p < 0.01$). Similar findings were found in blood ketone bodies' level, though the changes were not as high as in the brain (Fig. 3D; 25% increase; $p < 0.05$).

3.4. CR preserved mitochondrial metabolites

We further looked into metabolites related to TCA cycle in the cortex and hippocampus. We found that the level of citrate in the old CR animals was significantly higher ($p < 0.01$; Fig. 3E) compared with old control animals but was not significantly different compared with young controls. We also found significantly higher α-ketoglutarate (α-KG) in the old CR group relative to the old controls ($p < 0.001$; Fig. 3F), but indistinguishable from the young controls.

4. Discussion

In this study, we demonstrated that CR increases the level of ke-tone bodies and preserves CBF in aging F344BNF1 rats. We also observed that CR reduces glycolysis in the brain, including decreased glucose uptake, glucose 1-phosphate/D-fructose 6-phosphate level, and lactate concentration. These are consistent with previous findings that CR reduces the numbers of glucose transporter (e.g., GLUT1) and monocarboxylate transporter (e.g., MCT1) in aged rats (Roy et al., 2013). Despite the decrease in CMR_{Glc}, we previously showed that the mitochondrial TCA cycle flux is preserved in CR old rats (Lin et al., 2014). This could be due to compensatory increases in the metabolism of ketone bodies (Hasselbalch et al., 1996; Roy et al., 2013). Preserved metabolic and vascular functions were observed both globally and regionally including in the cortex, hippocampus, and hypothalamus. Because the cortex and hippocampus are highly associated with cognitive functions (e.g., memory, learning, and perceptual attention), our findings are in good agreement with literature that CR slowed cognitive decline in aging F344BNF1 rats (Markowska and Savonenko, 2002). Similar findings were also reported in humans (Witte et al., 2009). Because the hypothalamus has glucose-sensing neurons, reduced CMR_{Glc} could increase the electrical activity of hypothalamus orexin neurons (which are inhibited by glucose), but suppress the excitability of melanin-concentrating hormone neurons. The consequent increased release of orexin and decreased release of melanin-concentrating hormone stimulate wakefulness and activity (Burdakov et al., 2005). In line with this, CR-treated aging rats had higher physical activity than the age-matched controls (Carter et al., 2009). Collectively, this suggests that CR can preserve brain physiological, cognitive, and physical function in aging.

Based on our observations from the current and the previous study (Lin et al., 2014), we summarize the pathways in Fig. 4: CR reduces CMR_{Glc}, but enhances ketone bodies, both globally and regionally. Ketone bodies may come from blood stream (generated by liver) and the fatty acid oxidation in astrocytes (Guzman and Blazquez, 2001; Maalouf et al., 2009). We found that ketone bodies were much higher in the brain than in the blood in the old CR rats, suggesting that astrocytes, not blood, contributed to increased ketone bodies. Ketone bodies are then converted to acetoacetate, further metabolized to acetyl-CoA, and enter into the mitochondrial TCA cycle in neurons, thus preserving mitochondrial bioenergetics (Lanza et al., 2012; Lin et al., 2014; Lopez-Lluch et al., 2006; Maalouf et al., 2009). As a result, we found preserved citrate and α-KG levels induced by CR. α-KG is transaminated to form Glu and rapidly exchange with Glu (Hertz, 2013). Glu-Gln neurotransmitter cycle communicates between neurons and astrocytes to sustain neuronal activity, including maintaining the neurotransmitter trafficking and eliciting blood-flow response to energy substrate delivery (e.g., oxygen, glucose, and ketone bodies) (Kida et al., 2001). Our finding is consistent with the preserved Glu-Gln neurotransmission seen in CR-treated aging rats (Lin et al., 2014). Because neuronal transmission and levels of ketone bodies are highly associated with CBF (Fox and Raichle,1986; Fox et al., 1988; Hasselbalch et al., 1996; Lin et al., 2010; Linde et al., 2006) and we observed preserved CBF, we suggest that CR can preserve brain metabolism, neuronal activity, and CBF and thus slow cognitive decline in aging.

The shift from glucose metabolism to ketone bodies' utilization under CR is consistent with findings that ketone bodies are an alternative fuel for brain cells when glucose availability is insufficient; ketosis proportionally spares glucose utilization in the brain (Zhang et al., 2013). Ketone bodies can support most, if not all, of basal (housekeeping) neuronal oxidative metabolism. In a recent study, Chowdhury et al. reported that acetyl-CoA oxidation from ketone bodies accounted for approximately 62% of neuronal TCA cycle flux, while the remaining 38% was contributed by glucose, in awake rats under hyperketonemia (Chowdhury et al., 2014). Because both brain glucose utilization and neuronal activity decline during aging, the shifted metabolism from glucose to ketone bodies under CR may be able to sustain brain energy supply and functions in aging brain.

The shifted metabolism may also play an important role in preserving memory in aging since glucose plays a critical role in forming and enhancing memory (Dash et al., 2006). As mentioned above, glucose consumption declines with age and in an accelerated manner in AD. Without additional or alternative fuel substrates, it is not surprising that the ability for memory processing goes down with age and more significantly in AD. Emerging evidence shows that lactate could be an additional energy source when extracellular glucose levels are not sufficient to maintain optimal cognitive functions (Suzuki et al., 2011). During intense cognitive functions, for example, astrocytic glycogenolysis is activated to provide lactate, which is transported to neurons, converted to pyruvate, and enters the TCA cycle (Brown et al., 2004; Pellerin and Magistretti, 1994). Using intrahippocampal infusion of lactate, Newman et al. recently showed that rats had enhanced spatial working memory (Newman et al., 2011). In this study, we showed that both brain glucose and lactate levels were lower in the CR rats, and the brain used ketone bodies as an alternative fuel substrate instead. Similar to lactate, ketone is generated via astrocytes (through fatty acid oxidation pathway), indicating that astrocytes play an important role in supplying energy substrates to neurons when glucose level is insufficient. Moreover, since the glucose level has been chronically lower in the brain, CR rats may have not relied on glucose as the main energy source and thus been protected from the age-related glucose metabolism impairment (including reduced glucose uptake, increased glucose intolerance, and increased insulin resistance). The preserved memory functions found in aged CR rats may be associated with the shifted metabolism from glucose to ke-tone bodies.

In addition to sustaining neuronal activity and memory, metabolism of ketone bodies has neuroprotective effects and therapeutic potential in a variety of different common and rare disease states (Akram, 2013; Veech, 2004). The most well known is the treatment efficacy for refractory epilepsy (Kinsman et al., 1992). Epilepsy is caused by hyper-synchronous firing of neurons. Ketone bodies are able to maintain neurotransmission balance in epilepsy by either increasing inhibitory neurotransmitters (e.g., γ-aminobutyric acid) or inhibiting vascular glutamate transporters (McNally and Hartman, 2012). Others have shown that a ketogenic diet has therapeutic effects on diseases of insulin resistance (Veech, 2013), diseases resulting from free radical damage, and hypoxia (Maalouf et al., 2007; Sullivan et al., 2004). In line with this, ketogenic diets have demonstrated improved structural and functional outcome in traumatic brain injury models, mild traumatic brain injury/concussion models, and spinal cord injury in preclinical studies employing both pre- and post-injury implementation (Prins and Matsumoto, 2014). In animal models with neurodegenerative

disorders, increased ketone bodies metabolism (by administrating a ketone ester diet) exhibit anxiolytic and cognition-sparing properties, and lessen beta-amyloid and tau pathologies in mouse modeling human AD (Kashiwaya et al., 2013). This is consistent with the literature in that CR can reduce beta-amyloid and tau deposition, and restore memory in animals modeling human AD (Mouton et al., 2009).

Increased ketone bodies may also contribute to increased CBF. Two studies show that an acute increase in the concentration of ketone bodies by infusion of BHB increased the CBF without affecting the overall cerebral metabolic activity, suggesting that ketone bodies have directeffecton the cerebral endotheliumto increase CBF, independent of metabolic interactions (Hasselbalch et al., 1996; Linde et al., 2006). Mammalian target of rapamycin (mTOR) signaling may be involved in the process. Ketogenesis is associated with downregulated mTOR activity (Sengupta et al., 2010) and upregulated adenosine monophoshate–activated protein kinase (Blazquez et al., 1999). Both of these changes can activate endothelial nitric oxide synthase signaling and consequently increase CBF (Shafique et al., 2013). This is consistent with our previous findings that mTOR inhibition with rapamycin activates endothelial nitric oxide synthase and restores vascular functions (CBF and vascular density) in mice modeling human AD (Lin et al., 2013; Richardson et al., 2014). Thisisalso consistent withthe literature in that CR preserves vascular functions and vascular density in aging (Lynch et al., 1999; Ungvari et al., 2010).

Preserved levels of α-KG may also contribute to sustained brain viability. As mentioned above, α-KG involves regulating Glu-Gln neurotransmission cycling. In addition, a recent study showed that α-KG may mimic CR and play a key role in extending life span. Consistent with our observations in rats, Chin et al. reported that *Caenorhabditis elegans* had increased endogenous α-KG levels on starvation (Chin et al., 2014). They also found that the beneficial effects of α-KG depend on the target of rapamycin inactivation. Collectively, they suggested that α-KG is a key metabolite that mediates longevity by dietary restriction.

The goal of this study was to identify the chronic CR effects in normal aging brain physiology. In the future, it will be important to investigate the acute effects on young animals and to identify the age-dependent CR effects on brain functions as well as the correlation between the brain metabolism and peripheral energy metabolism. One limitation of our study is that we used a long-lived rodent model to investigate CReffects.Recent studies have shown that the life span response to a single level of CR (e.g., 40% CR) varies widely in mice with different genetic backgrounds (Liao et al., 2010). In some cases, CR shortened the life span in inbred mice. The main findings in the studies were that CR life extension correlated inversely with fat reduction–strains with the least reduction in fat were more likely to show life extension, and those with the greatest reduction were more likely to have shortened life span (Liao et al., 2011). As fatty acids in astrocytes are needed for ketone body metabolism, those with shorter life spans may not be able to upregulate ketone body utilization under CR. Reduction in CMR_{Glc} without elevated ketone bodies may lead to shorter life span. Brain metabolic and vascular functions were not examined in these studies. Therefore, it will be important in the future to determine if CR also has adverse

effects on brain functions during aging in rodent strains where deleterious effects on life span are observed.

Investigations of CR effects on brain functions are translatable. Previous studies show that CR can improve memory in humans (Witte et al., 2009). Because the PETand MRI imaging used in the study are readily used in humans, it would be important in future studies to identify CR-induced changes in metabolic and vascular functions in human brain aging using these multimetric imaging methods (Lin et al., 2012a; 2014; Uh et al., 2011).

In conclusion, we found that CR protects brain physiology in aging F344BNF1 rats. Upregulating ketone bodies metabolism, sustaining CBF and α-KG (all which involve in TOR pathway) may play a crucial role in preserving neuronal health span under CR. These results provide a rationale for CR-induced sustenance of brain health with extended life span. Understanding nutritional effects on brain function may have profound implications in human aging and other age-related neurodegenerative disorders.

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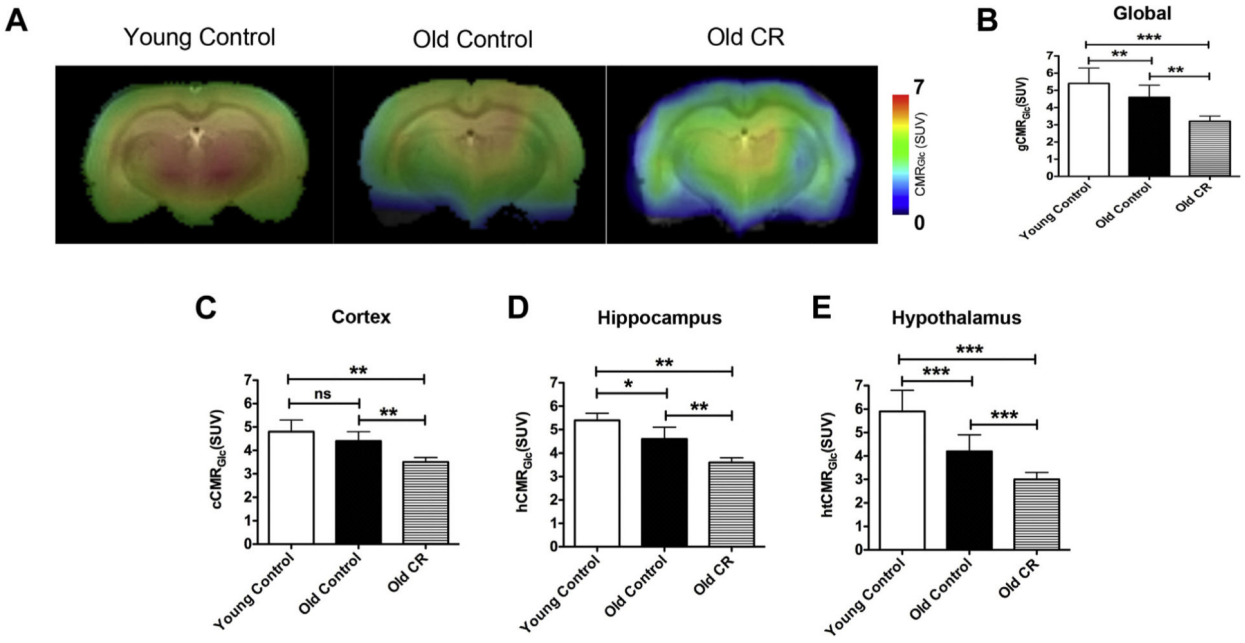

Fig. 1.

Caloric restriction reduced cerebral metabolic rate of glucose (CMR_{Glc}) (A) CMR_{Glc} of representative control and CR rats; (B) Quantitative global CMR_{Glc} ($gCMR_{Glc}$); (C) Quantitative cortical CMR_{Glc} ($cCMR_{Glc}$); (D) Quantitative hippocampal CMR_{Glc} (hCMRGlc); (E) Quantitative hypothalamic CMR_{Glc} ($htCMR_{Glc}$). N = 6 per experimental group. Data are presented as mean ± standard error of mean. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Abbreviations: CR, caloric restriction; SUV, standardized uptake value.

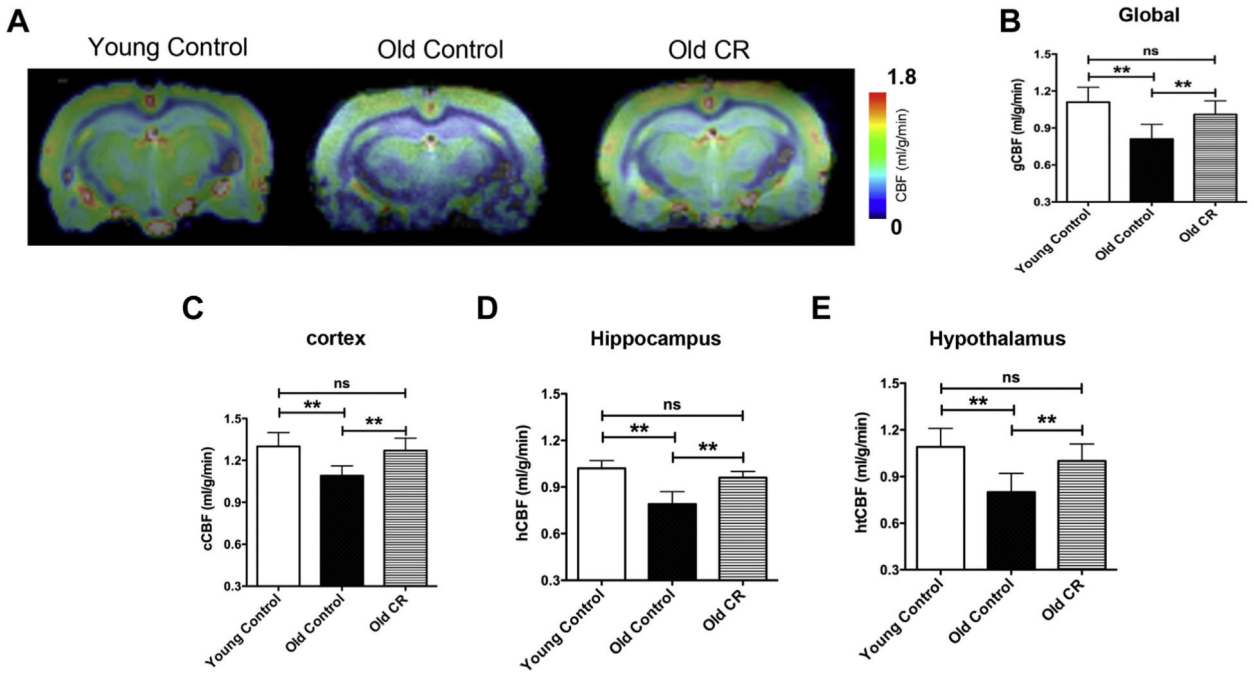

Fig. 2.

Caloric restriction preserved cerebral blood flow (CBF). (A) CBF maps of representative control and CR rats obtained by ASL; (B) quantitative global CBF (gCBF); (C) quantitative cortical CBF (cCBF); (D) quantitative hippocampal CBF (hCBF); and (E) quantitative hypothalamic CBF (htCBF). N = 6 per experimental group. Data are presented as mean ± standard error of the mean.**$p < 0.01$. Abbreviation: CR, caloric restriction.

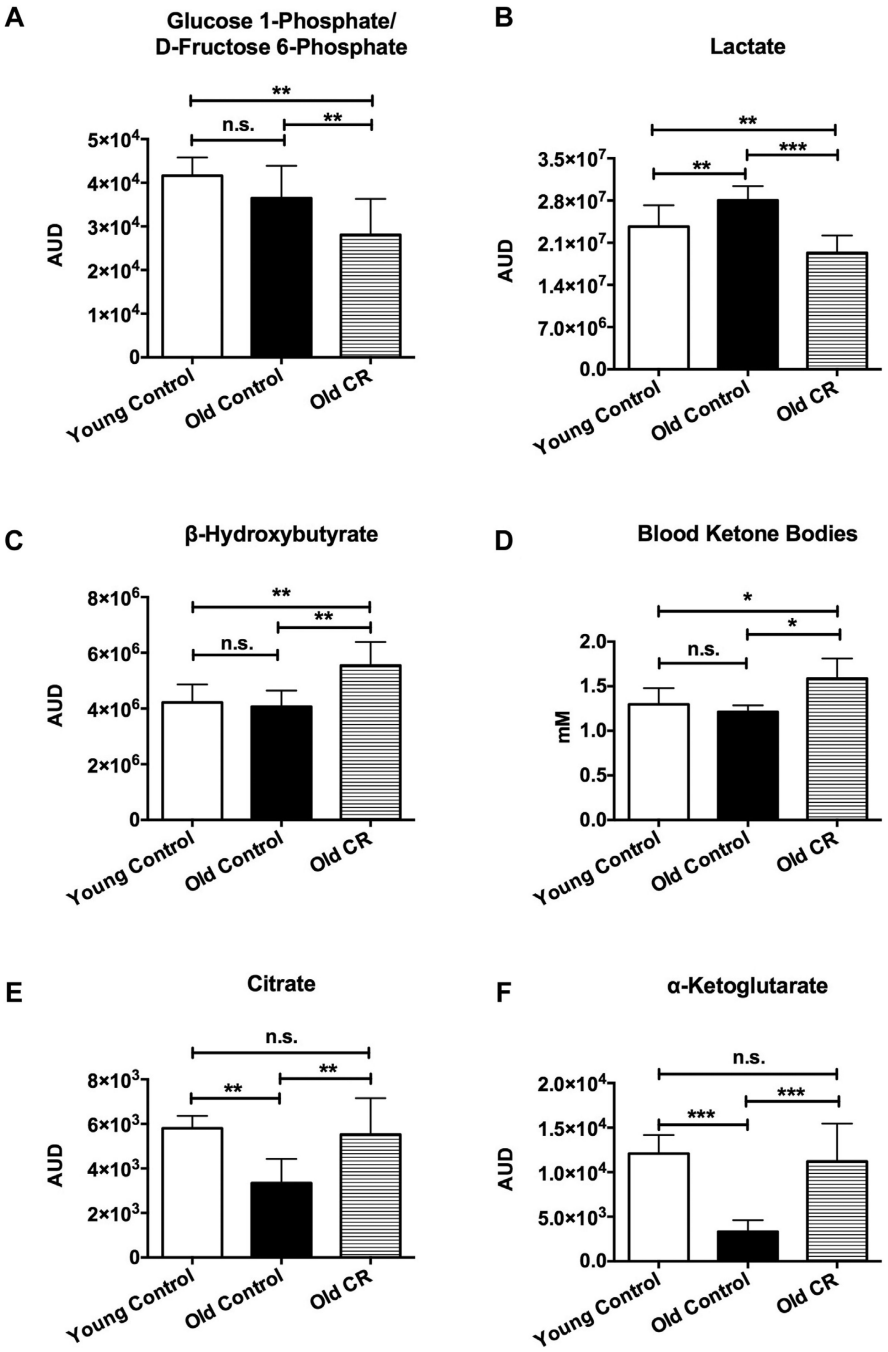

Fig. 3.

Caloric restriction reduced glycolysis, elevated ketone body metabolism, and preserved brain mitochondrial tricarboxylic acid (TCA) cycle metabolites. (A) Glucose 1-phosphate/D-fructose 6-phosphate, intermediates in the glycolytic metabolic pathway; (B) lactate, the end product of glycolysis; (C) brain ketone bodies, β-hydroxybutyrate (BHB); (D) blood ketone bodies; (E) citrate; and (F) α-ketoglutarate, 2 intermediates in the mitochondrial TCA cycle. N = 6 per experimental group. Data are presented as mean ± standard error of the mean. *p

< 0.05; **p < 0.01; ***p < 0.001. Abbreviations: AUD, area under the curve; CR, caloric restriction.

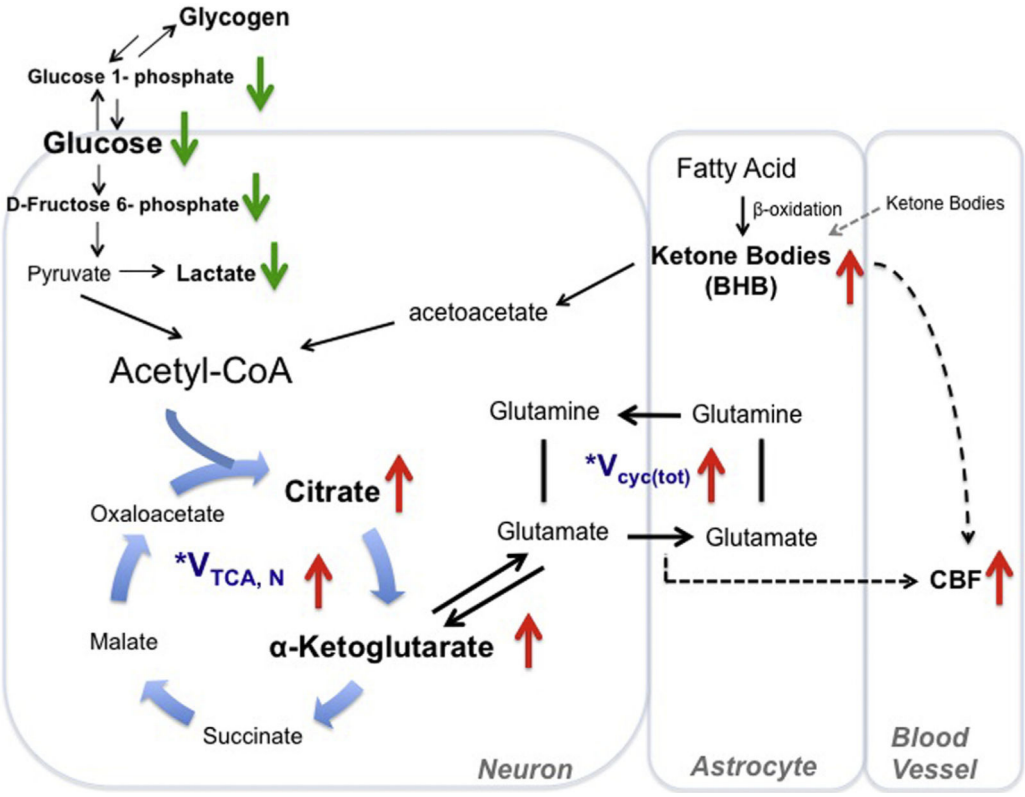

Fig. 4.

Proposed metabolic and hemodynamic changes induced by caloric restriction (CR). This figure shows the comparison between old control and CR rats. CR downregulated glucose metabolic pathway (with reduced glucose uptake, glycolysis, lactate, and glucose-glycogen recycling) but upregulated ketogenic pathway. The ketone bodies may come from astrocytic fatty acid oxidation or blood stream. Ketone bodies are converted to acetoacetate and then further metabolized to acetyl-CoA. The altered metabolic pathway resulted in enhanced TCA cycle flux and glutamate-glutamine recycling ($V_{cyc(tot)}$) between neurons and astrocytes. Elevated CBF may be due to enhanced neuronal activity and increased ketone bodies levels. *This was shown in our previous study (Lin et al., 2014). Abbreviations: BHB, β-hydroxybutyrate; CBF, cerebral blood flow; TCA, tricarboxylic acid.