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Neuroimaging Biomarkers of Caloric Restriction on Brain Metabolic and Vascular Functions

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Abstract

Purpose of review—Non-invasive neuroimaging methods have been developed as powerful tools for identifying *in vivo* brain functions for studies in humans and animals. Here we review the imaging biomarkers that are being used to determine the changes within brain metabolic and vascular functions induced by caloric restriction (CR), and their potential usefulness for future studies with dietary interventions in humans.

Recent findings—CR causes an early shift in brain metabolism of glucose to ketone bodies, and enhances ATP production, neuronal activity and cerebral blood flow (CBF). With age, CR preserves mitochondrial activity, neurotransmission, CBF, and spatial memory. CR also reduces anxiety in aging mice. Neuroimaging studies in humans show that CR restores abnormal brain activity in the amygdala of women with obesity and enhances brain connectivity in old adults.

Summary—Neuroimaging methods have excellent translational values and can be widely applied in future studies to identify dietary effects on brain functions in humans.

Keywords

caloric restriction; positron emission tomography (PET); magnetic resonance imaging (MRI); magnetic resonance spectroscopy (MRS); glucose metabolism; ketone bodies; cerebral blood flow; mammalian target of rapamcyin (mTOR); brain aging; Alzheimer's disease; memory; anxiety; translational research

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Compliance with Ethics Guidelines

Conflict of Interest

Ai-Ling Lin, Ishita Parikh, Jared D. Hoffman, and David Ma declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Brain metabolic and vascular integrity plays an important role in determining cognitive capability and mental health. Failure to maintain cerebral metabolic rate of glucose (CMRglc) has been shown to lead to cognitive impairment and brain volume atrophy, as observed in normal aging and in patients with diabetes and Alzheimer's disease (AD) [1]. Similarly, studies have shown that neurovascular risk is highly associated with accelerated decline in language ability, verbal memory, attention and visuospatial abilities [2, 3]. Reduced cerebral blood flow (CBF) is linked to anxiety and depression [4–6], and impaired blood-brain barrier is associated with neuroinflammation and synaptic dysfunction [7]. These metabolic and hemodynamic reductions precede brain structural alteration (gray matter and white matter atrophy) and cognitive impairment [8–10]. Therefore, preserving brain metabolism (i.e., glucose oxidative capacity) and hemodynamics are critical for optimizing our lifespan and healthspan [11].

Caloric restriction (CR), without malnutrition, has been repeatedly shown to extend life expectancy as well as enhance brain functions [12–15]. On biochemical and molecular level, CR shows to improve glucose homeostasis and insulin sensitivity [16, 17], up-regulate brain-derived neuro-trophic factor [18–20], and reduce oxidative stress and inflammation [21–24]. CR also shows beneficial effects on vascular system by decreasing blood pressure, atherogenic lipids, inflammatory cytokines and increased cellular stress resistance [25, 26]. In line with this, animals treated with CR had lower incidences of age-related neurodegenerative disorders and diabetes [27, 28]. However, experiments on biochemical and molecular levels may limit CR research to animal models, in conditions of in vitro or ex vivo, and their findings might not be fully translated to humans.

Noninvasive neuroimaging methods have been developed as powerful tools for identifying *in vivo* metabolic and vascular biomarkers, including positron emission tomography (PET), and magnetic resonance imaging (MRI) and spectroscopy (MRS) [29, 30]. Among these, PET with fluorine-18 (^{18}F)-labeled 2-fluoro-2-deoxy-d-glucose (^{18}FDG) tracer and proton (^{1}H) MRS are the well-established methods to quantify CMRglc and neural metabolites, respectively. Other novel techniques, including ^{1}H[^{13}C] proton-observed-carbon-edited (POCE) NMR, have also developed to determine brain bioenergetics in aging brain. MRI-based arterial spin labeling (ASL) uses arterial blood water as an endogenous tracer to determine quantitative cerebral blood flow (CBF), which refers to the rate of delivery of arterial blood to the capillary bed in brain tissue and is quantified in milliliters of blood per 100 g of brain tissue per minute. Table 1 summarizes the imaging techniques. These imaging methods have been widely used in humans, and have been "reversely" translated to be used animal studies. Using these advanced imaging techniques, we are able to identify the CR-induced changes in brain metabolic and vascular functions over time in studies related to aging and neurodegenerative diseases. Here we review the imaging biomarkers being used to determine the CR effects on brain metabolic and vascular functions, and their potential usefulness for future studies with dietary interventions in humans.

Neuroimaging Evidence of Preserving Brain Metabolic Functions by CR

Mitochondria are the powerhouse of the cells. Mitochondrial oxidative phosphorylation of glucose is the predominate mode of energy generation in mammalian species. CR effects have been extensively studied in isolated mitochondria, showing increased capacity for oxidative phosphorylation resulting from elevated mitochondrial respiration or increased mitochondrial biogenesis. Mammalian brain has the highest energy demand of any organ based on its size; mitochondrial function plays a critical role in supporting neuronal activity and functional processes of the brain.

Using advanced POCE techniques, we are able to trace *in vivo* mitochondrial activity [31]. With the infusion of ^{13}C- labeled glucose, the mitochondrial glucose oxidation rates in neurons can be measured ($CMR_{glc(ox),N}$; μmol/g/min). The POCE techniques also provide information for glutamate–glutamine neurotransmitter cycling (V_{cycle}; μmol/g/min), which allows us to determine neuronal activity at the same time. We have recently reported the of measure $CMR_{glc(ox),N}$ and V_{cycle} in aged rats chronically treated with CR diet (24 months of age). We compared the results from young *ad libitum* (AL; 5 months of age) and age-matched old AL (i.e., healthy aging) rats. We found that, compared with the young AL, neuronal energy production and neurotransmission rates were significantly reduced in healthy aging. However, old CR rats had similar levels of $CMR_{glc(ox),N}$ and V_{cycle} compared to those of young AL rats, suggesting preserved mitochondrial functions and neuronal activity (Fig. 1A). We calculated adenosine triphosphate (ATP) production rate for the three groups, and found that old CR rats also had comparable outcomes with the young AL.

^{1}H MRS is another *in vivo* imaging technique, which permits visualization of various markers for cellular integrity and function (Fig. 1B). In a recent study, we used ^{1}H-MRS to determine energy metabolites in hippocampus in young and old mice fed with either AL or 40% CR diet [32]. We found that young CR mice had significant increases of total creatine (TCr), a high-energy substrate, compared to young AL mice. Although TCr dropped dramatically when the CR mice were getting old, the level was still comparable to the young AL, and higher than that of the old AL. TCr is the sum of creatine and phosphorus creatine (plays a crucial role as an intracellular buffer during the production of ATP), suggesting that CR increases ATP production in young CR mice, and preserves ATP production in old CR mice. We also found significantly elevated Taurine in young CR compared to the young AL mice. As Taurine is associated with neurotransmitter modulation, this may indicate that young CR mice have an early enhancement of neuronal activity compared to the age-matched AL mice. Collectively, data from POCE and ^{1}H-MRS indicate that CR enhances brain metabolism and neuronal activity at early stage, and preserves these functions in aging.

As glucose is the predominate energy substrate for sustaining mitochondrial functions and neuronal activity, we further investigated the CR effects on brain glucose uptake using ^{18}FDG-PET. Based on the findings with POCE and ^{1}H-MRS, we expected that brain glucose uptake would also be enhanced at early stage and preserved with age. To our surprise, CR dramatically reduces brain glucose uptake in young mice, both globally and in the regions related to cognitive functions (e.g., hippocampus and frontal cortex) (Fig. 1C, *left*). In addition, the similar lower level of glucose uptake was maintained with age [32].

Similar results were also found in rats; old CR rats had significantly lower glucose uptake in the various brain regions compared to that of the age-matched AL rats [33]. These CMRglc changes are in line with the evidence of down-regulation mammalian target of rapamycin (mTOR) signaling by CR. mTOR is a nutrient sensor, a serine/threonine protein that is expressed at high levels in the brain. It serves as a potent neuronal survival and division signal, responding to different signals, such as nutrients, growth factors, and stress [34].

The PET imaging findings led us to speculate that brain may use alternative fuel subtracts to sustain mitochondrial activity and neuronal functions. In addition to glucose, brain uses ketone bodies as energy source [35]. We thus used mass spectroscopy to measure the levels of ketone bodies, beta-hydroxylbutyrate (BHB) from the brain tissue. We found that old rats (24months of age) with CR diet had significantly increased BHB level, compared with the age-matched and young (5 months of age) AL rats. Similarly, young and old CR mice had significantly higher ketone bodies in the blood compared to their age-matched AL mice [32]. Our findings suggested that CR induces a metabolic shift from glucose to ketone bodies utilization in the brain at very early stage.

Increased ketone bodies metabolism suggest increased oxidative metabolism. Utilization of ketone bodies significantly elevates the oxygen utilization in mitochondria through beta-oxidation of fatty acid [36, 37]. This is supported by evidence from isolated mitochondria, showing CR enhances mitochondrial function and induces bioenergetic efficiency [38]. This is also consistent with our previous imaging findings that old animals with chronic CR diet had preserved oxidative metabolism, mitochondrial functions (TCA cycle flux and ATP production), and neuronal activity (neurotransmission rate) compared to the old AL animals [31]. The increased oxidative metabolism, particularly in brain regions associated with cognition (e.g., frontal cortex and hippocampus), may also play a crucial role for neuronal and cognitive protections.

Previous studies showed that cognition-associated brain regions have non-oxidative glycolysis exceeding the required needs of oxidative phosphorylation, a phenomenon known as aerobic glycolysis (AG) [39]. Excessive AG (or the “Warburg effect”) is a key process that sustains T cell activation and differentiation, and involved in inflammatory-mediated conditions [40]. In line with this, the distribution of AG in normal young adults is spatially correlated with Aβ deposition in AD patients and cognitively normal individuals with elevated Aβ [41, 42]. Animal studies further demonstrated that Aβ plaque formation is an activity dependent process associated with AG [41, 43]. Therefore, increased oxidative metabolism in cognition-related regions may decrease AG and thus reduce the risk for AD, consistent with the findings in CR mice [44, 45]. Reduced AD risk was also found in rhesus monkeys, showing CR impedes age-related iron deposition in the brain, which consequently reduces the potential interaction between metal and Aβ, and thus decelerates the pathogenesis of AD [46, 47].

Taken together, using multi-modal imaging methods we can demonstrate that CR enhances oxidative metabolism at early stage and decelerates the decline with age; the preservation of brain oxidative metabolism may be contributed by the shifted utilization from glucose to ketone bodies.

Neuroimaging Evidence of Preserving Brain Vascular Functions by CR

CBF is highly correlated with local neuronal activity and metabolism, known as neurovascular coupling. Neurovascular coupling is used as a surrogate marker of brain function [48, 49]. In light of the metabolic preservation by CR, we further investigated CBF in rodents fed with AL and 40% CR diet. We found that CR significantly enhanced CBF when mice were young (Fig. 1C, right) and decelerated the rate of CBF decline in aging [50]. As a result, old CR mice (20–24 months of age) had similar levels of CBF compared to the young AL mice, indicating CBF preservation in aging. Similar results were found in rats, showing that old rats with chronic CR diet had much higher CBF compared to the age-matched animal, and had comparable CBF level compared to the young AL rats [33]. These findings indicate that CR had early enhancement on CBF and is preserved with age, which is consistent with the metabolic results.

We further identified the potential mechanism of the CR-induced changes on CBF. We previously showed that mTOR inhibited activated endothelial nitric oxide synthase (eNOS) and releases vasodilator nitric oxide, which results in increased CBF [51, 52]. To determine whether the enhancement of CBF in young adult mice is also associated with mTOR signaling, we measured the protein levels of mTOR and eNOS in capillaries isolated from young CR and AL mice. We found that, CR mice had significantly lower levels of mTOR compared to AL mice but higher level of eNOS, consistent with our previous findings [51]. In addition, mTOR-induced changes in metabolism (e.g., shift from glucose to ketone bodies metabolism) are also associated with CBF changes. Two studies show that an acute increase in ketone bodies concentration via infusion of BHB, increased CBF without affecting the overall cerebral metabolic activity; suggesting that ketone bodies have direct effect on the cerebral endothelium to increase CBF, independent of metabolic interactions [53, 54]. In addition, ketogenesis is also associated with downregulated mTOR activity [55] and upregulated adenosine monophosphate-activated protein kinase [56]. Both of these changes can activate endothelial nitric oxide synthase signaling and consequently increase CBF [57].

It has been shown that lifelong CR was able to mitigate the age-related endothelial dysfunction in conduit arteries and partially in cerebral resistance arteries. CR has been demonstrated to exert beneficial effects in the cerebromicrovascular endothelial cells of young and aged rats fed with CR diet compared to the control group, demonstrating that CR could help prevent endothelial dysfunction and alleviate the decrease in CBF that comes with age [58]. This is also consistent with the literature in that CR preserves vascular functions and vascular density in aging [59, 60].

Correlation of Neuroimaging outcomes with Cognition and Anxiety

CR has been repeatedly shown to improve memory in aging, both for studies in humans and animals [61–64]. We had similar observations in a recent study, showing CR had significantly protective effects on learning and spatial memory for old mice in a Radial Arm Water Maze task [50]. The cognitive outcomes are correlated with CBF in hippocampus and frontal cortex, the brain areas regulating learning and memory. The findings indicate that

level of CBF in cognition-associated brain regions may play a critical role for determining performances on learning and spatial memory.

In the same study, we used elevated plus maze (EPM) to evaluate anxiety of the mice. The EPM consists of two open and two closed arms. Mice with higher anxiety had the tendency to stay in the closed arms because closed arms are perceived as safe zones. We determined the anxiety-related behavior by measuring the time spent in the closed arms over the 5 min test session. For the young mice, we did not find significant differences between the CR and AL groups, though CR mice had a trend of less time in the closed arms. In contrast, old AL mice spent significantly longer time in the closed arms compared to the old CR mice, indicating higher anxiety of the old AL mice. Consequently, when comparing the age-related performances, the AL group showed higher increases in anxiety (31.43% group averaged) compared to the CR mice (12.54% group averaged). Our findings are consistent with those in middle-aged rhesus monkeys, showing CR potentially alters their reaction to stressful situations. The enhanced resilience to stress in the monkeys has been linked to preserved brain volume and density in the emotional-control core and endocrine axis [65].

We further identified that anxiety level in the mice had significant and inverse correlations with CBF in hippocampus and in frontal cortex [50]. These findings indicate that preservation of CBF with age is pivotal for sustaining memory and mental health. More importantly, this positive impact on cognitive functions may also be attributed to early-life changes in neurovascular and neurometabolic functions. A recent study suggested that neuroprotective mechanisms play a major role during early stages and compensatory mechanisms in later stages of neurological diseases [66]. This is consistent with our imaging findings that CR induces early enhancements, and later preservation, on brain metabolic and vascular physiology.

Neuroimaging Biomarkers for Human Aging and CR Interventions

The non-invasive metabolic and vascular imaging methods have been extensively applied in humans for studies related to normal cerebral physiology [49, 67], aging, and neurodegenerative disorders [29, 30]. Consistent with what we found in the animal models, metabolic and vascular functions decline were also observed in human aging brain.

Cross-sectional ^{18}FDG-PET studies have repeatedly shown that brain glucose metabolism decreases with age in frontal cortex, anterior cingulate cortex, ventral and dorsal lateral prefrontal/inferior frontal cortex, medial prefrontal areas and precentral and perisylvian areas [68–70]. These regional changes are paralleled by global CMRglc reductions of approximately 6% per decade [71]. Interestingly, this rate of CMRglc decrease is consistent with decreased brain structural integrity and the estimated rate of decrease in synaptic density measured postmortem in healthy elderly brains [72, 73]. Reduced synaptic density is associated with cognitive impairment [74]. In line with this, longitudinal ^{18}FDG-PET studies that monitored changes in cognitive performance in healthy aging subjects showed that CMRglc changes in the parieto-temporal cortex correlate with cognitive decline over time. Studies show that CMRglc in memory and learning-related brain regions among normal aging subjects, including hippocampus and entorhinal cortex, accurately predict future

decline from normal cognition to mild cognitive impairment (MCI) and AD [75, 76]. With a 6–14 years of follow-up scans, the rate of hippocampal CMRglc reduction in healthy elderly individuals was less than 1% per year, whereas patients who decline to MCI and AD had significantly higher rates of CMRglc reductions of 2.4–4.4% per year, respectively.

Using combined infusions of [1-^{13}C] glucose and [2-^{13}C] acetate with POCE, Boumezbeur *et al.* identified the difference of *in vivo* mitochondrial function between a healthy group of elderly subjects and a group of young adult controls [77]. They found that, compared with young subjects, neuronal mitochondrial metabolism (TCA cycle) and glutamate/glutamine cycle flux was 24–28% lower in elderly subjects. The reduction in individual subjects correlated strongly with reductions in NAA (a neuronal biomarker) and glutamate concentrations consistent with chronic reductions in brain mitochondrial function. In contrast, in the elderly, glial mitochondrial metabolism increased 30% compared with that of young subjects, indicating age-related changes in glial mitochondrial metabolism [78].

The decline of brain glucose metabolism in aging brain needs ketone bodies (KB) as an alternative fuel substrates to sustain brain functions. However, unlike in the condition of CR, the aging brain does not have sufficient KB production or storage to cope with the bioenergetics deficits. Instead, it uses catabolism of myelin lipids to generate KB as an adaptive response to address brain fuel and energy demand [79]. This is consistent with findings that white matter degeneration is as a pathological hallmark in aging and neurodegenerative disorders; and an increase in fatty acids and mitochondrial fatty acid metabolism machinery is coincident with a rise in brain ketone bodies and decline in plasma ketone bodies. This mechanistic pathway links mitochondrial dysfunction early in aging with later age development of white matter degeneration [80].

Neurovascular functions also decline with age. CBF of the cerebral cortex and the basal forebrain correlated significantly with advancing age [81]. Vascular pathological burden as hypoperfusion and neurodegeneration both precede and parallel cognitive decline [82]. In the elderly without dementia but with memory dysfunction, cerebral circulatory and vascular abnormalities have been suggested to contribute to MCI and AD [83]. The neurovascular risk is highly associated with accelerated decline in executive functions and cognition, including language, verbal memory, attention and visuospatial abilities [2, 3]. Mental health, such as anxiety and depression, is also link to reduced CBF [4–6], similar to our observations in animal models [50].

Neuroimaging studies indicate that rodents and humans have similar patterns for vascular and metabolic changes in brain aging. As we have seen neuroprotective effects of CR in rodents, we expect that effects would also be seen in humans. However, quantitative metabolic and vascular measurements induced by CR using neuroimaging have not been reported in human studies. Instead, functional MRI (fMRI), a qualitative determination, has been applied in humans for CR-related studies (Table 1). fMRI is a functional neuroimaging procedure using MRI technology that measures brain activity by detecting changes associated with CBF, with an assumption that CBF and brain activity are tightly coupled [84, 85].

Using fMRI and structural measurements, Witte et al. reported potential effects on memory performance in older adults using resveratrol supplement, a promising CR-mimetic nutrient [86]. Before and after the intervention/control period, they let the participants undergo memory tasks and neuroimaging to assess volume, microstructure, and functional connectivity (FC) of the hippocampus, a key region implicated in memory functions. They also assayed their anthropometry, glucose, lipid metabolism, inflammation, neurotrophic factors, and vascular parameters. They observed a significant effect of resveratrol on word retention for words over 30 min period compared with placebo. In addition, resveratrol led to significant increases in hippocampal FC and leptin levels, and decreases in glycated hemoglobin (HbA1c) and body fat compared with placebo. Increases in FC between the left posterior hippocampus and the medial prefrontal cortex correlated with increases in retention scores and with decreases in HbA1c. This study provides initial evidence that supplementary resveratrol improves memory performance in association with improved glucose metabolism and increased hippocampal FC in older adults.

In another study, using fMRI, Jakobsdottir et al. reported that CR can reverse abnormal brain activity in brain areas (e.g., amygdala) involved in processing visual food related stimuli of postmenopausal women with obesity [87]. They observed that before weight loss, increased activation of the right amygdala was seen in response to food stimuli, and free fatty acids and glucose levels correlated with activity in various areas involved in food reward processing. In the first week of CR, obese postmenopausal women had various metabolic changes occur before clinically relevant weight loss was achieved. After weight loss, fasting ghrelin and sated leptin levels correlated with activity in these areas. Activity in the amygdala region and correlations with metabolic factors differ substantially before and after weight loss.

Collectively, the fMRI studies in humans indicate that CR may have profound impact on brain activity and functional connectivity in the context of brain aging and obesity. However, whether CR has protective effects on brain metabolic and vascular functions as we saw in animal models remain unknown. In the future, it would be important to use quantitative neuroimaging methods (e.g., ^{18}FDG-PET, POCE, ^{1}H-MRS, and ASL) to determine the metabolic and vascular changes induced by CR, and monitor effects over time, in humans.

Conclusion

CR has shown to have various effects in protecting brain functionality, including slowing down brain aging and reducing risks for neurodegenerative disorders. Using multi-modal, non-invasive neuroimaging methods, the beneficial effects of CR on brain metabolic and vascular functions can be detected *in vivo*. In this review, we discuss the imaging evidence of early enhancement and later preservation of metabolic and vascular functions induced by CR, as well as correlation between CBF and improved cognitive functions and reduced anxiety in animal models. As the quantitative neuroimaging methods used in animal models can also be used in human studies, they will have tremendous usefulness in future studies to identify CR and other dietary effects on brain metabolism and vasculature in humans. Understanding nutritional effects on brain function may have profound implications in human aging and age-related neurodegenerative disorders.

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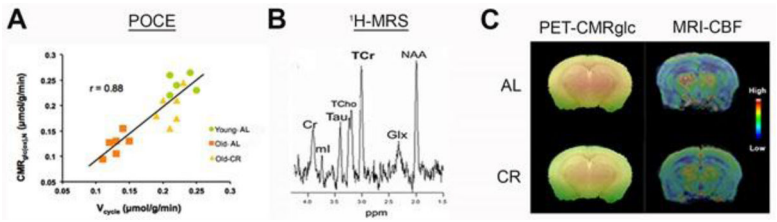

Figure 1.

(A) The POCE results showing the correlation between mitochondrial activity in neurons ($CMR_{glc(ox),N}$) and the glutamate-glutamine neurotransmission rate (V_{cycle}). Old rats treated with CR (Old-CR; 24 months of age) showed levels of $CMR_{glc(ox),N}$ and V_{cycle} comparable to those of the Young-AL (6 months of age). In contrast, Old-AL had significantly lower $CMR_{glc(ox),N}$ and V_{cycle}. Published data from [31]. **(B)** A representative ^{1}H-MRS spectrum, showing choline (Cho), total creatine (TCr), taurine (Tau), glutamate-glutamine complex (Glx), myo-inositol (mI), *N*-acetylaspartate (NAA), in parts per million (ppm). Published data from [32]. **(C) (left)** CMRglc visual maps from PET ^{18}FDG imaging, showing young CR mice had significant lower CMRglc compared to the young AL mice; **(right)** CBF visual maps from MRI-ASL imaging, showing young CR mice had significant higher CBF compared to the young AL mice. The color code indicates the CMRglc and CBF in a linear scale. Published data from [32, 50]. (See description of each imaging method in Table 1).

Table 1

List of discussed neuroimaging methods

Imaging methods	Measurements	Quantitative vs. Qualitative
^{18}FDG- PET	Glucose metabolism (CMRglc)	Quantitative
POCE	Mitochondrial function; Neurotransmission rate; neuronal and glial activities	
^{1}H-MRS	A variety of essential brain metabolites	
ASL	Cerebral blood flow (CBF)	
fMRI	Neuronal activity in relation to CBF and blood oxygenation	Qualitative

18 FDG: fluorine-18 (^{18}F)-labeled 2-fluoro-2-deoxy-d-glucose; **PET:** positron emission tomography; **POCE:** ^{1}H[^{13}C] proton-observed-carbon-edited NMR; **^{1}H-MRS:** proton magnetic resonance spectroscopy; **ASL:** arterial spin labeling; **fMRI:** functional magnetic resonance imaging