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The ketogenic diet increases brain glucose and ketone uptake in aged rats: A dual tracer PET and volumetric MRI study

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ABSTRACT

Despite decades of study, it is still unclear whether regional brain glucose uptake is lower in the cognitively healthy elderly. Whether regional brain uptake of ketones (β-hydroxybutyrate and acetoacetate [AcAc]), the main alternative brain fuel to glucose, changes with age is unknown. We used a sequential, dual tracer positron emission tomography (PET) protocol to quantify brain ^{18}F-fluorodeoxyglucose (^{18}F-FDG) and ^{11}C-AcAc uptake in two studies with healthy, male Sprague-Dawley rats: (i) Aged (21 months; 21M) versus young (4 months; 4M) rats, and (ii) The effect of a 14 day high-fat ketogenic diet (KD) on brain ^{18}F-FDG and ^{11}C-AcAc uptake in 24 month old rats (24M). Similar whole brain volumes assessed by magnetic resonance imaging, were observed in aged 21M versus 4M rats, but the lateral ventricles were 30% larger in the 21M rats ($p=0.001$). Whole brain cerebral metabolic rates of AcAc (CMR_{AcAc}) and glucose (CMR_{glc}) did not differ between 21M and 4M rats, but were 28% and 44% higher, respectively, in 24M-KD compared to 24M rats. The region-to-whole brain ratio of CMR_{glc} was 37–41% lower in the cortex and 40–45% lower in the cerebellum compared to CMR_{AcAc} in 4M and 21M rats. We conclude that a quantitative measure of uptake of the brain's two principal exogenous fuels was generally similar in healthy aged and young rats, that the % of distribution across brain regions differed between ketones and glucose, and that brain uptake of both fuels was stimulated by mild, experimental ketonemia.

Abbreviations: AD (Alzheimer's disease), KD (ketogenic diet); PET (positron emission tomography), ^{18}F-FDG (^{18}F-fluorodeoxyglucose); ^{11}C-AcAc (^{11}C-acetoacetate), CMR_{AcAc} (cerebral metabolic rate of acetoacetate); CMR_{glc} (cerebral metabolic rate of glucose), MRI (magnetic resonance imaging); WB (whole brain), Cx (cortex); Hp (hippocampus), St (striatum); Cb (cerebellum), BBB (blood–brain barrier); VOI (volume of interest), Gd-DTPA (gadolinium-diethylene-triaminopentaacetic acid)

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1. Introduction

Alzheimer's disease (AD) is associated with an overall reduction in brain glucose uptake of ~25%, but whether this reduction is a consequence of the disease or could be contributing to it is unclear. For example, regional brain hypometabolism can be present in young adult carriers of apolipoprotein E ε4 allele (Reiman et al., 2004) or in those with a maternal family history of AD (Mosconi et al., 2006). In both cases, brain glucose hypometabolism can be present three to four decades before the typical age of onset of AD, thereby potentially contributing to AD neuropathology. It is also unclear whether brain glucose uptake is decreased in the cognitively healthy elderly; over the last 30 years, about ten studies have found no difference with age, while about the same number of studies reported a lower global brain glucose uptake in the elderly (Cunnane et al., 2011). Methodological differences between studies seem to contribute to the different outcomes so, at present, it is unclear whether or not brain hypometabolism is part of normal aging or is part of a neurodegenerative process associated with aging.

Under conditions of glucose deficit, i.e., fasting, ketones (acetoacetate [AcAc] and β-hydroxybutyrate) become the principle alternative brain energy substrates to glucose in the circulation and can furnish up to 70% of the brain's energy requirement (Cahill, 2006; Owen et al., 1967). Ketones are synthesized from free fatty acids primarily in the liver but *in vitro* studies suggest astrocytes could also be a site of ketogenesis (Auestad et al., 1991).

Similar to fasting, blood ketones are raised by the very high-fat, low carbohydrate ketogenic diet (KD). The KD has been used for nearly a century to treat drug-resistant childhood epilepsy (Freeman et al., 2006; Wilder and Winter, 1922). The KD also has neuroprotective effects and reduces amyloid pathology in a mouse model of AD (Van der Auwera et al., 2005) and in aged dogs (Studzinski et al., 2008). In humans, mild, experimental ketonemia induced by the KD or a ketogenic food supplement given over a period of up to 90 days reportedly improve memory in mild cognitive impairment (Krikorian et al., 2012) and in AD (Henderson et al., 2009; Reger et al., 2004). The mechanism of the beneficial effects of the KD on memory in these studies is unknown, but one possibility is that mildly elevated plasma ketones increases brain ketone uptake which may partially compensate for glucose brain hypometabolism, thereby improving fuel supply to the brain.

The brains two main fuels (glucose, ketones) are transported into the brain by different transporters and are metabolized to acetyl CoA by different pathways. Our recent development of ^{11}C-AcAc as a brain PET tracer (Bentourkia et al., 2009; Pifferi et al., 2011; Tremblay et al., 2007) therefore provides an opportunity to assess for the first time how aging itself or aging plus the KD affect brain uptake of these two key brain fuels in the rat. Specifically, male Sprague-Dawley rats were used in two studies: (i) Across age; 4 month old (young; 4M) versus 21 month old (aged; 21M) rats, in which brain ketone (^{11}C-AcAc) and glucose (^{18}F-fluorodeoxyglucose; ^{18}F-FDG) uptake were measured using a sequential dual tracer PET protocol in each rat. Regional brain volumes were also assessed using magnetic resonance imaging (MRI), as well as blood–brain barrier (BBB) permeability using the contrast agent gadolinium-diethylene-triaminopentaacetic acid (Gd-DTPA). (ii) Regional brain ^{11}C-AcAc and ^{18}F-FDG uptake in 24 month old rats on a standard diet (24M) or on a high-fat KD (24M-KD) for 14 days before the dual tracer PET experiment.

2. Results

2.1. Aging study

2.1.1. Physiological variables in aged rats

Compared with the 4M group, the 21M group was 41% heavier (p=0.0002) but both groups matched the standard growth curve for male Sprague-Dawley rats (Harlan Laboratories technical data). The 21M group had 66% higher plasma insulin compared to the 4M group (p=0.030), but brain weight, and plasma lactate, free fatty acids, glucose, ketones and triglycerides were not significantly different between the two groups (Table 1).

2.1.2. Brain volume and BBB permeability in aged rats

T_2-weighted images revealed no difference in whole brain volume between 4M and 21M rats (2.55±0.09 cm^3 for 4M). Individual whole brain volumes were positively correlated

Table 1 – Weight and plasma metabolic parameters in 4 month (4M) and 21 month (21M) old rats fasted for 18 h.

	4M	21M
Body weight (g)	448 (39)	631 (41)***
Brain weight (g)	2.27 (0.14)	2.19 (0.07)
Glucose (mM)	6.9 (1.4)	9.0 (2.3)
Acetoacetate (μM)	751 (219)	710 (197)
β-hydroxybutyrate (μM)	1657 (498)	1372 (544)
Lactate (mM)	1.1 (0.2)	1.1 (0.4)
Free fatty acids (mM)	1.8 (1.0)	2.3 (1.0)
Triglycerides (mM)	1.3 (0.5)	1.9 (0.6)
Insulin (μU/ml)	8.2 (2.8)	13.6 (4.8)*

Mean (SD); n=6/group.
* $p<0.05$.
*** $p<0.001$.