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Brain Research

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.
* $p<0.05$.
*** $p<0.001$.

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with whole brain weight ($r=0.96$; $p<0.0001$; data not shown). Hippocampus, cortex, striatum and cerebellum volume did not differ between the 4M and 21M groups (128±8, 546±34, 59±6 and 306±14 mm^3, respectively, for the 4M group). However, lateral ventricle volume was 30% higher in 21M compared to 4M rats ($p=0.001$; Fig. 1A and B). There was a trend towards higher whole brain uptake of the contrast agent Gd-DTPA in the 21M compared to 4M rats (Fig. 1C; $p=0.061$).

2.1.3. *Brain ^{11}C-AcAc and ^{18}F-FDG uptake in aged rats*

Whole brain cerebral metabolic rate of AcAc (CMR_{AcAc}; μmol/100 g/min) was similar in 4M and 21M rats (2.9±0.5 μmol/100 g/min for 4M; Fig. 2A). No difference in CMR_{AcAc} with age was seen for cortex, hippocampus, striatum or cerebellum. CMR_{AcAc} was similar across brain regions in 4M. Plasma AcAc was significantly positively correlated with whole brain CMR_{AcAc} ($r=0.50$, $p=0.030$). Whole brain cerebral metabolic rate of glucose (CMR_{glc}) was also similar in 4M and 21M rats (23.7±10.3 μmol/100 g/min for 4M; Fig. 2B). The only age-related difference observed was for the hippocampus in which the CMR_{glc} was 51% higher in the 21M group ($p=0.031$).

Using regional brain volumes obtained by MRI, the CMR/region could be calculated as well as age-related differences in the relative distribution of CMR_{AcAc} and CMR_{glc} across specific brain regions. Expressed as nmol/region/min, the cortex had the highest CMR_{AcAc} and CMR_{glc}, followed by cerebellum, hippocampus and striatum (Fig. 2C and D). Whole brain CMR_{AcAc} and CMR_{glc} (nmol/min) were 75±12 and 605±264, respectively, in 4M rats and were similar for 21M rats.

The contribution of ^{11}C-AcAc to brain energy substrate uptake (^{11}C-AcAc+^{18}F-FDG) was 13% in the whole brain of 4M rats. The contribution of ^{11}C-AcAc to brain energy substrate uptake was 38% lower in the whole brain, 45% lower in the hippocampus and 52% lower in the striatum of the 21M compared to 4M rats ($p=0.032$, 0.017, and 0.032, respectively; Fig. 3).

In comparing the regional brain uptake of the two fuels, in 4M rats, ^{18}F-FDG distribution to the cortex and cerebellum was about 40% lower than for ^{11}C-AcAc ($p=0.014$ and 0.047, respectively), but no difference between the two fuels was observed for the hippocampus or striatum (Fig. 4A). At 4M, the remainder of the brain accounted for 49% of whole brain CMR_{AcAc} and 68% of whole brain CMR_{glc} (data not shown; $p=0.032$). Similar differences between CMR_{AcAc} and CMR_{glc} were found in 21M rats: ^{18}F-FDG brain uptake was 37% less distributed to the cortex and 45% less to the cerebellum than for ^{11}C-AcAc ($p=0.016$ and 0.036, respectively; Fig. 4B). At 21M,

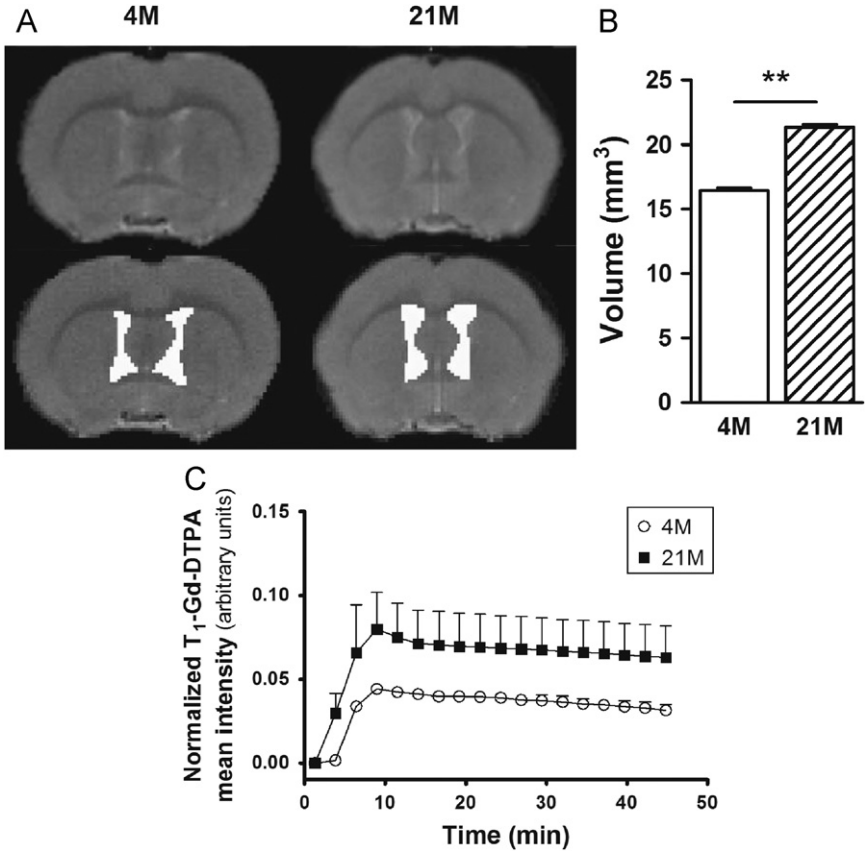

Fig. 1 – Magnetic resonance images of the brain of 4 month (4M) and 21 month (21M) old rats. Representative axial views of T_2-weighted images showing lateral ventricles segmentation in white (A). Lateral ventricle volumes are in mm^3 ((B); mean±SEM; $^{**}p<0.01$). Blood–brain barrier permeability was assessed by dynamic contrast-enhanced T_1-weighted imaging following gadolinium-diethylene-triaminopentaacetic acid injection (C). Gd-DPTA data are mean±SEM normalized using the first time frame; 4M not statistically different from 21M ($p=0.061$).

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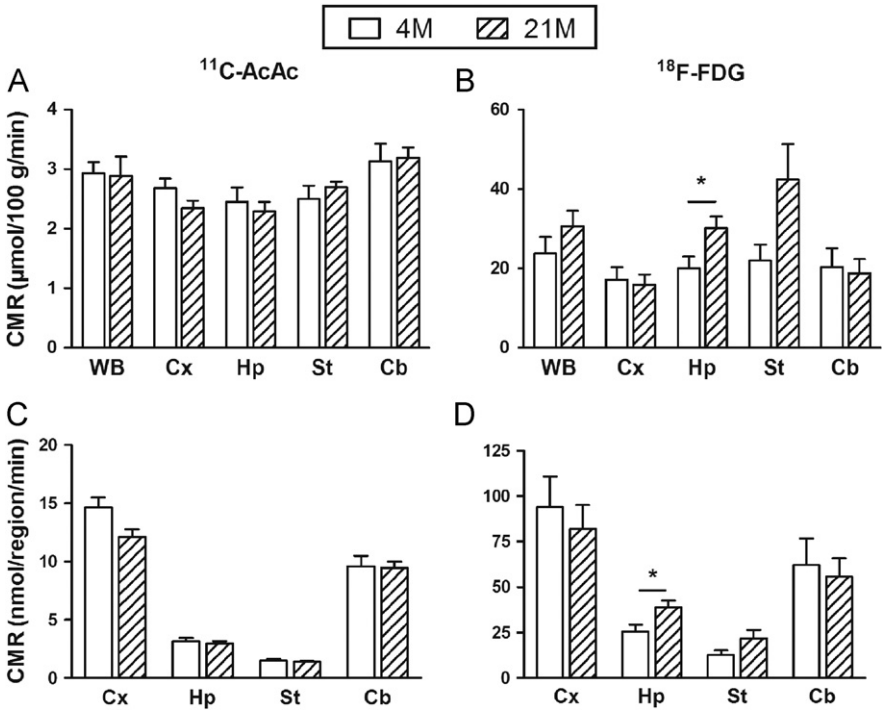

Fig. 2 – Cerebral metabolic rate (CMR) of ^{11}C-AcAc (A and C) and ^{18}F-FDG (B and D) in the whole brain (WB), cortex (Cx), hippocampus (Hp), striatum (St) and cerebellum (Cb) of 4 month (4M) and 21 month (21M) old rats. Data are expressed as µmol/100 g/min (A and B) and nmol/region/min (C and D). Data are mean ± SEM (*$p < 0.05$).

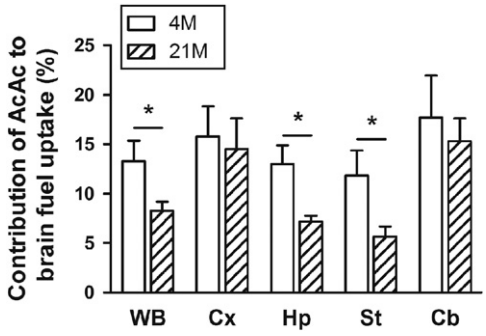

Fig. 3 – Percentage contribution of ^{11}C-AcAc to total brain energy substrate uptake (^{11}C-AcAc+^{18}F-FDG) in the whole brain (WB), cortex (Cx), hippocampus (Hp), striatum (St) and cerebellum (Cb) of 4 month (4M) and 21 month (21M) old rats. Data are mean ± SEM (*$p < 0.05$).

the remainder of the brain accounted for 53% of whole brain CMR_{AcAc} and 66% of whole brain CMR_{glc} (data not shown; $p=0.057$).

2.2. *Ketogenic diet study*

2.2.1. *Physiological variables on the KD*

The 24M-KD group was 7% heavier than the 24M group ($p=0.026$; Table 2). The 24M-KD group was in mild ketosis, as shown by the higher plasma β-hydroxybutyrate and AcAc compared to the 24M group (+90% and +63%; $p=0.062$ and 0.251, respectively). Brain weight, and plasma glucose, l actate, free fatty acids, triglycerides and insulin were not different between the two groups.

2.2.2. *Brain ^{11}C-AcAc and ^{18}F-FDG uptake on the KD*

CMR_{AcAc} (µmol/100 g/min) was 28% higher in the whole brain, 45% higher in the hippocampus and 44% higher in the striatum of the 24M-KD vs. 24M rats ($p=0.026$, 0.009, and 0.008, respectively; Fig. 5A). Whole brain and regional CMR_{glc} were significantly higher in 24M-KD compared to 24M rats: +44% in whole brain ($p=0.030$), +54% in cortex ($p=0.030$), +58% in striatum ($p=0.035$) and +78% in cerebellum ($p=0.029$; Fig. 5B).

The same significant differences in CMR were observed when the data were expressed as nmol/region/min (Fig. 5C and D). Whole brain CMR_{AcAc} in 24M and 24M-KD rats was 60 ± 8 and 76 ± 9 nmol/min, respectively ($p=0.026$), and whole brain CMR_{glc} was 800 ± 171 and 1152 ± 274 nmol/min, respectively ($p=0.030$). The contribution of ^{11}C-AcAc to whole brain energy substrate uptake (^{11}C-AcAc+^{18}F-FDG) was 6–7% in 24M and 24M-KD rats and was similar across brain regions (data not shown).

In 24M rats, there was no significant difference in the brain uptake of the two fuels as a % of whole brain CMR (Fig. 4C). However, in 24M-KD rats, compared to ^{11}C-AcAc uptake, ^{18}F-FDG uptake by the cortex was 25% lower but 35% higher in the striatum ($p=0.030$ and 0.036, respectively; Fig. 4D).

3. Discussion

Our main observations are that in male Sprague-Dawley rats, CMR_{AcAc} and CMR_{glc} were similar in aged (21M) compared to young (4M) rats and that the high-fat KD increases both CMR_{AcAc}

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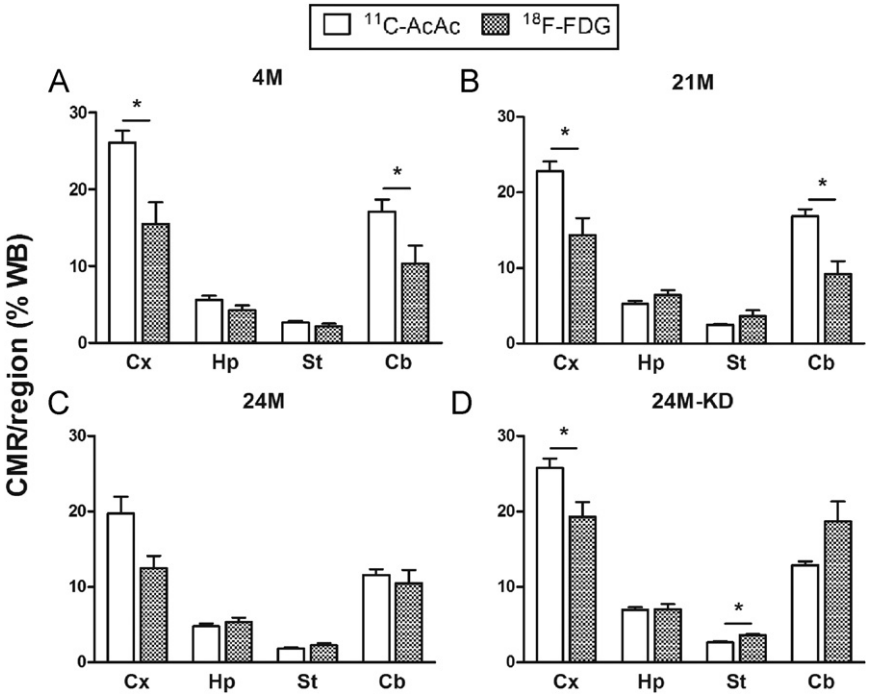

Fig. 4 – Comparison of cerebral metabolic rate (CMR) of ^{11}C-AcAc to ^{18}F-FDG in the cortex (Cx), hippocampus (Hp), striatum (St) and cerebellum (Cb) of 4 month old rats (4M; A), 21 months (21M; B), 24 months on a standard diet (24M; C) or on a ketogenic diet (24M-KD; D). CMR data are expressed per region as percentage of whole brain (WB) values. Mean ± SEM (*$p < 0.05$).

Table 2 – Weight and plasma metabolic parameters in 24 month old rats on a standard diet (24M) or high-fat ketogenic diet (24M-KD) and fasted for 18 h.

	24M	24M-KD
Body weight (g)	630 (27)	672 (22)*
Brain weight (g)	2.23 (0.06)	2.25 (0.09)
Glucose (mM)	9.0 (1.5)	9.7 (2.4)
Acetoacetate (μM)	477 (263)	779 (271)
β-hydroxybutyrate (μM)	719 (198)	1366 (455)
Lactate (mM)	1.2 (0.5)	1.5 (0.4)
Free fatty acids (mM)	1.7 (1.6)	0.8 (0.4)
Triglycerides (mM)	2.5 (1.0)	1.3 (0.7)
Insulin (μU/ml)	16.1 (10.6)	21.0 (8.8)

Mean (SD); $n = 6$/group.
* Significant difference between the two groups ($p < 0.05$).

and CMR_{glc} in 24 month old rats. Since the region-to-whole brain ratios differ significantly between CMR_{AcAc} and CMR_{glc}, it may therefore be more appropriate to refer to conditions inducing lower brain FDG uptake as brain *glucose* hypometabolism rather than the more general term–brain hypometabolism.

3.1. *Aging study*

Despite similar whole brain volume in the 21M versus 4M rats, the former had 30% larger lateral ventricles (Fig. 1A and B). To the best of our knowledge, this is the first study to report differences in brain ventricle volume associated with age in the rat. Though well within the detection limits of MRI, this larger lateral ventricle volume represents 0.2% of whole brain volume in the rat, thereby having no significant effect on overall brain volume. Hippocampus volume was similar in the 21 and 4M rats, which contrasts with observations from Driscoll et al. (2006), who reported smaller hippocampus volume in 12 and 24 month old female FBNF1 rats. These differences might be explained by sex and strain differences because Sprague-Dawley rats tend to live longer than Fischer 344 rats (Altun et al., 2007; Charles River Laboratories, 1982).

BBB permeability to the contrast agent, Gd-DTPA, was 87% higher in the 21M rats (Fig. 1C), which agrees with previously described age-related structural changes in the BBB (Shah and Mooradian, 1997). An age-related decrease in rat brain endothelial cell number and capillary diameter has been previously reported (Barr, 1978). Furthermore, lower occludin, a structural protein critical for BBB tight junctions function, was observed in 24 month old rats (Mooradian et al., 2003). Whether increased BBB permeability to Gd-DTPA influenced CMR remains to be determined; this is a possibility given than Gd-DPTA permeability was 87% higher and that regional CMR_{glc} was also 51–93% higher (hippocampus and striatum) in the 21M rats. However, regional CMR_{AcAc} was no more than 8% higher in the striatum of 21M rats, which would be inconsistent with higher non-specific transport across the BBB.

The 4M group had a mean whole brain CMR_{glc} of 23.7 μmol/l00 g/min, which is similar to a comparable study in normal adult male Sprague-Dawley rats fasted 16 h before the ^{18}F-FDG PET scan (28.1 μmol/100 g/min; (Shimoji et al., 2004). CMR_{glc} was not different in our 21M versus 4M rats (Fig. 2B), an observation that differs somewhat with the literature except that the methodology was also somewhat different; Le Poncin-Lafitte et al. (1983) reported lower ^{14}C-2-deoxyglucose uptake in the hippocampus and striatum of

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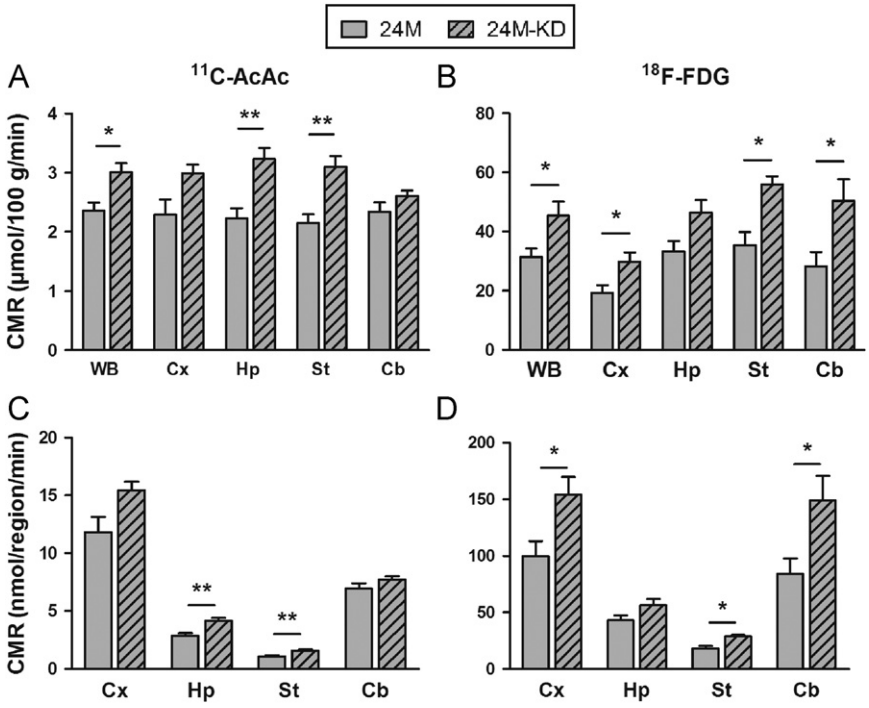

Fig. 5 – Cerebral metabolic rate (CMR) of ^{11}C-AcAc (A and C) and ^{18}F-FDG (B and D) in the whole brain (WB), cortex (Cx), hippocampus (Hp), striatum (St) and cerebellum (Cb) of 24 month old rats on a standard diet (24M) or on a ketogenic diet (24M-KD). Data are expressed as µmol/100 g/min (A and B) and nmol/region/min (C and D). Mean ± SEM (*$p<0.05$; **$p<0.01$).

aged rats. A recent study reported lower brain ^{18}F-FDG uptake by semi-quantitative PET imaging in 23 month old female Wistar rats (Lopez-Grueso et al., 2010). Female rats may undergo earlier age-related brain glucose hypometabolism than male rats (Higuera-Matas et al., 2008), which were used in the present study. As well, anesthesia with isoflurane can significantly decrease ^{18}F-FDG brain uptake (Matsumura et al., 2003), but it seems unlikely that that explains the similar ^{18}F-FDG brain uptake we observed across groups because the same anesthesia and PET protocols were followed in all our rats. In humans, brain glucose uptake is variously reported to be unchanged or lower in the healthy elderly (Curiati et al., 2011; Kochunov et al., 2009; Yanase et al., 2005); there again, methodological differences appear to play a key role in the disparity between studies (Cunnane et al., 2011).

To the best of our knowledge, this is the first report in which PET was used to quantify brain ketone uptake in aged rats. We used the Patlak kinetic model which, for brain ketone uptake, gives identical results to the one- and two-tissue compartment models (Blomqvist et al., 1995; Blomqvist et al., 2002). We observed a positive correlation between brain ketone uptake and plasma ketone concentration, as expected and previously reported in adult rats (Daniel et al., 1971; Pifferi et al., 2011; Ruderman et al., 1974). Indeed, CMR_{AcAc} is usually considered to be primarily regulated by arterial AcAc concentration. As in the present study, CMR_{AcAc} measured by arteriovenous differences in humans was previously shown not to differ in elderly versus young adults (Gottstein et al., 1971; Lying-Tunell et al., 1980).

Previous studies of autoradiography in rats have also shown that regional brain utilization of ketones differs from that of glucose (Hawkins and Biebuyck, 1979; Hawkins et al., 1986). Here, we confirm those results by PET imaging (Fig. 4); we show that CMR_{glc} was lower in the cortex and cerebellum compared to CMR_{AcAc}. In the present study, CMR_{AcAc} itself did not differ between our young and aged rats, but the tendency towards higher CMR_{glc} in the aged rats (Fig. 2B–D) resulted in AcAc contributing 38–52% less to whole and regional brain fuel uptake (glucose+AcAc) in the aged rats (Fig. 3). This preliminary observation suggests that significant aged-related changes in brain fuel metabolism come to light when comparing brain uptake of glucose and ketones in a sequential dual tracer PET experiment that would not be possible to observe with one fuel tracer alone. This observation also depended on being able to quantify CMR, which required measurement of input functions and volumes of interest by MRI; whether this differential quantitative effect of age on brain fuel metabolism is present in humans or is linked to differences in behaviour or cognition remains to be determined.

3.2. *Ketogenic diet study*

We have previously reported increased brain uptake of ^{18}F-FDG and ^{11}C-AcAc in young rats on a KD for 14 days (Pifferi et al., 2011). Those data were expressed using semi-quantitative 'standardized uptake values' since the plasma time-activity curves necessary to calculate CMR were not available. Here we confirm that our previous results apply when the CMR_{AcAc} or CMR_{glc} are calculated, and that 28–78% higher CMR_{AcAc} and CMR_{glc} on the KD seem to be generalized across different brain regions (Fig. 5).

During ketosis, ketones are preferentially used by neurons (Jiang et al., 2011). Ketosis also increases Krebs' cycle flux because of AcAc transformation directly into acetyl-CoA (Melo

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et al., 2006; Yudkoff et al., 2004). Therefore, increased mitochondrial metabolism during mild ketosis may increase brain glucose uptake (hence CMR_{glc}) in order to satisfy the increased need for oxaloacetate and maintain postsynaptic ion gradients. In that case, higher CMR_{glc} would mainly occur in astrocytes, which prefer glucose as an energy substrate (Bouzier-Sore et al., 2006). Nuclear magnetic resonance spectroscopy studies also showed a higher brain ^{13}C-glucose uptake in ketotic mice (Yudkoff et al., 2005) and in the Strasbourg strain of rats with genetic absence epilepsy on a KD (Melo et al., 2006).

Nevertheless, not all rat studies of CMR_{glc} during experimental hyperketonemia are consistent with our present results; some suggest there may be no change in CMR_{glc} (al-Mudallal et al., 1995; Linde et al., 2006), or that CMR_{glc} may be lower in the frontal cortex and cerebellum (LaManna et al., 2009). The results of LaManna et al. (2009) do not necessarily contradict ours because they induced a much higher ketonemia (5 mM versus 1.3 mM β-hydroxybutyrate in the present study), which could result in 3-4 fold higher brain ketone uptake (Cunnane et al., 2011).

3.3. Conclusion

In summary, the present study shows that there are subtle regional and whole brain differences in CMR_{AcAc} and CMR_{glc} between young and aged male Sprague-Dawley rats. Hence, ^{11}C-AcAc is a useful new PET tracer permitting better definition of how much brain fuel metabolism may change under different experimental conditions. If translatable to humans, our present data imply that brain glucose hypometabolism in the elderly may be more related to disease-associated processes and is not necessarily part of normal brain aging. One potentially significant observation is that mild ketonemia induced by the high-fat KD increased both CMR_{AcAc} and CMR_{glc} in aged rats (Fig. 5), leading us to suggest that the cognitive benefits associated with a mild experimental ketonemia in AD (Henderson et al., 2009; Reger et al., 2004), mild cognitive impairment (Krikorian et al., 2012), and in diabetic patients (Page et al., 2009) may occur at least in part via improved brain uptake of both ketones and glucose. Further experiments are required to better understand brain substrate utilization by neurons and astrocytes in aged rats in the context of a KD.

4. Experimental procedures

4.1. Study design

Male Sprague-Dawley rats (Harlan Laboratories, Montreal, Canada) were used in two studies: (i) Across age; 4 month old (young; 4M) versus 21 month old (aged; 21M) rats, and (ii) Effect of a KD in 24 month old rats fed a standard diet (24M) or fed a high-fat KD (24M-KD) for 14 days before the PET experiment. There were six rats/group, which was sufficient to show statistically significant differences in the PET data in our previous work (Pifferi et al., 2011). Aged rats were obtained at 12 months of age and housed in our animal facility. 4 and 21M groups underwent an MRI scan approximately 1 week before the double tracer PET study.

The KD had a ratio of fat to protein and carbohydrate of 3.5:1.0 (Diet no. 180478; Dyets Inc, Bethlehem, PA, USA). Fourteen days on the high-fat KD is sufficient to induce mild ketonemia in rats (Pifferi et al., 2011) but the ketonemia can be transitory even though the rats are continuously on the KD. After the MRI and PET scans, all rats were sacrificed. The experimental protocol was approved by the Institutional Animal Research Ethics Review Board and all handling of animals was conducted in accordance with the Animal Care and Use Committee at the Université de Sherbrooke.

4.2. MRI

MRI was performed using a 7 Tesla small-animal scanner (Varian, Palo Alto, CA, USA) equipped with 205/120 Magnex gradient coils and a 63 mm volume coil (Varian, Palo Alto, CA, USA). Rats were anesthetized with 2% isoflurane and a polyethylene catheter was installed in the tail vein. Body temperature and respiration rate were monitored to ensure stable physiological conditions throughout the acquisitions. T_2-weighted images were acquired using a fast spin-echo pulse sequence (repetition time/effective echo time: 3500/12 ms; number of averages: 8; field of view: 5×5 cm^2; matrix: 256×256; 0.2×0.2 mm^2 in-plane resolution; 35 slices of 1 mm). These images were used to assess volumes of the whole brain, cortex, hippocampus, striatum, cerebellum and lateral ventricles in the 4 and 21M rats. Volumes of interest (VOIs) were manually drawn on the axial view and volumes were computed in mm^3, using *ITK-SNAP* software (Yushkevich et al., 2006). A second set of T_1-weighted images were acquired with the contrast agent – gadolinium-diethylene-triaminopentaacetic acid (Gd-DTPA [Magnevist], 0.9 kDa; Bayer HealthCare Inc.) – to determine BBB permeability (Cote et al., 2010). A bolus of Gd-DTPA was injected into the caudal vein (143 mM, 600 μl over 1 min). Gd-DPTA injection was done after 2 time frames (308 s), with uptake determined in the whole brain. Overall, 18 images were acquired at intervals of 154 s, for a total acquisition time of 46.2 min (repetition time/effective echo time: 300/2.5 ms; number of averages: 4; field of view: 5×5 cm^2; matrix: 128×128; 0.4×0.4 mm^2 in-plane resolution; 35 slices of 1 mm). Data was normalized using the first time frame.

4.3. PET

Prior to undergoing the PET protocol, all rats were fasted for 18 h. They were imaged on a small-animal PET scanner with a 7.5 cm axial field of view, an isotropic spatial resolution of 1.2 mm, and an energy window setting of 250–650 keV (LabPET/-Triumph; Gamma Medica, Northridge, CA, USA). Before the PET scan, rats were anesthetized with 2% isoflurane and a polyethylene catheter was installed in the tail vein for tracer injection. ^{11}C-AcAc was synthetized as previously described (Tremblay et al., 2007). ^{18}F-FDG was prepared using a TRACERlab FDG-MX synthesis unit (GE Healthcare, Waukesha, WI, USA). The double tracer PET protocol was as follows: (i) ^{11}C-AcAc injection (~50 MBq) with a 20 min dynamic scan, (ii) a 15 min delay, and (iii) ^{18}F-FDG injection (~50 MBq) with a 40 min dynamic acquisition. Both tracers were injected in a volume of 300 μl 0.9% sodium chloride solution at 1 ml/min followed by a 300 μl flush at 1 ml/min. ^{18}F-FDG acquisition was started 30 s before injection to be able to correct for residual radioactivity

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from ^{11}C-AcAc acquisition. Body temperature and respiration rate were monitored to ensure stable physiological conditions throughout the PET acquisitions.

4.4. Analysis of PET data

The PET images were reconstructed in 2D using 15 iterations with the following frames sequence: 1×30; 12×5; 8×30; $n\times300$ s, where $n=3$ for the ^{11}C-AcAc acquisition and $n=7$ for the ^{18}F-FDG scan. Voxel size was $0.5\times0.5\times1.2$ mm^3. PET scans were co-registered with the T_2-weighted MR images using PMOD software (PMOD Technologies Ltd, Zurich, Switzerland). A representative MR scan from the 4 and 21M groups were used to co-register the PET images. Tracer uptake was determined in whole brain and four brain regions: hippocampus, cerebral cortex, striatum and cerebellum. No correction for partial volume effect was made as the chosen VOIs had a substantial size (Phelps, 2006). The smallest VOI was the striatum, with a mean volume of 59 mm^3. Plasma time-activity curves for ^{11}C-AcAc and ^{18}F-FDG acquisitions were determined using an image-derived input function (Bentourkia et al., 2009; Liistro et al., 2010; Menard et al., 2010; Tantawy and Peterson, 2010). A VOI was drawn on the left ventricular cavity blood pool on summed ^{18}F-FDG PET images acquired during the first 90 s following injection. Image-derived input functions were corrected using two plasma samples taken at 15 and 18 min for ^{11}C-AcAc acquisitions and 30 and 35 min for ^{18}F-FDG scans. Radioactivity was measured in a gamma counter (Packard Cobra, GMI, USA). Regional CMR_{AcAc} and CMR_{glc} were determined using a multiple-time graphical analysis method (Patlak et al., 1983; Patlak and Blasberg, 1985); Eq. (1)), where the measured PET activity (C_{Tissue}) is divided by plasma activity (C_P) and plotted at a normalized time. After steady state is achieved, as determined by a 5% maximal deviation from the Patlak regression line, the plot results in a straight line, where the slope K represents brain influx and V is the tracer's distribution volume.

$$\frac{C_{Tissue}(t)}{C_P(t)} = K\frac{\int_0^t (C_P(t)dt)}{C_P(t)} + V \quad (1)$$

CMR_{glc} was calculated using Eq. (2), where [glucose] is the plasma glucose concentration. A lumped constant (LC) of 0.48 was used as previously reported in rats (Sokoloff et al., 1977).

$$CMR_{glc} = \frac{K\times[\text{glucose}]}{LC} \quad (2)$$

CMR_{AcAc} was calculated from the following equation (Blomqvist et al., 1995):

$$CMR_{AcAc} = K\times[\text{AcAc}] \quad (3)$$

4.5. Blood analysis

After the PET scan, 5 ml of blood was collected by cardiac puncture before euthanasia. Blood samples were kept on ice and then centrifuged (6000 RPM; 5 min; 4 °C) to collect plasma. Samples were kept at −80 °C until analysed using a clinical chemistry analyzer (Dimension Xpand Plus, Siemens Healthcare Diagnosis Inc, Deerfield, IL, USA) with kits for glucose (DF40), lactate (DF16), triglycerides (DF69A) and free fatty acids (NEFA-HR2, Wako Ltd, Richmond, VA, USA). Plasma AcAc and β-hydroxybutyrate were measured with an open channel of the Dimension analyzer as previously described (Pifferi et al., 2011). Insulin levels were measured by enzyme-linked immunosorbent assay (80-INSRTU-E01, Alpco, Salem, NH, USA).

4.6. Statistical analysis

Statistical analyses were performed using non-parametric tests (Prism 5; GraphPad, La Jolla, CA, USA). Data show the comparison between the 4 and 21M groups, and between the 24M and 24M-KD groups (Mann–Whitney test). Correlations were established by Spearman's test. Two-tailed $p<0.05$ was considered statistically significant.

Disclosure statement

The authors declare no conflicts of interest.

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