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KETONES SUPPRESS BRAIN GLUCOSE CONSUMPTION

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Abstract

The brain is dependent on glucose as a primary energy substrate, but is capable of utilizing ketones such as β -hydroxybutyrate (BHB) and acetoacetate (AcAc), as occurs with fasting, prolonged starvation or chronic feeding of a high fat/low carbohydrate diet (ketogenic diet). In this study, the local cerebral metabolic rate of glucose consumption (CMRglu; $\mu\text{M}/\text{min}/100\text{g}$) was calculated in the cortex and cerebellum of control and ketotic rats using Patlak analysis. Rats were imaged on a rodent PET scanner and MRI was performed on a 7-Tesla Bruker scanner for registration with the PET images. Plasma glucose and BHB concentrations were measured and 90-minute dynamic PET scans were started simultaneously with bolus injection of 2-Deoxy-2-[¹⁸F]Fluoro-D-Glucose (FDG). The blood radioactivity concentration was automatically sampled from the tail vein for 3 min following injection and manual periodic blood samples were taken. The calculated local CMRglu decreased with increasing plasma BHB concentration in the cerebellum ($\text{CMRglu} = -4.07 * [\text{BHB}] + 61.4$, $r^2 = 0.3$) and in the frontal cortex ($\text{CMRglu} = -3.93 * [\text{BHB}] + 42.7$, $r^2 = 0.5$). These data indicate that, under conditions of ketosis, glucose consumption is decreased in the cortex and cerebellum by about 10% per each mM of plasma ketone bodies.

1. INTRODUCTION

Neurodegeneration after oxidative stress limits the recovery of tissue response and appears to be caused by impaired glycolysis. If indeed there is a defect in glucose metabolism it might be beneficial to supplement energy metabolism with an alternate substrate. It was suggested that brain can supplement glucose as the principal energy substrate with ketone bodies^{1–3} without altering oxygen consumption^{4,5}. Classic studies of ketosis induced by fasting or starvation in humans showed that brain function was maintained which was attributed to the utilization (oxidation) of ketone bodies as alternate energy substrates to glucose by the brain⁶. Rats that have been fasted for 2–3 days showed no difference in cerebral blood flow (CBF) or CMRO_2 ⁷.

One mechanism by which ketosis might be beneficial is through the metabolic step where ketones enter the TCA cycle at the level of citrate bypassing glycolysis, the step after pyruvate dehydrogenase complex where the enzyme activity is often impaired. Through feed-back regulation, ketones are known to down regulate glycolytic rates at various levels such as citrate, phosphofructokinase and/or hexokinase. In addition, particularly in brain, ketones are a carbon source for glutamate (anaplerosis) and thus help to balance glutamate/glutamine homeostasis through stabilization of energy metabolism in astrocyte following recovery from a hypoxic/ischemic event.

Based on our experiments and evidence in the literature, we have developed the hypothesis that ketones are effective against pathology associated with altered glucose metabolism, the

rationale being that ketosis helps to regulate glucose metabolism. In this study, the effects of ketosis on the local cerebral metabolic rate of glucose consumption (CMR_{glu}) were investigated in an *in vivo* rat model of ketosis using positron emission tomography (PET) with 2-[¹⁸F] fluoro-2-deoxy-D-glucose (FDG).

2. MATERIALS AND METHODS

Animal preparation and Diets

All procedures were performed in strict accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Adult male Wistar rats (final weights: 175–250 g) were allowed to acclimate in the CWRU animal facility for one week before experiments. All rats were housed two-three in a cage, maintained on a 12:12 light-dark cycle with standard/ketogenic rat chow and water available *ad libitum*.

Diet Protocols

The ketogenic (KG) diet was purchased from Research Diets (New Brunswick, NJ, U.S.A.) and standard (STD) diet, Teklab 8664, was provided by the CWRU animal facility (see table 1). The KG diet protocol was chosen for its proven effectiveness for inducing moderate and stable ketosis in young adult rats and for its palatability^{8–10}.

Surgical Procedures

On the day of the experiment, anesthesia was induced by gas mixture (3% isoflurane in O₂) and maintained with 1–2% isoflurane in O₂ through a nasal cone. Ketogenic and control rats underwent the same procedures. Cannulae were placed in: (i) a ventral tail artery using polyethylene tubing (PE-50, 0.023” i.d., 0.038” o.d.), plasma glucose, lactate and ketone concentrations (ii) an external jugular vein into the right atrium using a silastic catheter (0.025” i.d., 0.047” o.d.) for FDG injection. Lidocaine (1%) was infiltrated on the site. About 2cm incision was then made on the area to be cannulated and the tail artery was isolated. An intramedic(R) polyethylene tubing, size PE 50 with an I.D. of 0.58mm and O.D. of 0.965mm was then advanced into the artery. Two sutures were used to secure the catheter in place while the other end of the catheter was connected through a tubing to a Blood Pressure Analyzer for continuous recording and monitoring of the animal’s blood pressure. The neck area was also infiltrated with 1% lidocaine solution. An incision was made and the external jugular vein was isolated and catheterized with a silastic tubing (0.025” i.d., .047” o.d.) which was advanced into the right atrium. The catheter was secured and the incision wound closed with one or two sutures. The other end of the catheter was used for administration of the radioactive indicator to the animal. A small blood sample taken by tail stick from the KG group on the day of surgery was tested using a MediSense Precision Xtra ketone meter to document ketonemia (BHB > 1.0 mM).

MicroPET analysis

The animal was placed on a bed apparatus and moved into a 7-Tesla Bruker scanner for magnetic resonance imaging (MRI) to get anatomical reference for PET imaging. After MRI, the apparatus was moved into the gantry of a Concord Microsystems R4 microPET (Siemens, Knoxville, TN). A transmission scan was performed with a cobalt (⁵⁷Co) point source for attenuation correction. A 90-minute dynamic PET scan was started simultaneously with bolus injection of 10 MBq/100 g of FDG. Plasma glucose, lactate and BHB concentrations were directly measured. The blood radioactivity concentration was automatically sampled from the tail artery for 3 min using a blood acquisition monitor (BAM) and manual blood samples were taken at 3.5, 4, 4.5, 5, 7, 10, 15, 30, 45, 60, 75 and 90 min. The emission data were rebinned

into 17 time frames (3×20 sec, 2×1 min, 1×2 min, 6×5 min, 6×10 min). ROIs were manually defined on the frontal cerebral cortex and the cerebellum using the high resolution MRI images and mapped onto dynamic PET images. Patlak analysis was finally applied to extract the CMR_{glu} in both tissue types, assuming a lumped constant (LC) of 0.705^{11,12}.

3. RESULTS

The diet conditions did not affect the physiological parameters body weight, plasma BHB, plasma glucose and plasma lactate (Table 2). All rats in each of the groups gained weight consistently over the experimental period. Weight gains did not show any significant differences between diet groups as well as with plasma lactate and glucose. BHB levels in blood were significantly higher in KG compared to the STD diet group.

The CMR_{glu} calculated with Patlak analysis was plotted as a function of the measured blood BHB concentration (Figure 1). These data showed that CMR_{glu} decreases with increasing ketosis in brain of rats that were either short-term fasted or fed a KG diet for three weeks, (*the blood [BHB] "0" value represents non-ketotic rats*). The calculated CMR_{glu} linearly decreased with increasing blood ketone concentration in the frontal cortex ($\text{CMR}_{\text{glu}} = -3.9 * [\text{BHB}] + 42.7$; $R^2 = 0.484$) (data not shown) and in the cerebellum ($\text{CMR}_{\text{glu}} = -4.1 * [\text{BHB}] + 61.4$; $R^2 = 0.30$). A linear relationship between the CMR_{glu} in the frontal cortex and cerebellum was also observed ($R^2 = 0.881$). Most striking is that the data show a 10% decrease in CMR_{glu} for every 1mM increase in blood ketone (BHB) levels. These data are consistent with our previous studies that, in combination with increased ketosis and increased transport of ketone bodies at the blood brain barrier, there are more ketones available for brain metabolism¹⁰

Each rat was scanned on the PET scanner for 90 minutes post-injection and the list-mode data was rebinned in 17 timeframes. The PET and MRI images were registered and ROIs were drawn on the cerebellum and cortex (Figure 2). The micro PET data were used to detect upregulation of glycolysis and it provided insight into fates of glucose uptake and metabolism (CMR_{glu}) during acute and chronic ketosis in rat brain. Pet scans using ¹⁸FDG, were used to examine the relationship between glucose uptake (CMR_{glu}) and ketosis (ketone plasma levels). Our results showed differences in ¹⁸FDG uptake in ketotic vs non-ketotic rat brain.

4. DISCUSSION

From these data, we estimated that in the non-ketotic state with a blood concentration of 0.04 mM BHB and a cerebral blood flow of 1.0 ml/g/min, an extraction fraction of 8%¹⁰ is expected to result in a ketone influx rate of about 3 nmol/g/min. For ketone bodies to replace at least 10% of glucose oxidation an increase in blood ketone concentration and an up regulation of monocarboxylate transporters is required. The utilization of ketones has been shown to be proportional to the uptake^{3,13}. Thus, for a 1.0 mM blood BHB, a 12% extraction fraction and a cerebral blood flow of 1.0 ml/g/min a ketone influx of 120 nmol/g/min can be expected. Glucose consumption rates in the anesthetized rat cerebral cortex are approximately 500 nmol/g/min, thus there would be sufficient ketone flux to replace 10% of the glucose oxidation.

It is hypothesized that ketone bodies play a neuroprotective role through an improvement in metabolic efficiency, by sparing glucose, and the degradation of muscle-derived amino acids for substrates¹⁵. During hypoxia, ketone bodies have been shown to be neuroprotective^{16,17} by depressing glucose uptake and CMR_{glu} possibly due to metabolic blocks as a result of oxidative damage. Ketone bodies are thought to stabilize the lactate/pyruvate ratio and bypass the metabolic blocks associated with oxidative stress induced impairment of glucose metabolism.

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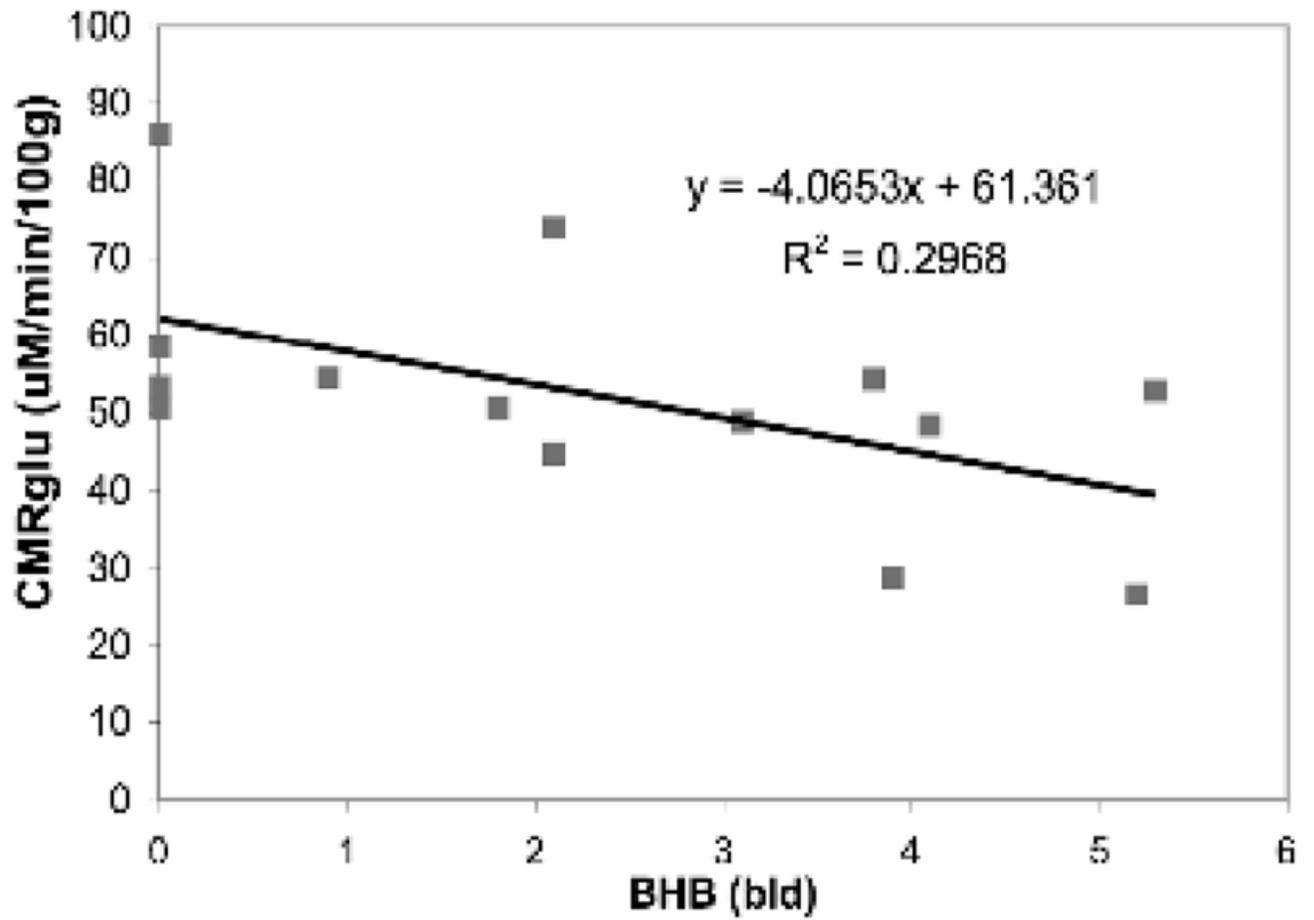


Figure 1.
Cerebellum CMRglu.

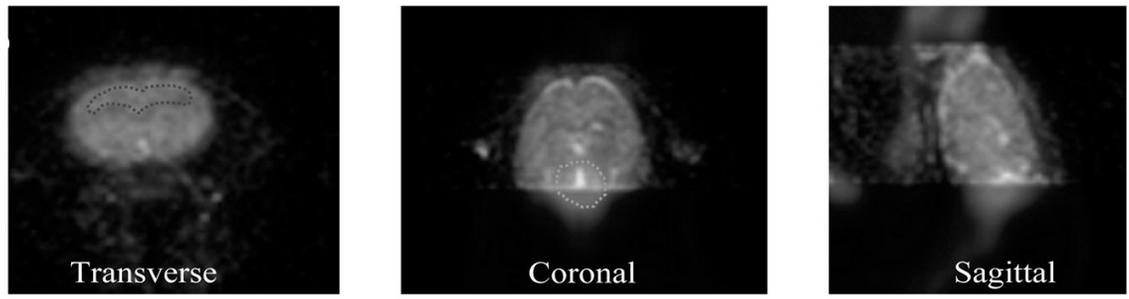


Figure 2.
MicroPET images of rat brain.

Table 1

Diet composition

	Fat (%)	Protein (%)	Carbohydrate (%)
Ketogenic (KG)	89.5	10.4	0.1
Standard (STD)	27.5	20.0	52.6

Table 2Physiological Parameters (mean \pm standard deviation).

	STD (n=6)	KG (n=10)
Blood BHB, mM	0.9 \pm 0.6	3.78 \pm 1.1 *
Plasma glucose, mM	10.73 \pm 3.78	10.63 \pm 1.55
Plasma lactate, mM	1.123 \pm 0.46	0.80 \pm 0.06
Weights, g	267.56 \pm 59.11	252.71 \pm 47.45

*
p < 0.05