

# Chapter 51

## Contribution of Brain Glucose and Ketone Bodies to Oxidative Metabolism

Yifan Zhang, Youzhi Kuang, Joseph C. LaManna, and Michelle A. Puchowicz

**Abstract** Ketone bodies are an alternative energy substrate to glucose in brain. Under conditions of oxidative stress, we hypothesize that ketosis stabilizes glucose metabolism by partitioning glucose away from oxidative metabolism towards ketone body oxidation. In this study we assessed oxidative metabolism in ketotic rat brain using stable isotope mass spectrometry analysis. The contribution of glucose and ketone bodies to oxidative metabolism was studied in cortical brain homogenates isolated from anesthetized ketotic rats. To induce chronic ketosis, rats were fed either a ketogenic (high-fat, carbohydrate restricted) or standard rodent chow for 3 weeks and then infused intravenously with tracers of [U-<sup>13</sup>C] glucose or [U-<sup>13</sup>C] acetoacetate for 60 min. The measured percent contribution of glucose or ketone bodies to oxidative metabolism was analyzed by measuring the <sup>13</sup>C-label incorporation into acetyl-CoA. Using mass spectrometry (gas-chromatography; GC-MS, and liquid-chromatography; LCMS) and isotopomer analysis, the fractional amount of substrate oxidation was measured as the M+2 enrichment (%) of acetyl-CoA relative to the achieved enrichment of the infused precursors, [U-<sup>13</sup>C]glucose or [U-<sup>13</sup>C] acetoacetate. Results: the percent contribution of glucose oxidation in cortical brain in rats fed the ketogenic diet was  $71.2 \pm 16.8$  (mean%  $\pm$  SD) compared to the standard chow,  $89.0 \pm 14.6$ . Acetoacetate oxidation was significantly higher with ketosis

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compared to standard chow,  $41.7 \pm 9.4$  vs.  $21.9 \pm 10.6$ . These data confer the high oxidative capacity for glucose irrespective of ketotic or non-ketotic states. With ketosis induced by 3 weeks of diet, cortical brain utilizes twice as much acetoacetate compared to non-ketosis.

**Keywords** Ketosis • Mass spectrometry • Rat cortex • Stable isotopes

## 1 Introduction

This study was developed on the basis that ketones are effective against pathology associated with altered glucose metabolism, such as with ischemia reperfusion injury and seizure disorders. Ketosis can be induced by prolonged fasting or ketogenic (KG) diet. We had previously reported neuroprotection by ketosis following recovery from transient focal ischemia [1]. Using a rat model of ketosis, we hypothesized that the cerebral metabolic rate for glucose (CMR<sub>glc</sub>) decreases with increasing ketosis. Thus, a shift of oxidative metabolism away from glucose towards ketone bodies may result in neuroprotection, irrespective of the mechanistic link. It has been described in humans and rodents that CMR<sub>glc</sub> decreases with ketosis [2, 3]. To show the partitioning of glucose metabolism towards ketone oxidation, one would need to simultaneously measure CMR<sub>glc</sub> under ketotic and non-ketotic conditions. CMR<sub>glc</sub> can be readily measured by a metabolic trapping mechanism using <sup>18</sup>Fluorodeoxyglucose (<sup>18</sup>FDG) tracer and Positron Emission Tomography (PET) imaging system [2]. However, the CMR<sub>ket</sub> cannot be reliably measured due to various constraints. These include (1) costly use of PET imaging systems, (2) lack of a trapping tracer for accurate measurements of ketone body utilization, (3) short half-life of the currently available tracers, and (4) relatively low sensitivity of nuclear magnetic resonance (NMR) [3–5].

To test in the cortical brain the partitioning of ketone utilization during ketosis, we designed a study using stable isotope tracers and mass spectrometry to estimate the fractional contribution of glucose or ketone bodies (acetoacetate) to oxidative metabolism. This enabled the implementation of a relatively inexpensive method (compared to PET), using an *in vivo* rat model of ketosis to study cortical brain glucose and ketone body metabolism. GC-MS and LC-MS systems are used for investigating intermediary metabolism, as they have high sensitivities to many analytes and metabolites. Compared to NMR methods, the use of small sample size therefore allows a smaller blood volume to sample. Stable isotopes of <sup>13</sup>C-labeled tracers were infused into anesthetized rats and assayed by mass spectrometry. This approach assumes that the <sup>13</sup>C-label incorporated acetyl-CoA is from the oxidation of the precursors, <sup>13</sup>C-glucose or <sup>13</sup>C-acetoacetate. Using isotopomer analysis, the M+2 enrichment of acetyl-CoA was measured and the fractional percent contribution of substrates (glucose or ketone bodies) to oxidative metabolism was calculated as the mole percent enrichment (MPE) of acetyl-CoA [6, 7].

## 2 Methods

The experimental protocol employed in this study was approved by the Institutional Animal Care and Use Committee (IACUC) at Case Western Reserve University.

### 2.1 *Animal Preparation and Diets*

Adult male Wistar rats (final weight: 310–440 g;  $n=20$ ) were purchased from Charles River and were allowed to acclimatize in the CWRU Animal Resource Center (ARC) for at least 1 week before feeding their respective diets. Rats were then fed either the ketogenic (KG) or Standard (STD) diets for 3 weeks prior to the experimental day [1, 2]. The KG diet was purchased from Research Diet (New Brunswick, NJ, USA) and the standard rodent chow (Teklab 8664) was provided by CWRU ARC. All procedures were performed with approval from the Case Western Reserve University IACUC. On the experimental day, both diet groups (KG and STD) underwent the same surgical procedures for the placement of jugular and arterial catheters and tracer infusions [2]. Rats were morning fasted for 4 h prior to tracer infusions prior to infusions. Anesthesia was induced with isoflurane balanced with a mixture of  $N_2/O_2$  and the rats were maintained under light anesthesia during the tracer infusions. The flow rates of the gases were manually adjusted to maintain breath rates (60–80 breath/min). Arterial blood gases were measured (ABL-5, Radiometer, Copenhagen) to confirm stable arterial blood pH.

### 2.2 *Experimental Design, Tracer Preparation, and Infusions*

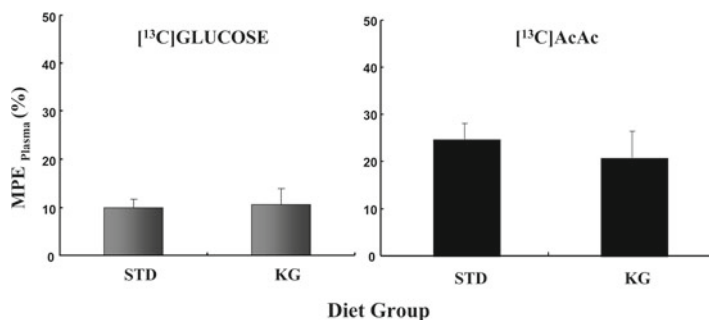
Four study groups were implemented: rats were infused with tracers of  $[U-^{13}C]$ glucose or  $[U-^{13}C]$ AcAc and fed either standard (STD) or ketogenic (KG) diets.  $[U-^{13}C]$  glucose (99.8 %) was solved in 0.9 % NaCl solution to a final concentration of 38.7 mM.  $[U-^{13}C]$ AcAc was derived from  $[U-^{13}C]$ ethyl-acetoacetate, as previously described [8] and concentrated to 137 mM. All chemicals were purchased from Sigma-Aldrich. Tracers were infused via the jugular vein catheter (0.50 or 1.0 mmol/kg/h) (Harvard Apparatus syringe pump-11 Plus) for 60 min. To verify steady-state conditions, blood samples (100–200  $\mu$ L) were taken from the tail artery at time point 0 (pre-infusion), and at 15, 30, 40, 50, and 60 min (during infusion), immediately centrifuged and the plasma frozen for GC-MS analysis of the  $[U-^{13}C]$  precursor enrichments and concentrations of glucose and acetoacetate. At the end of infusion, the rats were decapitated; the brains were dissected immediately, frozen in liquid nitrogen, and stored at  $-80$  °C. Cortical sections ( $\sim$ 200 mg tissue) were then dissected under frozen conditions and homogenized using a specific organic solvent mixture designed for isolation of acyl-CoAs and related metabolites [6, 7].

### 2.3 Estimation of the Contribution of Acetoacetate and Glucose to Oxidative Metabolism

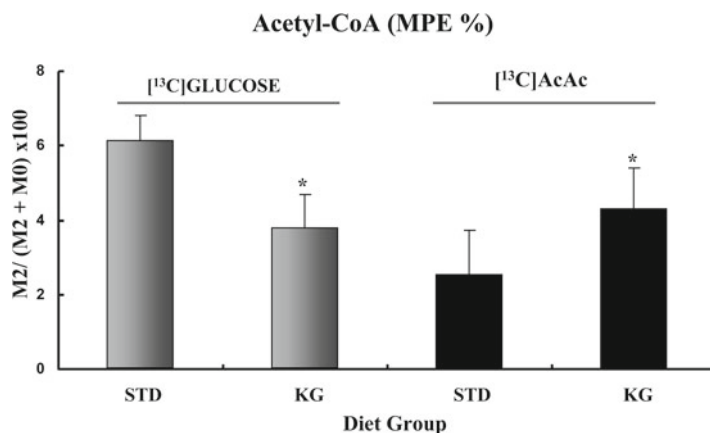
Cortical brains were processed for  $^{13}\text{C}$ -acetyl-CoA (M+2) enrichments (MPEs) using LC-MS, a similar method as previously described [6, 7]. The plasma MPE of  $^{13}\text{C}$ -glucose and  $^{13}\text{C}$ -AcAc was measured using GC-MS methods [6, 7]. After background correction, the MPEs of the precursor  $^{13}\text{C}$ -substrates and the oxidative product (acetyl-CoA), were calculated from the measured ion masses (M+4, [ $^{13}\text{C}$ ] AcAc; M+6, [ $^{13}\text{C}$ ]glucose; M+2, [ $^{13}\text{C}$ ]acetyl-CoA) to the unlabeled (M0, endogenous intermediate); e.g., acetyl-CoA (M+2) MPE =  $[\text{M}2/(\text{M}2 + \text{M}0) \times 100]$ . The percent fractional contribution of glucose or AcAc to oxidative metabolism in cortical brain was estimated from the MPE of acetyl-CoA relative to the plasma MPE of the  $^{13}\text{C}$ -infused substrates and calculated: Substrate contribution to oxidative metabolism (%) =  $[(\text{brain acetylCoA MPE} \times 2)/(\text{plasma glucose or AcAc MPE})] \times 100$ . All data are expressed as mean  $\pm$  SD. Statistical analyses were performed using a two sample *t*-test. Significance was considered at the level of  $p < 0.05$ .

## 3 Results and Discussion

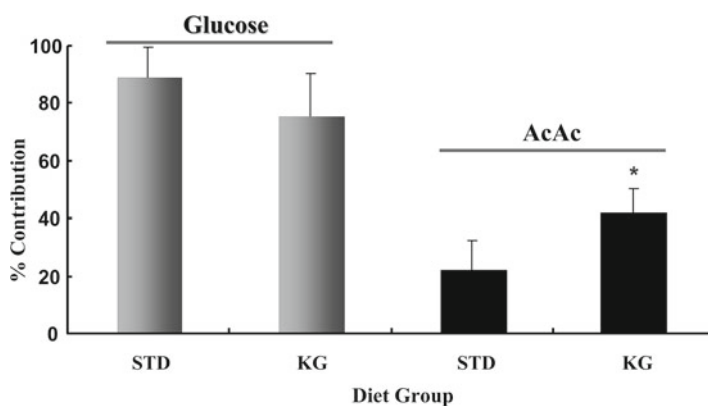
The fractional contribution of glucose or AcAc to cortical brain oxidative metabolism was estimated in anesthetized ketotic rats using stable isotope mass spectrometry analysis. The plasma MPE tracer dilution profiles of  $^{13}\text{C}$ -glucose and  $^{13}\text{C}$ -AcAc reached steady-state conditions by 50 min (time course not shown). Ketosis induced by KG diet did not significantly affect plasma  $^{13}\text{C}$ -glucose or  $^{13}\text{C}$ -AcAc MPE compared to STD groups ( $9.8 \pm 1.0$  % vs.  $9.2 \pm 0.5$  % and  $20.9 \pm 5.5$  vs.  $24.7 \pm 3.3$ , respectively) (Fig. 51.1). Cortical oxidative metabolism was significantly altered by



**Fig. 51.1** Plasma molar enrichment (MPE %) at  $t=50$  min. Tracers of [ $^{13}\text{C}$ ]glucose and [ $^{13}\text{C}$ ] AcAc (acetoacetate) were infused in two diet groups, standard (STD) and ketogenic (KG). Steady-state MPE was achieved by  $t=50$  min (time course not shown).  $^{13}\text{C}$ -glucose infusions resulted in a 10 % plasma MPE in both diet groups. As a result of an increase in infusion rate of [ $^{13}\text{C}$ ]AcAc in the KG diet group compared to STD diet, a twofold increase in the  $^{13}\text{C}$ -AcAc plasma MPE was observed (mean  $\pm$  SD;  $*p < 0.05$ )



**Fig. 51.2** Acetyl-CoA MPE in cortical brain. Rats fed STD or KG diets were infused with either [<sup>13</sup>C]glucose or [<sup>13</sup>C]AcAc tracers. Ketosis resulted in decreased glucose MPE with a parallel increase in AcAc MPE



**Fig. 51.3** Contribution of glucose and AcAc to oxidative metabolism. Percent contribution of glucose oxidation in cortical brain decreased with ketosis. A significant increase in percent contribution to AcAc oxidation with ketosis was also observed

ketosis (Figs. 51.2 and 51.3). With glucose oxidation, a 30 % decrease in acetyl-CoA MPE was observed (Fig. 51.2, see STD and KG groups given tracer infusions of [<sup>13</sup>C]glucose), whereas with AcAc oxidation (see [<sup>13</sup>C]AcAc), acetyl-CoA MPE increased about 40 % with ketosis (STD vs. KG groups). These data show a partitioning of brain glucose oxidation towards ketone body oxidation with chronic ketosis. When estimating the percent contribution of glucose to oxidative metabolism, ketosis (KG) resulted in a decrease in glucose oxidation which was not significantly different from the STD diet group (Fig. 51.3). These data confirm the high oxidative capacity for glucose in cortical brain irrespective of ketosis or non-ketosis. With respect to percent contribution of ketone body oxidation, ketosis

resulted in an increase in oxidative metabolism, as shown by the twofold increase in AcAc percent contribution compared to STD diet (Fig. 51.3). Consistent with our hypothesis, ketosis induced by diet plays a role in cortical brain utilization of AcAc. These findings demonstrate the ability of brain to switch towards ketone body oxidation with ketosis (Figs. 51.2 and 51.3) [4, 5]. This model appears to overestimate oxidative metabolism by about 15 %. The sum of the percent contribution of glucose and AcAc to oxidative metabolism exceeds 100 % (Fig. 51.3). Indeed, in healthy non-ketotic mammals, glucose contribution to oxidative metabolism in brain is about 90 %. So we suspect that the fraction of ketone contribution to oxidation metabolism is overestimated by about 15 %. The reason for this overestimation remains to be determined. One explanation is the precursor pool of  $^{13}\text{C}$ -AcAc enrichment in brain tissue differs from plasma; an underestimation of the precursor MPE could account for this discrepancy.

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