PET study of $^{11}$C-acetoacetate kinetics in rat brain during dietary treatments affecting ketosis

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Bentourkia M, Tremblay S, Pifferi F, Rousseau J, Lecomte R, Cunnane S. PET study of $^{11}$C-acetoacetate kinetics in rat brain during dietary treatments affecting ketosis. Am J Physiol Endocrinol Metab 296: E796 –E801, 2009. First published January 27, 2009; doi:10.1152/ajpendo.90644.2008.—Normally, the brain’s fuel is glucose, but during fasting it increasingly relies on ketones (β-hydroxybutyrate, acetoacetate, and acetone) produced in liver mitochondria from fatty acid β-oxidation. Although moderately raised blood ketones produced on a very high fat ketogenic diet have important clinical effects on the brain, including reducing seizures, the precise ketone metabolism by the brain is still poorly understood. The aim of the present work was to assess brain uptake of carbon-11-labeled acetoacetate ($^{11}$C-acetoacetate) by positron emission tomography (PET) imaging in the intact, living rat. To vary plasma ketones, we used three dietary conditions: high carbohydrate control diet (low plasma ketones), fat-rich ketogenic diet (raised plasma ketones), and 48-h fasting (raised plasma ketones). $^{11}$C-acetoacetate metabolism was measured in the brain, heart, and tissue in the mouth area. Using $^{11}$C-acetoacetate and small animal PET imaging, we have noninvasively quantified an approximately seven- to eightfold enhanced brain uptake of ketones on a ketogenic diet or during fasting. This opens up an opportunity to study brain ketone metabolism in humans. positron emission tomography; ketogenic diet; ketone bodies

Normally, glucose is the human brain’s main fuel with use of only a small amount of ketone bodies (or ketones) in a ratio of ~97:3. However, during short-term fasting, the ratio of energy supplied by glucose vs. ketones becomes ~70:30 and is reversed to ~30:70 during starvation (13, 37, 41). Thus, in adults, ketones are a key replacement fuel for glucose. However, in the fetus and infant, they are essential brain fuels and lipid substrates and appear to be important for the brain’s early development (8). Unlike the heart and other tissues, which readily utilize fatty acids as alternate energy substrates to glucose, the brain specifically needs ketones to replace low availability of glucose. Both neurons and glia utilize ketones.

There are three ketones, β-hydroxybutyrate, acetoacetate, and acetone, that are produced by β-oxidizing fatty acids in the liver mitochondria before their release into the blood and subsequent transport to the brain and elsewhere. Once in the brain, β-hydroxybutyrate and acetoacetate are converted to acetoacetyl-CoA and then to acetyl-CoA, which enter the tricarboxylic acid pathway for ATP generation through the same final pathway as glucose (11, 17, 18, 22, 23, 24, 29). Ketones have other functions in the brain, such as to provide substrates for the synthesis of several molecules including cholesterol in myelin (24).

Children with refractory seizures, i.e., those that are not responsive to antiepileptic drugs, commonly have fewer seizures while on a very high fat ketogenic diet (Ref. 12). Ketones also prevent symptoms of experimental hypoglycemia in adults, including anxiety, fainting, hunger, irritability, and tremor (1, 39). Hence, in various clinical situations, ketones appear to have important “protective” effects on the brain.

The very high fat KD was introduced in the 1920s in replacement of fasting, which was used as antiseizure remedy. At that time, epilepsy was thought to be caused by intestinal intoxication, and a diet consisting of water only for 30 days was shown to reduce some refractory seizures (38). Fasting liberates fatty acids from fat stores, and similarly, the KD decreases ketone and free fatty acids in blood. Moreover, because of the calorie restriction of the KD, blood glucose is significantly reduced (9).

The high fat/carbohydrate KD was found to produce mild ketosis accompanied by weight loss and high levels of plasma saturated fats, which also provoke a decrease of hepatic gluconeogenesis from amino acids (38). In patients with astrocytoma, the KD decreases tumor glucose uptake. It also reduces tumor mass and vascularity by 80% in mice, thereby inhibiting angiogenesis (25, 27).

The present project aimed to use positron emission tomography (PET) imaging of $^{11}$C-acetoacetate metabolism to develop a better understanding of ketone metabolism in the brain and to identify potential applications of ketones in neurodegenerative diseases. In this study, rats consumed one of the following three types of diets: carbohydrate-rich control diet (low plasma ketones), very high fat, very low carbohydrate KD (moderately high plasma ketones), or total fasting for 48 h (moderately high ketones). We report here $^{11}$C-acetoacetate uptake by the brain, and, for comparison, we also show the results from the heart and tissue around the mouth, the latter to assess the degree of possible $^{11}$C-acetoacetate spillover from surrounding tissues into the brain PET images.

Theory

Ketones and brain metabolism. The blood-brain barrier monocarboxylic acid transporter 1 (MCT1) is a common carrier for ketones, lactate, pyruvate, and α-keto acids. Pyruvate, lactate, β-hydroxybutyrate, and acetoacetate are transported across the blood-brain barrier by a combination of passive and...
facilitated diffusion. MCT1 is expressed in endothelial cells and pericytes, while MCT2 is expressed in neurons and astrocytes. β-Hydroxybutyrate and acetoacetate are rapidly taken up by the brain in proportion to their concentration in the blood (22, 28).

In the brain, ketones are metabolized in cytosol and mitochondria. β-Hydroxybutyrate is successively degraded in mitochondria by three enzymes: β-hydroxybutyrate dehydrogenase and 3-ketoacid-CoA transferase, which are located in mitochondria, and acetoacetyl-CoA thiase, which is found in mitochondria and cytosol. The reactions induced by these three enzymes are freely reversible. The end product of these reactions is acetyl-CoA, the first intermediate in common with the glucose metabolism pathway. Acetoacetate is also metabolized in the mitochondria and is oxidized by the same pathway as β-hydroxybutyrate, entering the pathway at the second step. Acetoacetate can also be metabolized in cytosol to acetoacetyl-CoA by acetoacetyl-CoA synthetase in the presence of ATP. This reaction is unidirectional in favor of acetoacetyl-CoA synthesis. Therefore, the cytosolic metabolic pathway is similar to the mitochondrial one. The mitochondrial pathway of ketone body metabolism serves oxidative purposes and generates acetyl-CoA molecules for amino acid biosynthesis, whereas the cytosolic route is involved prominently in lipid and cholesterol biosynthesis. In the liver, β-hydroxybutyrate dehydrogenase functions in the reverse direction compared with brain and converts acetoacetate to β-hydroxybutyrate (22, 28).

**Kinetic modeling.** Since 11C-acetoacetate and 11C-acetate follow the same pathway through acetyl-CoA (3, 4, 18, 19, 20, 29, 30, 32, 40), we used the same three-compartment model as used for 11C-acetate kinetics (Fig. 1; Eq. 1; Ref. 5).

The three-compartment model is also used in the studies by Blomqvist et al. (3, 4) in a PET study of R-β-[1-13C]-hydroxybutyrate metabolism in humans. When R-β-[1-13C]-hydroxybutyrate is injected into a subject, it rapidly equilibrates with 13C-acetate and both of these tracers can be found in the blood. Moreover, the two radiotracers cross the blood-brain barrier at different rates, so an overall metabolic rate of the two tracers combined was estimated (3, 4).

The same three-compartment model has also been used for several other radiotracers in PET imaging, including 11C-acetate (5, 6, 33), 13N-ammonia (26), and 18F-fluorodeoxyglucose (18F-FDG; Refs. 14, 31). In the present work, the model shown in Fig. 1 can be expressed as follows (5):

$$C_{PET}(t) = \left[ A_1 \cdot \exp(-k_2 \cdot t) + A_2 \cdot \exp(-k_3 \cdot t) \right] \times C_t(t) + TBV \cdot C_{inf}(t)$$

where $C_{PET}$ is the measured radioactivity in the tissue region of interest of the PET image, $k_1$-$k_4$ are the four rate constants between compartments, TBV is the tissue blood volume, which accounts for the blood component in the tissue, and $C_p(t)$ and $C_{inf}(t)$ are the plasma and whole-blood time-activity curves (TACs). The symbol $\times$ represents the operation of convolution. The rate constants $K_1$ (influx) and $k_2$ (efflux), each expressed in min$^{-1}$, are correlated to tracer perfusion and oxygen consumption, respectively (5, 6, 33).

The plasma TAC $C_p(t)$ is used as the input function and is required for quantification. It was extracted from the whole blood TAC $C_{inf}(t)$ derived from the heart blood pool in the PET images. The correction that needs to be made for the presence of 11C-acetate metabolites in blood, i.e., 11C-CO$_2$, was computed as follows (5):

$$C_p(t) = \left[ 1 - a \cdot \left[ 1 - \exp\left(-\frac{\text{Log}(2)}{m}\right) \right] \right] \cdot C_{inf}(t)$$

where $a$ and $m$ are the metabolite parameters that account for the presence of 11C-CO$_2$ in blood. The four rate constants $K_1$-$k_4$, TBV, and the metabolite parameters $a$ and $m$ are all determined by fitting the model to the PET data $C_{PET}(t)$. In our case, the input curve to the brain, $C_{pb}(t)$, was derived from the input curve $C_{pb}(t)$, which was determined using a region of interest (ROI) on the blood pool in the heart’s left ventricle, by normalizing to the injected doses of brain to heart imaging:

$$C_{pb}(t) = C_{pb}(t) \cdot \frac{\text{dose}_{brain}}{\text{dose}_{heart}}$$

The ketone metabolic rate (MR$_{kb}$) was calculated as (3, 4):

$$MR_{kb} = \left[ C_{acac} \right] \frac{K_1 k_3}{k_2 + k_3}$$

where $C_{acac}$ (nmol/ml) is the concentration of unlabeled acetoacetate in the plasma (3, 4). The rate constants are in
min⁻¹, and therefore, MR_{kb} has units of nanomoles per milliliter per minute.

Although ¹¹C-acetoacetate was the tracer injected into the rats, the body reversibly converts it to β-hydroxybutyrate. Therefore, [C_{acac}] used in Eq. 4 should include the plasma concentration of β-hydroxybutyrate [C_{βHB}]. The blood β-hydroxybutyrate values measured in our study correlated well with those reported elsewhere under similar experimental conditions (2, 7, 15, 16, 34), so the [C_{acac}] we report here was taken from these references and combined with the [C_{βHB}] we measured in this study.

MATERIALS AND METHODS

Animal model. Sprague-Dawley rats (Charles River Breeding Laboratories, ~300 g; n = 4/group) were given one of the following three diets for 2 wk: a carbohydrate-rich control diet, a very high fat-rich KD, or they were fasted for 48 h (Table 1). All rats had free access to water throughout the study. The experiments were performed following a protocol devised according to the recommendations of the Canadian Council on Animal Care and approved by the ethics committee of the Faculty of Medicine and Health Sciences of the Université de Sherbrooke.

PET measurements. The PET data were acquired in list mode with the Sherbrooke small animal scanner (21). The rats were anesthetized with isoflurane (2.5% isoflurane in medical O₂) delivered through a nose cone. A butterfly cannula (Butterfly-25 Short; Venisystems) was placed in the tail vein for the administration of the radiotracer through an automatic injection pump (model 210; Lomir Biomedical) during 25 s. The position of the rats was adjusted at the center of the scanner axial field of view with the help of laser lines. Vital signs (body temperature, heart beat, and respiration frequency) were monitored and recorded throughout the scanning times to ensure stable physiological status at all times. ¹¹C-acetoacetate was synthesized as previously described (36).

Heart scans were accumulated for 30 min, and the subsequent brain scans were acquired for 20 min. A 10-min delay between heart and brain scans was necessary for animal and acquisition preparation.

Fig. 2. Rat brain image obtained with MRI (A), PET (B), and coregistered magnetic resonance imaging (MRI) and PET images (C). Landmarks are clearly seen on both MRI and PET images as indicated by 3 arrows, and brain and mouth tissue regions of interest were added on coregistered image. In C, the ¹⁸F-FDG-PET landmarks are overlaid on those of Gd-DTPA-MRI landmarks as indicated by the 3 arrows.

Fig. 3. Blood glucose and ketone concentrations in rats on a control diet, ketogenic diet or fasted for 48 h (means ± SD; n = 4/group). Blood samples were obtained before the PET scans.
Before each scan, a capillary blood sample was obtained from a hind foot pad and analyzed for glucose and 3-hydroxybutyrate with a glucose and ketone test strip (MediSense Precision Xtra; Abbott). Each scan was preceded by a 30-s data acquisition before tracer injection to evaluate the residual radioactivity from the previous injection. The mean radioactivity estimated from this frame was removed from all the decay-corrected subsequent frames before the kinetic model was applied. Subtraction of the background radioactivity from the subsequent brain PET data was justified both by the low residual radioactivity from the first injection (which lasted almost 2 times the half-life of 11C isotope) and by the higher amount of 11C-acetoacetate injected for brain imaging, which was almost twice the dose used for heart imaging (2.4 vs. 1.3 mCi, respectively). The images were then reconstructed with the maximum likelihood expectation maximization algorithm (35) in the following sequence: 1 × 30 s, 12 × 5 s, 8 × 30 s, n × 300 s, where n = 3 for the 20-min scans and n = 5 for the 30-min scans.

For better anatomical localization of the PET scans, the brain was also imaged by magnetic resonance imaging (MRI) with three markers containing 18F-FDG and Gd-DTPA set around the head of the rats for PET-MRI image coregistration.

Data analysis. The PET and MRI images were first resized on a common grid to match the voxel size in the PET images. Based on the landmarks in PET and MRI, the brain images were coregistered and the whole brain was delimited with an ROI on the MRI image. This ROI was then reproduced on the entire brain PET dynamic images. Another ROI was also drawn around the mouth area in the MRI and copied onto the PET images. The mouth area was used to assess the effect of contamination or spillover from the radioactively ‘hot’ regions in the rat head on the kinetic values measured in the brain, because 11C-acetoacetate uptake was much lower in the brain than in...
the adjacent mouth area. The heart was used to extract the input function and to compare tracer rate constants and ketone metabolism between the heart and the brain.

Statistical analysis. We performed a one-way ANOVA on the values of influx \( (K_1) \), eflux \( (k_2) \), and ketone metabolism \( (MR_{kb}) \) to compare, two-by-two, the brain, mouth tissue and heart data. We also compared the effects of the diets on these parameters within each tissue.

RESULTS

Figure 2, A and B, depicts MRI and PET images in the same rat. The two images were coregistered with the help of the three markers placed around the head of the rat (Fig. 2C). An example of ROIs drawn around the brain and mouth tissue used in the present analyses is also shown in Fig. 2C. Measurement of blood glucose and \( [C_{\text{BM}}] \) before the PET scans confirmed the significant change \( (P < 0.05) \) in blood glucose and ketone concentration in the fasted and KD groups compared with controls (Fig. 3).

The kinetic model applied to \( ^{11}\text{C}-\text{acetooacetate} \) uptake in the brain, mouth tissue, and heart for the three diets fitted the TACs translating the biochemical transformations of the substrate in the tissue. Figure 4 displays two examples of brain and heart TACs with fits.

Tracer rate constants, influx \( (K_1) \) and efflux \( (k_2) \), were calculated for the brain, mouth tissue and heart (Figs. 5 and 6). Of the three diets, only the KD appeared to favor \( K_1 \) and \( k_2 \) in the brain. In the mouth tissue, \( K_1 \) and \( k_2 \) behaved differently from their values in the brain. In each of the three diet groups, \( K_1 \) differed statistically in the brain and mouth tissue \( (P = 0.0303) \), while the \( P \) values in the comparison of brain to heart, and mouth tissue to heart were 0.1016 and 0.3551, respectively. Importantly, these differences suggest that there was no contamination (spillover) of \( ^{11}\text{C}-\text{acetooacetate} \) from the mouth region to the brain.

Since \( MR_{kb} \) depends on all the rate constants directly involved in the TAC fitting and on blood ketone concentrations \( (Eqs. 1 \text{ and } 4) \), the effect of the three diets on \( MR_{kb} \) presents a different tissue pattern from that of \( K_1 \) and \( k_2 \) (Fig. 7). \( MR_{kb} \) values in rats on all three types of diet were not significantly different for brain vs. mouth tissue, brain vs. heart, or mouth tissue vs. heart \( (P = 0.3263, 0.1755, 0.1040, \text{ respectively}) \). After removal of the values for the control rats that were mostly using glucose and had lower blood ketones, \( MR_{kb} \) differed significantly in brain vs. mouth tissue \( (P = 0.0436) \), in brain vs. heart \( (P = 0.0352) \), and in mouth tissue vs. heart \( (P = 0.0193) \). Compared with the control rats, the brain uptake of ketones was enhanced by seven- to eightfold in rats on the KD.

DISCUSSION

Our results show that rats in mild to moderate ketosis have changes in plasma glucose and ketones that vary depending on the form of ketosis, i.e., (glucose was significantly higher and ketones were significantly lower on the KD than with the 48-h fasting). The \( ^{11}\text{C}-\text{acetooacetate} \) PET measurements analyzed with the three-compartment kinetic model show that the brain acquires and uses ketones differently from the heart or mouth tissue. With fasting, the rate constants \( K_1 \) and \( k_2 \) were not proportional to ketone availability in the blood because the 48-h-fasted rats had higher plasma ketones but lower brain \( k_2 \) than the rats on the KD. The higher metabolic utilization rate of \( ^{11}\text{C}-\text{acetooacetate} \) by the brain, which was proportional to blood ketone availability, suggests that ketones were in fact used as efficiently by the brain during 48-h fasting as in the rats on the high fat KD, irrespective of the availability of glucose. Since blood glucose was lower during fasting than on the KD, in the former group, the brain might have continued to use significant amounts of glucose, possibly derived from gluconeogenesis.

The \( MR_{kb} \) values in mouth tissue were about twice as high as those in the brain under all three dietary conditions (Fig. 7). The brain ketone utilization values in the present study were 2.6, 18.7, and 21.2 nmol·ml\(^{-1}\)·min\(^{-1}\), respectively, for controls, ketogenic and fasted rats. Daniel et al. (10) reported very similar ketone influxes of 2.5 and 30.2 nmol·ml\(^{-1}\)·min\(^{-1}\) in fed and 96-fasted rats, respectively, where nmol·ml\(^{-1}\)·min\(^{-1}\) equals tissue density \( [g/ml] \times \text{nmol·ml}^{-1} \cdot \text{min}^{-1} \). Hawkins et al. (16) reported rat brain uptake of \( \beta[3-\text{14C}]\text{hydroxybutyrate} \) by quantitative autoradiography and found the values varied depending on the brain region with an average over the whole brain of ~6.0 for fed rats and 35.0 nmol·ml\(^{-1}\)·min\(^{-1}\) for 2-day-starved rats. Hence, our present PET data for the rate of ketone utilization by the rat brain under three dietary conditions representing a physiological range of blood glucose and ketone values are in good agreement with published values obtained using very different methodologies. Nevertheless, our present \( MR_{kb} \) values cannot presently be confirmed independently and might include partial volume and spillover effects.

In conclusion, using small animal PET imaging, we show here for the first time that \( ^{11}\text{C}-\text{acetooacetate} \) utilization by the rat brain increases approximately seven- to eightfold under dietary conditions raising plasma ketones. Combining PET methodology and dietary approaches to manipulate ketone production provides a minimally invasive approach to studying brain energy metabolism and has potential application in studies of brain ketone metabolism in human health and disease.

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