Spatiotemporal Uptake Characteristics of [18]F-2-Fluoro-2-Deoxy-d-Glucose in a Rat Middle Cerebral Artery Occlusion Model

Hong Yuan, PhD; Jonathan E. Frank, MS; Yonglong Hong, MD; Hongyu An, DSc; Cihat Eldeniz, BS; Jingxin Nie, PhD; Adomas Bunevicius, MD, PhD; Dinggang Shen, PhD; Weili Lin, PhD

Background and Purpose—Alterations of cerebral glucose metabolism are well anticipated during cerebral ischemia. However, detailed spatiotemporal characteristics of disturbed cerebral glucose metabolism during acute ischemia remain largely elusive. This study aims to delineate spatiotemporal distributions of [18]F-2-fluoro-2-deoxy-d-glucose (FDG) uptake using positron emission tomography imaging, particularly at the peri-ischemic zone, and its correlation with tissue outcome.

Methods—The intraluminal suture middle cerebral artery occlusion model was used to induce focal cerebral ischemia in rats (n=48). All animals underwent sequential MRI and FDG positron emission tomography imaging at different times (30–150 minutes) after middle cerebral artery occlusion. MR and positron emission tomography images were coregistered. FDG uptake in the peri-ischemic zone was assessed in relation to middle cerebral artery occlusion duration, cerebral blood flow, apparent diffusion coefficient, and 24-hour T2 lesions.

Results—Elevated FDG uptake was consistently observed at the peri-ischemic zone surrounding the presumed ischemic core with low FDG uptake. Both the spatial volume and the uptake level of the hyper-uptake region were inversely correlated with the duration of middle cerebral artery occlusion. The hyper-uptake regions exhibited a mild reduction of cerebral blood flow (28.2±3.2%) and apparent diffusion coefficient (9.1±1.4%) when compared with that in the contralateral hemisphere. Colocalization analysis revealed that, with reperfusion, an average of 12.1±1.7% of the hyper-uptake volume was recruited into final infarction.

Conclusions—Elevated FDG uptake at the peri-ischemic zone is consistently observed during acute cerebral ischemia. The region with elevated FDG uptake likely reflects viable tissues that can be salvaged with reperfusion. Therefore, acute FDG positron emission tomography imaging might hold promise in the management of patients with acute stroke. (Stroke. 2013;44:2292-2299.)

Key Words: acute ischemic stroke ■ cerebral glucose metabolism ■ FDG PET

During cerebral ischemia, reduction of cerebral blood flow (CBF) directly results in concurrent reduction of oxygen and glucose supply to the brain, which in turn leads to disturbed glucose metabolism and subsequent cellular dysfunctions attributed by the loss of ATP.1,2 Depending on the extent to which CBF is reduced, alternations of glucose metabolism could vary both spatially and temporally. Specifically, it has been widely and consistently reported that glucose metabolism is greatly suppressed in the ischemic core because of severe flow reduction in animals and patients with stroke using either 14C-2-deoxyglucose autoradiography3—4 or positron emission tomography (PET) imaging with [18]F-2-fluoro-2-deoxy-d-glucose (FDG).5—8 In contrast, results on the spatial and temporal patterns of glucose metabolism in regions with moderate CBF reduction are somewhat inconsistent. Using autoradiography, increased glucose uptake was observed near the ischemic border 30 minutes and 1 hour after middle cerebral artery occlusion (MCAO) in animals.3—5 However, Belayev et al9 reported a heterogeneous distribution of usage of glucose in the peri-ischemic zone with sporadic loci of focally elevated or depressed glucose uptake 2 hours after MCAO in a rat ischemic stroke model. As a result, no significant quantitative changes were observed in glucose metabolism between the 2 hemispheres. Using PET, an increased uptake of FDG in regions with mild reduction of CBF was also reported in rats 75 minutes after MCAO.7 However, this hyper-uptake pattern...
no longer existed 3 hours after MCAO in another study. Nasu et al11 imaged patients with stroke 1 to 7 days after cerebral ischemia using FDG PET, and hyper-accumulation of FDG was observed around the depressed uptake core in 7 of 20 patients. Collectively, although severely diminished glucose metabolism is consistently observed in ischemic core, characteristics of glucose uptake at the peri-ischemic zone remain controversial. Although several factors, including species, MCAO models, and imaging analysis approaches might contribute to the reported inconsistent FDG uptake patterns at the peri-ischemic regions, one of the most plausible explanations may be the different time intervals between the onset of MCAO and time of glucose metabolism assay or FDG PET imaging. Furthermore, studies investigating quantitative association between FDG hyper-uptake and final tissue outcome are lacking. To this end, this study aimed to characterize spatiotemporal glucose uptake patterns during acute cerebral ischemia using FDG PET in a rat MCAO model. Time intervals between FDG injection and onset of MCAO were varied ranging from 30 to 150 minutes after MCAO. FDG uptake in the peri-ischemic zone was assessed in relation to MCAO duration, CBF, and apparent diffusion coefficient (ADC) for all animals, and to 24-hour T2 lesions in relation to MCAO duration, CBF, and apparent diffusion coefficient (ADC) for all animals. Time of glucose metabolism assay or FDG PET imaging, MCAO. FDG uptake in the peri-ischemic zone was assessed in relation to MCAO duration, CBF, and apparent diffusion coefficient (ADC) for all animals, and to 24-hour T2 lesions for reperfused animals. Delineation of the spatiotemporal characteristics of FDG uptake during acute ischemia may offer insights into dynamic alterations of glucose metabolism, which may shed light on tissue viability.

Methods
A total of 48 male Long Evans rats (Charles River, Wilmington, MA) weighing between 250 and 350 g were included. All animal protocols were approved by the Institutional Animal Care and Use Committee. Animals were divided into 5 groups on the basis of when FDG was injected in relation to MCAO onset time, including 30 (n=5), 60 (n=13), 90 (n=13), 120 (n=12), and 150 minutes (n=5) after MCAO. MRI were acquired using a Bruker 9.4T scanner (BioSpec 94/30; Bruker BioSpin, Inc, Billerica, MA) and a phased array surface coil.

Surgical Procedures
The intraluminal MCAO method was adopted to induce focal cerebral ischemia.13 The suture was placed into the internal carotid artery via the external carotid artery stump and advanced for 21 mm until resistance was felt. For reperfusion, anesthesia was briefly discontinued when animals were transported from the PET scanner to the surgical table where isoflurane anesthesia was restored. Reperfusion was accomplished by withdrawing the suture from the internal carotid artery to the external carotid artery to restore blood flow. More information is provided in Surgical Procedures in the online-only Data Supplement.

Imaging
MRI were acquired using a Bruker 9.4T scanner (BioSpec 94/30; Bruker BioSpin, Inc, Billerica, MA) and a phased array surface coil. T1-weighted, T2-weighted (T2w), and echo planar imaging diffusion-weighted images were acquired and ADC maps were calculated. The dynamic susceptibility contrast approach using a single-shot echo planar imaging sequence was used and a singular value decomposition method was used for obtaining CBF maps.12 All imaging parameters are provided in the online-only Data Supplement.

FDG PET imaging was performed using a small animal PET/computed tomography (CT) scanner (Vista eXplore, GE Healthcare, Inc) with a center resolution of 1.2- and 46-mm axial field of view. A 7-minute CT scan was first acquired for subsequent attenuation correction and anatomic registration. FDG (39.4±2.2 MBq) diluted in saline was injected via the tail vein at the predefined time after MCAO. With the exception of the 30-minute group, dynamic PET acquisition was performed for 40 minutes (Figure I in the online-only Data Supplement). The list-mode PET data were binned 3×10, 3×30, 1×180, 3×300, and 2×600 s. For the 30-minute group, FDG was injected during MRI, and PET images were acquired for 10 minutes using the static acquisition mode. Data were reconstructed using the 2-dimensional ordered subset expectation–maximization algorithm with random, scatter, and attenuation correction.

Autoradiography
With the exception of the animals in the reperfusion subgroup, animals were euthanized after PET (Figure I in the online-only Data Supplement) and brain tissues were collected and snap-frozen in liquid nitrogen. Three coronal sections of 14 µm thickness were cut at 4.5, 6, and 8 mm below the brain anterior apex. Six of 28 animals had poor cutting qualities (wrinkles and tissue lost, etc.) and were excluded from the final analyses (more information is provided in Autoradiography in the online-only Data Supplement).

Image Analysis
All MRI from the same animals were first registered using the FLIRT15 in FSK 3.2 (FMRIB, Oxford, United Kingdom). Skull bone structure was extracted from CT images using a threshold method. All MRI from the same animals were first registered using a rigid-body registration scheme. The transformation matrices were subsequently used to register all MRI onto CT images. PET images were registered to CT images using a mutual-information–based registration method.

FDG hyper-uptake volume (V hyper) was obtained as the brain regions in the ipsilateral hemisphere that exhibited a standardized uptake value (SUV) level greater than the mean+2SD of that in the contralateral hemisphere from PET images that were obtained 30 to 40 minutes after FDG injection. In addition, V hyper fraction (F hyper) was calculated as the V hyper divided by the ipsilateral hemispheric volume. Similar criteria were applied on autoradiography images to obtain volume with hyper-FDG uptake and F hyper averaged from 3 sections was calculated for each animal.

Ischemic core volume was manually traced from each animal in the reperfusion subgroup using the 24-hour T2w images. Volume measurements were obtained from 14 coronal slices of the registered PET or MRI (from bregma +3.7 mm to bregma −7 mm and 0.775 mm per slice) covering the MCA territory. Both the FDG hyper-uptake and ischemic core volumes were overlaid onto the corresponding registered CBF and ADC maps acquired before PET imaging of the same animal. Subsequently, the ratios of CBF and ADC in both the hyper-uptake and the ischemic core regions to that of the homologous regions in the contralateral hemisphere were obtained for different MCAO groups, respectively. To determine the extent to which the FDG hyper-uptake regions were recruited into the infarction, an overlap volume ratio (OV R) was calculated as the ratio of the overlap volume between V hyper and infarction to V hyper.

Statistical Analysis
Data are presented as mean±SEM unless specified. Differences across groups were compared using 1-way ANOVA for the F hyper and SUV.
ratios. Multiple comparisons between groups were made using the Bonferroni test. Normality test was passed for all data sets. Pearson correlation analysis was performed using GraphPad Prism software. \( P < 0.05 \) was considered significant.

**Results**

The exact time intervals between MCAO and FDG injection for the 5 groups were 30 minutes (35.6±7.1 minutes), 60 minutes (60.8±9.0 minutes), 90 minutes (88.2±4.9 minutes), 120 minutes (124.7±7.3 minutes), and 150 minutes (153.6±5.9 minutes), respectively. Spatiotemporal dynamics of FDG uptake of a representative rat is shown in Figure 1. A brain region with extremely low FDG uptake is clearly visible shortly after FDG injection, most likely reflecting the ischemic core. An elevated FDG uptake region (arrows) becomes visible 1200 s and more apparent 1800 s after FDG injection, which continues to increase throughout the PET imaging session. Regardless of MCAO durations, averaged time uptake curves (Figure 1, bottom) demonstrate that the hyper-uptake region always exhibits a lower FDG uptake initially but continuously increases, surpasses normal tissue at around 10 to 20 minutes, and reaches significant differences at 30 to 40 minutes after injection.

The spatial characteristics of FDG uptake in relation to MCAO duration are shown in Figure 2. Evidently, a larger \( V_{\text{hyper}} \) is observed in animals with a shorter MCAO duration when compared that with a longer MCAO duration (Figure 2A). Specifically, \( F_{\text{hyper}} \) is inversely correlated with MCAO duration (Figure 2B; \( r = -0.62; P < 0.001 \)). Similarly, an inverse relationship also exists between the SUV ratio of the hyper-uptake and normal-uptake regions and the MCAO duration with an \( r \) of −0.46 (Figure 2C; \( P < 0.05 \)). Similar findings as those using PET are also observed using autoradiography (Figure 3), where the \( F_{\text{hyper}} \) is inversely correlated with the MCAO duration (\( r = -0.45; P < 0.05 \)), further confirming the validity of the PET findings.

The extent of ischemic injury in the hyper-FDG uptake regions was evaluated in the context of ADC and CBF reduction (Figure 4). The ischemic core exhibits 71.9±1.0% and 32.5±0.7% reduction of CBF and ADC, respectively (averaged from all animals), suggesting less severe ischemic injury in this region when compared with that in the ischemic core. In addition, the extent to which ADC and CBF is reduced in both the hyper-FDG uptake regions and the ischemic core seems independent of MCAO durations among the 5 groups (\( P > 0.05 \)).

The OVR (Figure 5) measured from animals in the reperfusion subgroup is small (Figure 5B) and statistically not different among groups (16.0±5%, 12.7±2.8%, 10.7±3.1%, and 9.1±2.9% for 30, 60, 90, and 120 minutes groups, respectively; \( P = 0.55 \)), leading to an overall average OVR of 12.1±1.7% from all animals in the reperfusion subgroup. In addition, the 24-hour T2 lesion, the \( F_{\text{hyper}} \) and the overlap volumes (mm\(^3\)) are provided in the Figure II in the online-only Data Supplement. These results demonstrate that the majority of the FDG hyper-uptake tissue was not recruited into 24-hour T2w lesion. Additional boundary analysis to determine the spatial relation between regions of FDG hyper-uptake and 24-hour T2 lesion is provided in Table I in the online-only Data Supplement.

**Discussion**

Spatiotemporal dynamics of FDG uptakes during acute cerebral ischemia were evaluated in a MCAO rat model. In addition to the anticipated reduction of FDG uptake in the ischemic lesions, an elevated FDG uptake is present at the peri-ischemic regions, consistent with that observed using autoradiography. The \( F_{\text{hyper}} \) inversely correlates with the duration of MCAO (Figure 2), suggesting that the spatial extent of the observed elevated FDG uptake may be modulated by the severity of ischemic injury. Temporally, independent of time interval between MCAO and FDG injection, the SUV in the hyper-uptake regions surpasses the normal brain area 10 to 20 minutes after FDG injection and persists throughout the remaining PET imaging session (Figure 1). Mild reductions of CBF and ADC are found in the hyper-uptake regions when compared with that in the contralateral hemisphere (Figure 4). More importantly, the majority of the hyper-FDG uptake volume is not recruited into infarction in the reperfused animals (Figure 5), suggesting that the hyper-FDG uptake regions may represent viable tissue that can be salvaged after reperfusion.

Although an increased accumulation of glucose around the ischemic border was first reported by Ginsberg et al \(^8\) in 1977 using 2-deoxy-d-glucose in a 60-minute MCAO cat model, this finding has not been consistently observed in subsequent reports by other groups.\(^8\)\(^–\)\(^10\) We postulate that the discrepancy of time intervals between MCAO onset and assessments of brain glucose metabolism among the reported studies can be the major factor contributing to the inconsistent findings in the literature. Although our results suggest that the hyper-FDG uptake at the peri-ischemic region is consistently observed between 30 and 150 minutes after MCAO, an inverse relation between \( F_{\text{hyper}} \) and time is observed. This finding suggests that (1) beyond 150 minutes after MCAO, the volume of hyper-FDG uptake is small and (2) imaging spatial resolution can be essential to delineate the presence or the absence of hyper-FDG uptake. Specifically, a dedicated high-resolution animal PET system was not available until recently. Therefore, it is conceivable that hyper-FDG uptake in the peri-ischemic region was present, but the lack of dedicated small animal PET prevented it from conclusively observed.

Several potential mechanisms may account for the observed hyper-uptake FDG in the peri-ischemic zone. Using a permanent MCAO rat model and FDG PET, Schroeter et al \(^7\) found increased glucose metabolism and concurrently increased neuroinflammation (PK-11195 PET) in the peri-ischemic regions 7 days after stroke. Similarly, Fukumoto et al \(^8\) reported neuroinflammation-related hyper-FDG uptake 7 days after photothrombosis ischemia but not before day 7. However, inflammation processes are unlikely to explain findings in our study because PET images were obtained within 30 to 150 minutes after MCAO when significant neuroinflammation is not expected. The expression of glial fibrillary acidic protein only starts to increase its mRNA 6 hours after occlusion in
and transmigration of blood-borne leukocytes is expected in more delayed phases of ischemic injury. Alternatively, our results may suggest an elevated glycolysis at the peri-ischemic regions during acute cerebral ischemia to compensate the loss of ATP. Lactate bioluminescence imaging showed a higher lactate content in regions similar to the elevated FDG uptake area, indicating glucoytic glucose metabolism in this area (Lactate bioluminescence imaging: Figure III in the

Figure 1. Top, Coregistered cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) maps superimposed on the computed tomographic (CT) images, and dynamic positron emission tomographic (PET) images from a rat with [18F]-2-fluoro-2-deoxy-D-glucose (FDG) injected at 58 min after middle cerebral artery occlusion. Temporal and spatial FDG uptake distributions before (0 s) and after FDG injection (30–2400 s) are shown. Diminished FDG uptake in the right side of the brain indicates the ischemic lesion. Hyper-uptake of FDG (arrows) is clearly visible in the peri-ischemic region at 30 and 40 min. Dynamic uptake curves from a cortical region in the contralateral hemisphere (dashed lines) and peri-ischemic region (solid lines) are shown in the bottom. The peri-ischemic region was defined from the last PET frame showing elevated FDG uptake. FDG uptake in the peri-ischemic region at 30 to 40 min after the injection was significantly higher than that in the contralateral hemisphere (**P<0.01; *P<0.05). SUV indicates standardized uptake value.
Whether this increased glucose metabolism represents aerobic or anaerobic glycolysis is still under debate. Several early studies attributed the increased glucose accumulation in the peri-ischemic region to anaerobic glycolysis; however, other studies seem to argue against this conclusion. Wise et al conducted a serial PET study with measurements of both oxygen cerebral metabolic rate (CMR) and cerebral glucose metabolic rate (CMRglu) in patients with stroke 1 to 31 days after stroke. They found a lower oxygen extraction in the ischemic lesion, meaning adequate oxygen supply, and the ratio of oxygen consumption and glucose metabolism was only one third of that in normal brain tissue, indicating aerobic rather anaerobic glycolysis in recovering ischemic tissue. In our study, only mild reduction of CBF was found in the observed hyper-uptake regions, suggesting certain availability of oxygen in those regions, and thus possible aerobic glycolysis, the conversion of glucose to lactate in the presence of oxygen. Even though glycolysis is not energy efficient, it might be effective in protecting cells from depolarizing because of its fast turn-around rate and its role as a membrane energy provider. Therefore, it is possible that cells near the peri-ischemic zone use aerobic glycolysis as the main energy production source to meet major cellular needs.

It is worth noting that elevated FDG uptake in the peri-ischemic region might not be equivalent to increased glucose metabolism because of potential alteration of the lumped constant (LC). The LC is the ratio between FDG and glucose metabolic rates, accounting for differences of transportation, phosphorylation, and volume of distribution between these 2 compounds. Although Hawkins et al reported that there were no or very small LC variations between ischemic and nonischemic tissues, more recent studies seem to suggest a significant increase of the LC in ischemic or hypoperfused tissues, ranging from 20% to 78%. Additional studies will be needed to further determine whether regional LC changes are present in the MCAO model used in our study.

The majority of the peri-ischemic regions exhibiting elevated FDG uptake seems to survive in the reperfused animals (Figure 5), suggesting that acute PET imaging may offer insights into tissue viability during cerebral ischemia. Specifically, a comprehensive study determining the relation among CBF, CMRglu, ATP, and nicotinamide adenine dinucleotide in a gerbil stroke model was conducted by Paschen et al. A 2-fold increase of CMRglu above the normal level was observed for CBF between 20 and 35 mL/min per 100 g but declined sharply for CBF <20 mL/min per 100 g. More importantly, the CBF threshold for CMRglu reduction coincided with the initial ATP reduction as CBF decreased. Paschen et al thus concluded that tissues with an increased CMRglu during acute cerebral ischemia may represent penumbral tissues. Our findings seem highly consistent with that reported by Paschen et al. In addition to the fact that only a small fraction of the hyper-uptake regions was recruited into infarction, the inverse relation between Fhyper and the SUV ratio and the MCAO duration is also consistent with the anticipated reduction of penumbral volume with MCAO duration. Nevertheless, there are several fundamental methodological differences between their and our studies. In particular, FDG uptake might not fully represent CMRglu. Future studies focusing on the potential usage of CMRglu may be warranted.

Notice our experimental protocol, the FDG was given at a fixed time in relation to reperfusion (40 minutes prior) independent of MCAO duration (Figure I in the online-only Data Supplement). This experimental design most likely leads to
the observed relatively stable OVR across groups (Figure 5). A future alternative approach to further confirm our current findings is to administer FDG at a fixed time in relation to the onset of MCAO, that is, at 30 minutes after MCAO, followed by PET imaging, while varying the durations of MCAO before reperfusion across different animals. In so doing, one would expect a positive correlation between OVR and MCAO duration.

Our study also consists of 2 major shortcomings. First, it has been demonstrated that infract volume may continue to

Figure 3. High-resolution autoradiography images from a control and 4 representative middle cerebral artery occlusion (MCAO) rats demonstrate [¹⁸F]-2-fluoro-2-deoxy-o-glucose (FDG) distribution in relation to MCAO duration. Time on the upper left corner of each image is the time interval between MCAO and FDG injection. The hyper-uptake volume fraction reduces as MCAO duration increases. The dotted line is the fitted linear regression line.

Figure 4. A, Registered positron emission tomographic (PET)/computed tomographic, cerebral blood flow (CBF), and apparent diffusion coefficient (ADC) maps from a representative animal with [¹⁸F]-2-fluoro-2-deoxy-o-glucose (FDG) injected at 120 min after middle cerebral artery occlusion (MCAO). Dotted lines indicate the regions with hyper-FDG uptake. The CBF (B) and ADC (C) ratios of the hyper-uptake tissue (□) or the ischemic core (●) to the contralateral region are shown for different MCAO groups, respectively. Boxes indicate 75th and 25th percentile levels.
most of the hyper-uptake regions were not recruited into the infarction. Future work will focus on validating whether the region of hyper-FDG uptake is indeed ischemia penumbra by further extending MCAO >150 minutes and including a permanent occlusion model. Considering the wide availability of FDG PET imaging capability in most of the medical centers, our results underscore the potential clinical usage of FDG PET in the management of patients with acute stroke.

Acknowledgments
The authors thank the UNC BRIC Small Animal Imaging Facility for imaging support and Dr Schroeder from the Department of Radiation Oncology, Duke University Medical Center, for helps on the lactate bioluminescence study.

Sources of Funding
The work was supported by National Institutes of Health (3R01NS054079-04S).

Disclosures
None.

References


Spatiotemporal Uptake Characteristics of $^{18}$F-2-Fluoro-2-Deoxy-d-Glucose in a Rat Middle Cerebral Artery Occlusion Model

Hong Yuan, Jonathan E. Frank, Yonglong Hong, Hongyu An, Cihat Eldeniz, Jingxin Nie, Adomas Bunevicius, Dinggang Shen and Weili Lin

*Stroke*. 2013;44:2292-2299; originally published online June 6, 2013;
doi: 10.1161/STROKEAHA.113.000903

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/8/2292

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2013/06/06/STROKEAHA.113.000903.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Spatiotemporal Uptake Characteristics of [18]F-FDG in a Rat Middle Cerebral Artery Occlusion Model

Hong Yuan, PhD\textsuperscript{1,2}, Jonathan E. Frank, MS\textsuperscript{2}, Yonglong Hong, MD\textsuperscript{2}, Hongyu An, DSc\textsuperscript{1,2}, Cihat Eldeniz, BS\textsuperscript{3}, Jingxin Nie, PhD\textsuperscript{1,2}, Adomas Bunevicius, MD, PhD\textsuperscript{2}, Dinggang Shen, PhD\textsuperscript{1,2}, and Weili Lin, PhD\textsuperscript{1,2, *}

\textsuperscript{1}Department of Radiology, University of North Carolina at Chapel Hill
\textsuperscript{2}Biomedical Research Imaging Center, University of North Carolina at Chapel Hill
\textsuperscript{3}Department of Biomedical Engineering, University of North Carolina at Chapel Hill

Corresponding author (*): 
Weili Lin, Ph.D.
University of North Carolina at Chapel Hill
Biomedical Research Imaging Center, CB\#7513
Chapel Hill, NC 27599
E-mail: Weili_lin@med.unc.edu
Phone: (919) 843-8120, Fax: (919)843-4456

Cover title: FDG PET imaging in acute ischemic stroke
Key words: Cerebral glucose metabolism; FDG PET; Acute ischemic stroke
**Experimental Protocols**

Figure I provides detailed experimental protocols employed for different groups of animals.

**Surgical Procedures**

The intraluminal MCAO method was adopted to induce focal cerebral ischemia. Rats were first anesthetized with isoflurane (5% for induction and 2% for maintenance) mixed with medical air. Body temperature was maintained at 37°C using both water heating pad and heating lamp with feedback controller connected to a rectal thermometer. Occluding suture was made from 4-0 monofilament suture coated with silicon (GE Silicone II) and cured for one week before surgery. The suture was placed into the internal carotid artery (ICA) via the external carotid artery (ECA) stump and advanced for about 21mm until resistance felt. For reperfusion, anesthesia was briefly discontinued when animals were transported from the PET scanner to the surgical table where isoflurane anesthesia was restored. Reperfusion was accomplished by withdrawing suture from ICA to ECA to restore blood flow.

**MR Imaging**
MR image acquisitions included four imaging sequences: T1-weighted imaging, T2-weighted imaging, diffusion weighted imaging, and perfusion weighted imaging sequences. T1-weighted images were acquired using a 2D Fast Low Angle Shot (FLASH) sequence with TE/TR= 2.7/270 msec, 60° flip angle, 256x256 matrix size, FOV of 32x32mm², and 1 mm slice thickness. T2-weighted images were acquired using a Rapid Acquisition with Refocused Echoes (RARE) sequence with TE/TR = 67/4000 msec, 256x256 matrix size, FOV of 32x32mm², and 1 mm slice thickness. Diffusion weighted imaging used an Echo-planar imaging (EPI) sequence with TR/TE=6250/33 msec, b=0, 1000 sec/mm², 128x128 matrix size, FOV=32x32mm², and 1 mm slice thickness. For the dynamic susceptibility contrast imaging (Perfusion weighted imaging), a bolus of gadolinium contrast agent (Omniscan, GE Healthcare Inc.) was injected through the tail vein within 2 second with a dose of 0.2 mmol/kg in 0.2 ml volume. Baseline images were acquired for 6 sec before the injection of the contrast agent and continued for 2 min. The imaging parameters for the EPI sequence were TR/TE=300/12 msec, FOV=32x32mm², 96x96 matrix size, and slice thickness=1mm.

**Autoradiography**

With the exception of the animals in the reperfusion subgroup, animals were euthanized after PET (Fig. I) and brain tissues were collected and snap-frozen in liquid nitrogen. Three coronal sections of 14 micron thickness were cut at 4, 5, and 6 mm below the brain anterior apex. Slices were air dried and exposed to a high resolution/sensitivity phosphor screen overnight. The screen was then scanned, after fully decay of radioactivity, using a digital phosphor imager (Cyclone Plus, PerkinElmer Inc.). Six out of 28 animals had poor cutting qualities (wrinkles and tissue lost etc.) and were excluded from the final autoradiography analyses.

**Volume measurement**

Volumes of regions with elevated FDG uptake, the infarction regions at 24hrs, and finally the overlap regions were measured in reperfusion animals. The absolute volume measurements were shown in Figure II.

![Figure II](image-url)

Figure II. Volumes of regions with hyper-FDG uptake, infarction at 24hrs, and their overlap regions in reperfusion animals.
**Boundary Analysis**

To demonstrate the spatial relation between the region with elevated FDG uptake ($V_{\text{hyper}}$) and the region of infarct tissue ($V_{\text{infarct}}$), measurements of boundary distance between two sets of volumes were conducted. First, the volumes with elevated FDG uptake and infarct tissue were demarcated from the two registered PET and T2w images, respectively, and formed two surfaces (Surface A for regions in PET images and Surface B for regions in T2w images). For each point $x$ on Surface A, corresponding distances to the boundary points on Surface B were mapped out from minimum to maximum, and binned to different levels of minimum distance, denoted by $D_1(\alpha)$, where $\alpha$ represents the percentile of distance distribution. Similarly, for each point $y$ on surface B, identical procedures as that provided above for point $x$ were employed to obtain $D_2(\alpha)$. We then derived the following: $D(\alpha) = (D_1(\alpha) + D_2(\alpha))/2$ as the boundary distance between two VOIs for a given $\alpha$. This boundary analysis was conducted in eight randomly selected animals.

With the boundary distance measure, the distance distribution for the two VOIs was obtained, and different percentiles of the minimum distances for six different levels of $\alpha$ are provided in Table I. A distance of zero indicates overlap between the two VOIs. The average distance at 5% level was $0.018 \pm 0.015$ mm. The results indicated that these two regions were spatially close to each other.

<table>
<thead>
<tr>
<th>A Level (percentile)</th>
<th>Rat-1</th>
<th>Rat-2</th>
<th>Rat-3</th>
<th>Rat-4</th>
<th>Rat-5</th>
<th>Rat-6</th>
<th>Rat-7</th>
<th>Rat-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0025</td>
<td>0.0000</td>
</tr>
<tr>
<td>1</td>
<td>0.0000</td>
<td>0.0013</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0052</td>
<td>0.0000</td>
</tr>
<tr>
<td>5</td>
<td>0.0109</td>
<td>0.0446</td>
<td>0.0068</td>
<td>0.0147</td>
<td>0.0114</td>
<td>0.0122</td>
<td>0.0386</td>
<td>0.0081</td>
</tr>
<tr>
<td>10</td>
<td>0.0513</td>
<td>0.1089</td>
<td>0.0207</td>
<td>0.0473</td>
<td>0.0287</td>
<td>0.0474</td>
<td>0.0863</td>
<td>0.0325</td>
</tr>
<tr>
<td>25</td>
<td>0.1727</td>
<td>0.3453</td>
<td>0.1030</td>
<td>0.1419</td>
<td>0.1246</td>
<td>0.1602</td>
<td>0.2605</td>
<td>0.1416</td>
</tr>
<tr>
<td>50</td>
<td>0.3817</td>
<td>0.7145</td>
<td>0.2253</td>
<td>0.2945</td>
<td>0.2769</td>
<td>0.3930</td>
<td>0.5547</td>
<td>0.3614</td>
</tr>
</tbody>
</table>

**Lactate bioluminescence imaging**

Lactate bioluminescence imaging was carried out in two rats with FDG injected at 60min (rat#1), and 90min (rat#2). Lactate bioluminescence imaging was carried out on the three brain slides neighboring to the autoradiography slides, which included three coronal sections (20-micron thick) of 4, 5, and 6 mm below the brain anterior apex. The sections were heat fixed for 10 min before bioluminescence imaging. The bioluminescence reaction was carried out in a temperature-stabilized reaction chamber at 25°C, which was placed under a fluorescence microscope (Zeiss, Thornwood, NY) with a highly sensitive cooled 16-bit CCD camera in a dark room. The sections were brought into contact with reaction cocktail solutions for lactate, which
were prepared following the published protocol. Basically, the reaction cocktail solution contained the following compounds and substrates: 0.1 M phosphate buffer containing 50mM glutamate (pH=7.0), gelatine (60 g/l), glycerol (30 g/l), polyvinylpyrrolidone (30 g/l), 80 mM NAD+, 0.5 mM dithiothreitol, 8 mM decanal, lactate dehydrogenase and glutamate-pyruvate transaminase (460 U/ml and 70 U/ml, respectively), luciferase (0.013 U/ml) and FMN oxidoreductase (8 U/ml). Imaging was taken for 30 seconds immediately after reaction.

Lactate images and their corresponding autoradiography images are provided in Figure III. In both rats, qualitative visual comparisons showed certain degree of spatial correlation. In rat#2, a higher amount of lactate on the cortex corresponds to a higher uptake of FDG in the similar location. However, some portion of the lactate observed in the striatum might not correspond to the FDG uptake in the striatum region. We attribute this lack of correlation to the fact that lactate is much more diffusive compared to FDG which was trapped within cells once uptaken. Therefore, they may not match exactly, especially at the border zone. Also the lactate slide for Rat#2 was 3 slides away from the autoradiography slide (about 50µm away), which could also lead to discrepancy.

![Figure III](image-url)

Figure III. Lactate bioluminescence and corresponding bright field and autoradiography images. Left column is the images taken from 4x bright field microscope; the middle column is the lactate bioluminescence images with lactate content calibration bar; and the right column is the FDG autoradiography with intensity index. Spatial correlation between lactate content and FDG uptake is present.
Although the actual time for autoradiography and lactate measurement was different, this discrepancy should not affect the results, assuming freezing stopped further metabolism of lactate and FDG. Once tissue slides were fixed in heat, the lactate content should be kept in situ without change overtime, and can be measured at any time after. The main purpose of the lactate imaging at this point was for qualitative assessments. More experiments will be needed to quantitatively correlate lactate formation and glucose utilization.

**T2 lesion growth**

A pilot study was conducted where animals (n=5) were scanned at 24 and 48hrs after reperfusion, and T2 lesion was assessed in the same animals at both time points. Infarction regions were manually delineated on the T2w MR images and measured for both time points. For the 5 transient MCAO rats, two had 100min MCAO duration, and one for each with 70min, 130min, and 160min MCAO durations. The increase of infarct volume from 24hrs to 48hrs was rather small, 5.1±1.8%. Representative images from a rat with 130min MCAO and scanned at 24hrs and 48hrs after reperfusion are shown in Figure IV.

![Figure IV](image)

Figure IV. T2-weighted images taken at 24hrs and 48hrs after reperfusion (animal reperfused at 130min post MCAO). Lesion areas at 24hrs were similar to those at 48hrs on the serial sections.