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Dynamic Glucose Enhanced MRI of the Placenta in a Mouse Model of Intrauterine Inflammation

Dan Wu^{1,2}, Jiadi Xu^{2,3}, Jun Lei⁴, Michael Mclane⁴, Peter C. van Zijl^{2,3}, Irina Burd⁴

¹Key Laboratory for Biomedical Engineering of Ministry of Education, College of Biomedical Engineering & Instrument Science, Zhejiang University, Hangzhou, China

²Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

³F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland, USA

⁴Department of Gynecology and Obstetrics, Integrated Research Center for Fetal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Introduction: We investigated the feasibility of dynamic glucose enhanced (DGE) MRI in accessing placental function in a mouse model of intrauterine inflammatory injury (IUI). DGE uses the glucose chemical exchange saturation transfer (glucoCEST) effect to reflect infused D-glucose.

Methods: IUI was induced in pregnant CD1 mice by intrauterine injection of lipopolysaccharide (LPS) on embryonic day 17. *In vivo* MRI was performed on an 11.7T scanner at 6hrs after injury, and glucoCEST effect was measured using an on- resonance variable delay multi-pulse (onVDMP) technique. onVDMP acquisition was repeated over a period of 25 min, and D-glucose was infused 5 minutes after the start. The time-resolved glucoCEST signals were characterized using the normalized signal difference (S_N) between onVDMP-labeled and nonlabeled images.

Results: S_N in the PBS-exposed placentae (n=6) showed an initial drop between 1–3 minutes after infusion, followed by a positive peak between 5–20 minutes, the time period expected to be associated with the process of glucose uptake and transport. In the LPS-exposed placentae (n=10), the positive peak was reduced or even absent, and the corresponding area-under-the-curve (AUC) was significantly lower than that in the controls. Particularly, the AUC maps suggested prominent group differences in the fetal side of the placenta. We also found that glucose transporter 1 in the LPS-exposed placentae did not respond to maternal glucose challenge.

Correspondence to: Dan Wu, Ph.D., College of Biomedical Engineering & Instrument Science, Zhejiang University, Room 314, Zhou Yiqing Building, No. 38 Zheda Road, Hangzhou, 310027, China, danwu.bme@zju.edu.cn, Phone: (+86) 15825508242.

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Discussion: DGE-MRI is useful for evaluating placental functions related to glucose utilization. The technique uses a non-toxic biodegradable agent (D-glucose) and thus has a potential for rapid translation to human studies of placental disorders.

Introduction

Glucose, as the primary substrate for energy production and a mediator in many biosynthetic processes [1, 2], is crucial to fetal development [3]. The placental capacity to deliver glucose to the fetus is an important indicator of placental health. Placental utilization of glucose has been investigated for many years, for example, malfunction of glucose transporters was found to be a contributor in a range of placental disorders, such as preeclampsia [4, 5] and intrauterine growth restriction (IUGR) [6, 7]. However, such studies were performed using *in vitro* approaches in excised placental tissue slices, isolated placental villi, or perfused placenta [8]. Clinical measures are mostly limited to biochemical sampling of the maternal blood, e.g., hormonal factors in a glucose tolerance test [9]. Direct *in vivo* assessment of glucose utilization in the placenta would be optimal and clinically useful, but such techniques are currently not available.

Magnetic resonance imaging (MRI) has become an increasingly important imaging tool to evaluate placental anatomy and function, in addition to ultrasound [10], especially in complex cases. For example, dynamic contrast enhanced (DCE) MRI [11, 12], arterial spin labeling (ASL) [13, 14], and intravoxel-incoherent motion (IVIM) MRI [15–18] have been adopted to measure placental perfusion; and T2 and T2* mapping [19–21], and BOLD MRI [22, 23] have shown sensitivity to placental oxygenation. Traditionally, the detection of glucose concentration and metabolic rate is performed by MR spectroscopy [24–30], which is a slow and low-resolution technique. Recently, it was demonstrated that glucose in brain and body organs can be monitored with Chemical Exchange Saturation Transfer (CEST) MRI [31, 32], as well as relaxometry-based MRI contrasts, such as T1ρ [33, 34] and T2-weighted MRI [35]. Briefly, CEST MRI utilizes radiofrequency (RF) irradiation to selectively saturate exchangeable protons in the low-concentration solutes typically only measurable with MRS. This saturation is subsequently transferred to water protons through proton exchange, replaced by non-saturated protons and again saturated. Because of the rapid exchange of these protons, the process is repeated many times within a single MRI detection, resulting in the building up of a reduction in water signal intensity. Thus, CEST MRI can amplify the signal from low-concentration metabolites to be detected via the abundant bulk water protons, in a manner proportional to the concentration of these agents. By tuning the CEST frequency to the hydroxyl protons on D-glucose, called glucoCEST MRI, we and others have shown the detection of the presence of D-glucose or its derivatives using the MRI signal *in vivo* [36–38]. The measurement of time-resolved changes of glucoCEST MRI signal after a bolus intravenous infusion of glucose, referred to as Dynamic Glucose Enhanced (DGE) MRI, reports the changes in glucose concentration in biological tissues and thus contains information on glucose delivery, transport and metabolic kinetics. DGE MRI using continuous-wave (CW) or pulsed CEST has been used to image the glucose uptake in tumors and other tissues both in animals [36–41] and humans [42]. Feasibility of DGE in the placenta is not yet known.

An On-Resonance Variable Delay Multiple Pulse (onVDMP) technique [43] was recently developed to further enhance the sensitivity of glucoCEST. In contrast to CW-CEST that targets a single off-resonance frequency, the onVDMP method efficiently labels a range of fast-exchanging protons, covering multiple resonance frequencies of the five hydroxyl protons with exchange rates between 3–6kHz. It has been demonstrated that DGE signal changes in the brain detected by onVDMP were about two times higher than those measured by CW-CEST [44]. In this study, we investigated the feasibility of onVDMP-based DGE to monitor glucose kinetics in normal and injured placentae. The study was performed in an established mouse model of intrauterine inflammation (IUI) [45, 46], in which placental and fetal injury was induced by excessive maternal production of inflammatory cytokines due to lipopolysaccharide (LPS) administration. This model mimics the most common clinical scenario associated with preterm birth. In this model, we have previously identified acute maternal vascular malperfusion (MVM) with IVIM and immunohistochemistry measurements in the placenta [47], and thus, the model serves as an ideal test bed for potential clinical translation of DGE MRI to assess placental function. In this study, we performed DGE MRI to examine the time-resolved placental response to a glucose challenge in LPS-exposed placentae, in comparison with the controls.

Methods

Materials:

All experimental procedures were approved by the Animal Use and Care Committee at the Johns Hopkins University School of Medicine. Timed pregnant CD-1 outbred mice (Charles River Laboratories, Wilmington, MA) were used in this study. Intrauterine injury was performed on embryonic day 17 (E17, full gestation is 19 days) according to an established animal protocol [45, 46]. Briefly, mice were placed under isoflurane anesthesia and a mini-laparotomy was performed in the lower abdomen. LPS (Sigma, St. Louis, MO, Lot. 102M4017V) of 25 μ g in a 100 μ L phosphate-buffered solution (PBS) was injected between the first two gestational sacs in the lower right uterine horn. Control mice underwent the same procedure but were injected with 100 μ L PBS. MRI data from six PBS-exposed placentae from three pregnant dams (two placentae per dam) and ten LPS-exposed placentae from four dams (2–3 placentae per dam) were used in this study.

MR data Acquisition:

In vivo MRI scans were performed on an 11.7T horizontal bore Bruker scanner (Biospec, MA), with a 72mm quadrature transmitter coil and an eight-channel phased-array mouse body receiver coil. The onVDMP labeling module [44] consisted of a train of 8 binomial RF pulses, each binomial pair made of two block pulses of opposite phase with a width of 1.5 ms (3 ms for the pair) and a peak power of 15.6 μ T, corresponding to a flip angle of 360 degrees for each block pulse. The pulse train was played out with the offset on the water resonance and a 10 ms mixing time between pulse pairs was applied to remove unwanted traverse magnetization. After the labeling module, images were acquired using a Rapid Acquisition with Relaxation Enhancement with Short TE (RAREst) sequence with echo time (TE) / repetition time (TR) = 3/2500 ms, 10 echo trains, partial Fourier acceleration factor of 1.4, field of view of 32×32 mm², matrix size of 64×64 , and a single 2 mm slice.

1–3 placentae were captured in the single slice. Each onVDMP image was acquired in 10 seconds with the labeling pulses on (S1) or off, with the latter for the reference S0 image.

During a DGE experiment, five S1 images and one S0 image were acquired per 1 min acquisition block, and such blocks were repeated for a total duration of 25 min (Figure 1A). At 5 min after the start of acquisition, a bolus of 0.15 mL 50% glucose (0.5 g/mL, clinical-grade Dextrose, Hospira) was manually infused through the tail vein with a syringe to induce DGE contrast.

Data Analysis:

S1 images were first averaged over the 5 min baseline to obtain $\overline{S1}_{base}$. The change of the S1 signals was calculated as $\Delta S = \overline{S1}_{base} - S1$, which was then normalized to the S0 signals in the corresponding acquisition block as follows: $\Delta S_N = \frac{\overline{S1}_{base} - S1}{S0}$. $\overline{S1}_{base}$ was kept constant as a baseline reference, S1 was obtained every 10 sec time point, and S0 was updated every minute in the corresponding acquisition block to account for possible fluctuation in the water proton density after infusion. This definition of ΔS_N follows our previous publications on DGE MRI [39, 42, 44]. ΔS_N values acquired between 10–25 min after the start of the protocol (or 5–25 min post infusion) were summed as the area under the curve (AUC15min), as a surrogate marker for glucose uptake and transport in the placenta.

The whole placental region of interest (ROI), as well as the fetal and maternal parts of the placenta were manually identified on the first S1 image in ROEditor (www.mristudio.org), with reference to the co-registered T2-weighted anatomical image (Figure 1B). Time-resolved ΔS_N signals were first calculated in a voxelwise manner, and then averaged over the placenta ROI for statistical analysis in Prism Graphpad (www.graphpad.com/scientific-software/prism/). Whole-placenta averaged AUC15min were compared between the LPS and PBS groups at every 10 sec time point between 10–25 min from the start, using a two-tailed t-test with unequal variance, followed by false-discovery rate (FDR) correction. Whole-placenta averaged AUC15min values were compared between the LPS- and PBS-exposed placentae using two-tailed t-test with Welch's correction. Difference in AUC15min between the fetal and maternal ROIs and the effect of LPS-exposure were analyzed together using a two-way ANOVA.

Quantitative polymerase chain reaction (qPCR):

A separate cohort of mice underwent IUI followed by glucose infusion. After surgery, intraperitoneal (IP) injection of 200 mL of sterile 10% dextrose (Hospira, Inc, Lake Forest, Illinois) or PBS (vehicle) was given to the dams at 1, 2, 3, 4, and 5 hours after surgery. Dams were divided by three groups, injury followed by glucose infusion (LPS+glucose, n=5 dams), sham injury followed by glucose infusion (PBS+glucose, n=3 dams, positive control) or equivalent amount of PBS infusion (PBS+PBS, n=3 dams, naïve control).

RNA was prepared from the fresh frozen placentae harvested 6 hours after the surgery using RNEasy Mini Kit (Qiagen, Valencia, CA), and cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad). The following probes were obtained from Integrated DNA

Technologies (Coralville, IA): GLUT1, HS.PT. 58.25872862 and β -actin, Mm.PT. 58.33540333. 18S ribosomal RNA endogenous control primers were obtained from Life Technologies (Grand Island, NY). qPCR was performed to examine GLUT₁ expression at mRNA level with Universal Master Mix II (Life technologies) on a CFX384 Real-Time PCR Detection system (Bio-Rad). mRNA levels in the PBS+PBS, PBS+D- glucose, and LPS+D- glucose groups were compared with a one-way ANOVA, followed by pairwise post-hoc t-test with Bonferroni correction.

Results:

The glucoCEST effect in the placenta is shown in the S1 and S0 images in Figure 1C. The dynamic changes of S_N in each individual placenta over a period of 25 min are shown in Figure 2. In the PBS-exposed control group (Figure 2A), it was observed that S_N dropped briefly between 1–3 min after the bolus of glucose infusion, attributed to the increase of bulk water through perfusion. S_N subsequently increased above the baseline for the rest of experiment, likely due to the enhanced glucoCEST effect with glucose uptake. In contrast, the signal patterns in the LPS-exposed placentae (Figure 2B) were quite different. The initial drop of S_N was absent in the majority of the injured placentae, whereas three of the 10 placentae showed a hyperintense response. After 5 min post infusion, S_N in the injured placentae was less than 10% effect or even negative. Despite the large standard deviation, comparison of S_N at each time point between the PBS and LPS groups revealed significant differences at 11.6–17.8 min from the start, indicated by the green bar in Figure. 3C ($p < 0.05$, t-test followed by FDR correction).

Figure 3 shows voxelwise maps of S_N at the end of every 1-minute block during the DGE experiment, to illustrate the spatial distribution of S_N in the placenta. The increase of S_N after 10 min seemed to be more prominent in the fetal side of the placenta and fetal-maternal interface, compared to that in the maternal side. The same spatial variation was seen in the AUC_{15min} maps (Figure 3C). In the LPS-exposed placenta, much lower signal changes were observed in placenta across the entire experiment (Figures 3B), and the corresponding AUC map also revealed marked difference compared to the PBS-exposed one (Fig. 3C). Statistical analysis showed the whole-placenta averaged AUC_{15min} was significantly lower in the LPS group compared to controls (Figure 3D, $p < 0.01$ by t -test). When fetal and maternal AUC_{15min} were separately evaluated using two-way ANOVA, both the effect of fetal / maternal variation and the effect of LPS-induced placental injury were both found to be significant. Post- hoc t -test indicated significant difference between PBS and LPS groups in the fetal part ($p < 0.01$), while the maternal difference was marginally non-significant ($p = 0.051$) (Figure 3E).

At the mRNA level, GLUT1 was significantly increased in positive control placentae with glucose infusion (PBS+glucose), compared with naïve control without glucose infusion (PBS+PBS) (One-way ANOVA, $p < 0.05$). Glucose infusion in the LPS- exposed dams (LPS +glucose) did not initiate the reaction of GLUT1 as the positive control did (Figure 4, One-way ANOVA, $p < 0.05$), indicating that the placental capability in response to maternal glucose challenge was impaired.

Discussion:

We reported DGE MRI of normal and injured placentae using the glucoCEST technique. The dynamics of glucoCEST signal change after D-glucose infusion presumably reflect glucose uptake and transfer in the placenta. glucoCEST effects has been investigated previously in the brain, where increased glucoCEST contrast in tumors was found to relate to increased vascularity and blood-brain barrier break down [39, 42]. In our *in utero* study, we found significantly reduced contrast in the injured placentae at 6hrs after inflammatory injury in a mouse model of IUI, which is known to develop acute placental injury [47]. The lack of glucose transport capacity in the placenta may adversely affect fetal development, and our group has previously reported that maternal glucose supplementation after IUI could alleviate the fetal brain injury [49]. Therefore, *in vivo* evaluation of placental glucose utilization is important in prenatal monitoring.

Several interesting phenomena were observed in the glucoCEST signal kinetics during the DGE experiment. In the control group, a transient negative peak in S_N appeared upon glucose infusion, followed by a large positive peak that sustained after 5 min post infusion. Since the placenta is the interface between maternal and fetal circulation, interpretation of the peaks is not straightforward. While the initial dip might be related to bulk water increase with blood perfusion, the positive peak, which indicates increased glucoCEST effect, may be associated with glucose uptake by the placental tissue as well as the fetal-maternal exchange [50]. glucoCEST effect in the fetal side of the placenta could be a result of glucose transport through the transporters [4, 6]. In fact, we found the fetal part of LPS-exposed placentas exhibited a more significant reduction of the response, compared to the maternal side, which we interpret in terms of a deficiency in glucose transporter, supported by the impaired GLUT1 transporter regulation. Note that the GLUT1 expression was examined at mRNA level in this study, and the half-life of GLUT1 mRNA varies under different conditions, ranging from 30 min to hours [51, 52]. In order to avoid the possible influence of the half-life at the time of examination, we chose to perform IP injection repeatedly after surgery until the time of harvesting; whereas a dose of IV injection was used for the DGE experiment to induce fast and detectable changes in glucoCEST within a short time (25min).

Very recently, glucoCEST MRI was attempted in human placenta, by Luo et al. [53] and presented in the Placenta MRI workshop (www.ismrm.org/workshops/2018/Placenta). The study used conventional CEST acquisition with z-spectrum asymmetry analysis, which is hampered by difficulties in B_0 correction and low temporal resolution. Here, we instead adapted the onVDMP technique to bypass the aforementioned difficulties. In addition to not requiring the acquisition of a z-spectrum, the onVDMP method has advantages over single-frequency CEST in that it targets at multiple fast-exchange hydroxyl protons on D-glucose that inherently improves sensitivity [44]. Moreover, onVDMP uses a much shorter total labeling time than CW-CEST, and thus reduces the specific absorption rate (SAR), which is favorable in fetal-maternal applications when MRI safety is particularly important. Regarding the translational potential of onVDMP technique, the field strength difference between preclinical and clinical scanners imposes a significant impact both on the MRI acquisition and on the CEST labeling. Low field strength is challenging for conventional CEST labeling. At lower field, the peaks of the hydroxyl protons are partially coalesced with

the water peak, and therefore, the conventional CEST method will induce direct water saturation, which partially obscures the CEST contrast. The onVDMP-CEST method allows much higher labeling power by utilizing a train of short pulses, which leads to improved CEST signal for hydroxyl protons compared to conventional CEST method. In addition, lower field is favorable for placenta imaging compared to high field, due to the shortened T2 relaxation time of venous and arterial blood and increased field inhomogeneity at high field [54]. On clinical scanners, the lower MRI signals of the placenta due to the low field strength can be compensated by the much longer T2 relaxation time, improved field homogeneity, and the use of larger voxel size (3×3mm for human placenta [44] versus 0.5×0.5mm for mice). Considering the advantages of both MRI acquisition and onVDMP labeling at lower field, we expect the new method will offer a powerful tool for monitoring glucose uptake in human placenta studies.

Another important factor to be considered in CEST MRI is that its contrast is sensitive to pH, which might also be altered in the placenta after inflammatory injury. In order to investigate this, we examined the baseline CEST effect before glucose infusion. A significant difference in $S1_{\text{base}}/S0$ was found between the control (50%±5%) and injury group (41%±6%). This difference in baseline could be a mixed effect of both pH change and baseline glucose concentration difference. The changes in the normalized glucoCEST signals during the DGE experiment, on the other hand, are related to glucose uptake kinetics, with a reasonable assumption that the pH value did not change during the DGE experiment.

There were limitations for the current study. For example, the fetal and maternal parts cannot be easily separated based on onVDMP or T2-weighted images. Therefore, we presented results from both the whole-placenta and segmented sub-regions (maternal and fetal). Besides the current focus on the placenta, it would also be interesting to look into the glucoCEST effect in fetal organs, such as the fetal kidney, liver, and brain. We only collected a single slice in the current experiment, which did not consistently capture other organs. Nevertheless, our study shows the feasibility of DGE MRI in the placenta and its utility in detecting placental injury in the context of intrauterine inflammation. Given the accessibility of glucose tolerance tests used in maternal examinations and the fact that DGE studies are possible in humans in a manner similar to such tolerance tests [22], this preclinical study sets a first example for a potentially clinically useful and safe functional assessment of the placenta for a wide spectrum of pregnancy disorders, including preterm labor and preterm premature rupture of membrane.

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Feasibility of glucoCEST to access placental function was examined in a mouse model.
glucoCEST effect was obtained with an on-resonance variable delay multi-pulse method.
Time-resolved glucoCEST effect increased after glucose infusion in normal placenta.
Placental glucoCEST effect reduced acutely after intrauterine inflammation.
Glucose transporter 1 did not respond to glucose infusion in the injured placentae.

Figure 1:

(A) The experimental diagram: every acquisition block (1min) consists of five S1 images and one S0 image, which was repeated for 25 mins; and the D-glucose was infused at 5 min via the tail vein. (B) T2-weighted abdominal MRI of a coronal section of a pregnant mouse on embryonic day 17, with the placentae outlined in yellow. The inset shows the manually defined maternal and fetal parts of one placenta. (C) Comparison of an onVDMP labeled image (S1) in comparison with a nonlabeled reference image (S0).

Figure 2: Time-resolved dynamics of the normalized signal change (ΔS_N , %) over the 25min DGE experiment in all individual placentae in (A) the PBS-exposed control group (n=6 from 3 dams), and (B) the LPS-exposed group (n=10 from 4 dams). (C) Averages of the normalized signals in the PBS (blue) and LPS-exposed (red) groups. Shaded regions present the standard deviation of the curves. AUC_{15min} was calculated as the area under the curve between 10–15 min from the start (time points between the black dashed lines). Green dashed lines indicate the time points, at which, ΔS_N was significantly lower in the LPS group compared to the PBS group ($p < 0.05$, t-test followed by FDR correction).

Figure 3:

Voxelwise maps of the normalized signal change (ΔS_N , %) in a placenta acquired at every minute during the DGE experiment, in the PBS-exposed group (A, corresponding to placenta 5 in Figure 2A) or LPS-exposed group (B, corresponding to placenta 4 in Figure 2B). (C) Voxelwise AUC_{15min} maps of a PBS-exposed placenta and a LPS-exposed placenta, corresponding to the ones shown in (A) and (B), respectively. (D) Group analysis showing significantly lower AUC_{15min} in the LPS-exposed placentae compared to the PBS placentae ($p < 0.01$). (E) Further analysis of AUC_{15min} in the maternal and fetal parts of the placenta showed significant group difference in the fetal part ($p < 0.01$, two-way ANOVA followed by post-hoc t-test with Bonferroni correction).

Figure 4:
At the mRNA level GLUT1 was significantly increased in the PBS+glucose group compared to PBS+PBS and LPS+glucose groups ($p < 0.05$, one-way ANOVA followed by pairwise post-hoc t-test).