Evaluation the effect of acute hyperglycemia on cerebral tissue properties with diffuse optical imaging systems

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ABSTRACT
In this work, we demonstrate the application of two optical imaging systems namely, dual-wavelength laser speckle and integrated spatial frequency domain imaging systems to image the changes in mouse brain tissue properties following acute hyperglycemia. We assume that hyperglycemia alters brain function which in turn can be monitored using these two optical modalities. Hyperglycemia was induced by intraperitoneal injection of an anesthetic ketamine and xylazine combination which is found to increase blood glucose more than threefold relative to normoglycemia. A total of ten mice were used, randomized into two groups of normoglycemia (n = 4) and hyperglycemia (n = 6). Experimental results demonstrated reductions in cerebral blood flow, tissue oxygen saturation, and cerebral metabolic rate of oxygen following acute hyperglycemia. In addition, differences in both cerebral tissue optical properties (absorption and scattering) with increasing glucose level were observed. Overall, experimental result demonstrates the capability of both systems to provide and map various brain tissue metrics following hyperglycemia.

Keywords: Hyperglycemia, Structured illumination, Laser speckle imaging, Optical properties, Cerebral hemodynamics and metabolism.

1. INTRODUCTION
Hyperglycemia is characterized by an abnormally high concentration of glucose measured in circulating blood. It occurs when the body is unable to maintain consistent, normal blood glucose (BG) level as a result of either low insulin production by the pancreas or peripheral insulin resistance.1 Acute hyperglycemia is result of a high-carbohydrate meal, a missed dose of medicine, stress, or illness.2 Because the brain is dependent on a continuous supply of glucose as its principal source of energy and metabolism, changes in BG concentration directly affect cerebral function.3 Conventional neuroimaging techniques such as positron emission tomography (PET) with glucose tracers and magnetic resonance imaging (MRI) have been used to monitor variations in cerebral blood flow (CBF) during hyperglycemia. These techniques have revolutionized medical research, yet their routine use remains technically complicated and expensive. In contrast, optical tissue imaging techniques circumvent several limitations of conventional neuroimaging and offer unique advantages including their low-cost, ease of operation, portability, and freedom from the adverse effects of ionizing radiation.4 The work presented here utilizes the dual-wavelength laser speckle imaging (DW-LSI) and integrated spatial frequency domain imaging (iSFDI) systems to characterize brain hemodynamic and metabolic changes during hyperglycemia in an intact mouse head. Both setups are relatively inexpensive, simple to operate, wide-field and noncontact optical imaging platforms which capable of detecting and mapping: optical properties, hemodynamics and metabolism, therefore providing an effective platforms for the study of tissue function.5,6 iSFDI is an integrated system that combine SFDI and LSI as a dual-modality imaging platform.7 Hyperglycemia was induced in mice by intraperitoneal injection of a commonly used anesthetic drug combination of ketamine and xylazine8 which increases BG level to approximately 460 mg/dL within 60 minutes. During experiments, the head of the anesthetized mouse was irradiated and the captured images were analyzed in each system to examine the spatiotemporal characteristics of volume-averaged hemodynamic and optical parameters, respectively. Experimental results demonstrated reductions in cerebral blood flow, tissue oxygen saturation, metabolic rate of oxygen following acute hyperglycemia. Additionally, a difference in optical properties (absorption and scattering) was observed which can be attributed to variations in the biochemical composition and cell structure of brain tissue.
2. MATERIALS AND METHODS

2.1 Animal model

Adult male mice (C57/BL6, ~12 weeks old, ~30 g) randomized into two groups of normoglycemia (control, n=4) and hyperglycemia (n=6). Acute hyperglycaemia was induced by IP injection of anesthetic drug of mixture of ketamine and xylazine (KX combo). Ketamine (100 mg/ml) and xylazine (20 mg/ml) were mixed together (1:4 ratio) to a final volume of 100 μl which was then administrated. We found that this drug combination increases blood glucose (BG) level approximately from 150.1 ± 5.87 to 350 ± 15.6 mg/dL at 18 ± 1.74 min post injections. BG continued to increase throughout the test period, reaching an average of 463 ± 20.34 mg/dL. BG measurements were taken, in conjunction with imaging, from the mouse tail vein by finger-stick glucometer (Accu-Chek, Roche, Germany) every 10 min over the experiment period. In contrast, the control group (normoglycemia) was anesthetized by injection of sodium thiopental (90 μl, 25 mg/ml). The average BG level following this anesthesia was 110.86 ± 3.64 mg/dL. After anesthesia, mice were immobilized in a homemade head mount and scalp hair was removed using depilatory cream. The depth of both anesthetics was sufficient (usually after 15 min) to eliminate foot withdrawal to pinch, corneal reflex, and vibrissal movements. Animals remained anesthetized throughout the experiment and were euthanized upon completion.

2.2 Instrumentations

**DW-LSI:** The experimental setup of DW-LSI is displayed in Fig. 1(a). Two diode laser beams (λ₁=532nm and λ₂=660nm) are switched by an optical chopper such that only one wavelength was transmitted at a given time. The diffuse reflected light at each wavelength is recorded by a CCD camera (GuppyPRO F-031B) equipped with a macro zoom lens (Computar, MLH10X, F5.6-32). The captured images were stored automatically and sequentially for future image processing. Prior to data analysis, the collected diffuse images were first normalized to overcome the nonlinearity of the camera quantum efficiency at the above wavelengths, after which the images were digitally filtered (special, Matlab) to eliminate high frequency noise originating from the camera itself during recording.

**iSF DI:** Figure 1(b) depicts the optical configuration of iSF DI. The system integrates two channels: spatially modulated white illumination patterns emanating from a digital light projector device and a laser diode with a wavelength of 650 nm. These channels are projected alternately such that only one source illuminates the tissue at a given time, allowing no illumination crosstalk between the two instruments. The beam from neither the projector nor the laser reaches the tissue and the diffuse reflected light passes through a color wheel (Thorlabs, FW102C) and is directed onto the tissue field by the projector (PLUS, U5-112) at high and low spatial frequency of 0.29 and 0 mm⁻¹, respectively. When the four wavelengths are complete, the output of the projector is blocked and the wheel moves to its fifth position (open) and the laser beam illuminates the tissue. The diffuse backscattered laser light (speckles) is collected by the same CCD camera. and recorded images are stored automatically and sequentially for future image processing.

Both setups are controlled by Matlab software (MathWorks, MA), and imaging acquisition, synchronization, and data processing are implemented offline using Matlab scripts developed in-house.

2.3 Evaluation of cerebral tissue parameters

After data collection and preprocessing with the DW-LSI, a sliding-window algorithm (7×7 pixel dimension) centered at every pixel of the raw image was applied to convert each 660 nm raw speckle image to a speckle contrast image (Kᵢ) using the following equation,

\[ Kᵢ = \frac{\sigmaᵢ}{<I>} \times \frac{1}{\sqrt{<V>}} \]  \hspace{1cm} (1)

where \( \sigmaᵢ \) and \( <I> \) are image standard deviation and its mean intensity, respectively, and \( <V> \) is the mean cerebral blood flow (CBF) velocity. During post-processing, speckle contrast images were averaged together and then converted to a single flow image (V=Kᵢ⁻²). Addition to flow measurements, estimation of cerebral hemoglobin oxygenation, \( SO₂ \), was approximated according to the intensity of reflected signal at each employed wavelength, \( Rd(λ₁) \) and \( Rd(λ₂) \), calculated using:
where \( \varepsilon_{HbO2} \) and \( \varepsilon_{Hbr} \) are the known molar extinction coefficients of oxyhemoglobin (HbO2) and deoxyhemoglobin (Hbr), respectively, with the constant wavelength values of \( \lambda_1 = 532 \) nm and \( \lambda_2 = 660 \) nm. In parallel with flow and saturation measurements, assessment of cerebral metabolic rate of oxygen (CMRO2) can be obtained from the information regarding Hbr and CBF. At 660nm, the overwhelming majority of tissue absorption is attributed to Hbr. Thus, the ability to simultaneously monitor the spatiotemporal changes in diffuse reflected light at 660nm (Hbr sensitivity) and blood flow at fine spatial location allows for the estimation of CMRO2.

The following formulas was used to compute CMRO2,\(^{11}\)

\[
CMRO_2 \propto \begin{cases} 
\alpha \times CBF \times Hbr & : \text{normoglycemia} \\
4 \times d(Hbr)/dt & : \text{hyperglycemia}
\end{cases}
\]

where \( \alpha \) is a constant that relates CBF to absolute blood flow and was estimated during calibration with the control group.

![Schematic diagram of the DW-LSI (a) and iSFDI (b) systems.](image)

Since in iSFDI system the diffuse reflectance images of low and high spatial frequency at each wavelength are known, the diffusion equation in the spatial frequency domain\(^6\) was numerically solved in a pixel-by-pixel fashion; two equations at two-different frequencies with two unknown parameters (absorption and scattering) are solved per wavelength. By scanning the entire reflectance images at each wavelength pixel-by-pixel, two-dimensional, spatially resolved mapping of the tissue’s absorption and reduced scattering properties are concurrently constructed. The mean and standard error of each image map is then obtained providing a single mean value within the entire region per wavelength. Combining the obtained absorption value with the wavelength-dependent molar extinction coefficients, \( \varepsilon(\lambda) \), molar concentrations and maps of dominant tissue chemical constituents, \( C \), can be recovered by applying the Beer-Lambert law,\(^12\)

\[
\bar{c} = [\varepsilon(\lambda)]^{-1} \tilde{\mu}_s(\lambda)
\]

Similarly, once the reduced scattering coefficient at different wavelengths is known, \( \mu_s'(\lambda_0) \), the NIR scattering spectra can be generated using a power-law model,\(^13\)

\[
\mu_s'(\lambda) = A\lambda^{-b}
\]

The scattering power, \( b \), has been shown to be related to mean size-scale of the tissue features (membranes, nucleus, lysosomes, mitochondria, etc.) while the scattering amplitude (A) correlates with the number, density and distribution of these features, their radius, and the medium’s index of refraction.\(^14\) Hence, structural changes of tissue on a macroscopic level can be studied via these two parameters.
3. RESULTS

A comparison of CBF between control and hyperglycemic mice over time is presented in Figs. 2(a) and 2(b), respectively. Each data point on the graph represents an averaging of thirty images and indicates the mean flow value while the error bar is the variation in the calculated mean flow over the ROI. Presented on each graph, are the average BG level and CBF value. As shown, increase in glucose concentration causes decreased blood flow. Linear relationship plot between the two is present in Fig. 2(c) with logarithmic scale on both axes; the good correlation highlights the coupling between CBF and BG. Two representative maps of CBF at normoglycemia and hyperglycemia are shown in 2(d). Although the underlying mechanism of this change is not completely understood, it might be attributed to the direct effects of hyperglycemia on myogenic autoregulation response to the deleterious effects of hyperglycemia-associated oxidative stress or to other, undefined yet mechanisms, leading to increased contractility and higher vascular tone.15,16 The reduced CBF we observed is consistent with previously published acute hyperglycemia data.17-19

![Figure 2](image)

Figure 2. Time series of CBF for normoglycemia (a) and hyperglycemia (b). As depicted, in contrast to normoglycemia where no changes in the flow is presented, gradual decrease following hyperglycemia is clearly seen. (c) CBF vs BG; flow is varied inversely with glucose concentration. The line is the best-fit linear regression of the data (diamond) with high negative correlation \( r = 0.89 \) between the two parameters. (d) Representative false color maps of CBF at normoglycemia (top) and hyperglycemia (bottom). The color bar above indicates the corresponded value of each pixel in the map, such that higher index values correspond to brighter pixels.

Maps of \( \text{SO}_2 \) and \( \text{CMRO}_2 \) from normoglycemia to hyperglycemia are presented in Fig. 3, respectively. These maps together with that of CBF (Fig. 2d) demonstrate DW-LSI system to provide profound insight regarding spatial variation in the hemodynamics of cerebral tissue following increase blood glucose. In general, under decreased CBF brain cells undergo a series of pathophysiological changes further complicated by disrupted oxygen balance and consumption (Fig. 3) leading to disrupt extracellular ion concentrations, increased lactate levels, decreased extracellular pH, and more.20

![Figure 3](image)

Figure 3. Two-dimensional false color maps of \( \text{SO}_2 \) (left) and \( \text{CMRO}_2 \) (right) demonstrate changes in both metrics from normoglycemia to hyperglycemia. Presented on each map is the BG level (142 to 481 mg/dl). These changes suggest possible metabolic suppression, mitochondrial dysfunction, hypothalamus signaling variations, and potential disruptions in neuronal function. The color bar above the maps indicates the range of each cerebral metric from low to high.
The resulted absorption and scattering spectra in normoglycemic and hyperglycemic conditions obtained with the iSFDI system are shown in Figs. 4(a) and 4(b), respectively. Clear differences in both optical properties with increasing glucose level is shown, which can be attributed to variations in the biochemical composition and cell structure of the tissue. The progression from normoglycemia to hyperglycemic in scattering (4b) is marked by more than a 24% decrease in both amplitude and scattering power, reflecting the morphological and functional changes occurring in the brain. Maps of the recovered absorption and scattering coefficients at a representative wavelength of 650 nm were built and can be seen in Fig. 4(c), demonstrating the spatial variation of both parameters across the observed area. Differences in color across the map highlight, indirectly, the changes in tissue hemodynamic parameters and structure following glucose elevation. It should be said that, the behavior of blood flow and saturation with the iSFDI system (not shown) were similar to that observed above in Figs. 2 and 3 by the DW-LSI system.

4. SUMMARY

This work demonstrates the use of DW-LSI and iSFDI to evaluate the effect of acute hyperglycemia in mouse brain. Hyperglycemia was induced by injection of an anesthetic ketamine and xylazine combination which is found to increase BG more than threefold relative to baseline. Acute hyperglycemia was found to cause changes in hemodynamic and metabolic as well as in tissue cerebral optical properties. Since the brain is dependent on a continuous supply of glucose as its principal source of energy and metabolism, changes in BG concentration rapidly affect cerebral function. The necessity to analyze the hemodynamics and functionality of the brain following acute hyperglycemia in simple manner was the motivation of this work. Taking our results together, we postulate that following acute hyperglycemia, brain cells undergo a series of pathophysiological and morphological changes further complicated by the decreased oxygen supply reflected by lowered oxygen saturation and decreased scattering properties. This leads to an overall decrease in oxygen consumption and, ultimately, global energy failure. Overall, this work reveals that acute hyperglycemia (1) modifies the hemodynamic response, (2) induces significant changes in blood flow, (3) causes variation in cerebral tissue optical properties, and (4) effect intracranial volume compartments presumably leads to increase in internal brain pressure.21,22

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