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**MAJOR PAPER**

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## Oxygen and Glucose as Stimulation Agents for BOLD Functional MR Imaging of Rabbit Liver: A Feasibility Study

Fang Yuan, Bin Song\*, Zixing Huang, Xijiao Liu,  
and Chunchao Xia

**Purpose:** To assess the feasibility of using oxygen and glucose as stimulating agents in blood-oxygen-level-dependent (BOLD) Functional Magnetic Resonance Imaging (fMRI) of rabbit liver and analyze the impacts by blood flow.

**Methods:** Pure oxygen inhalation, intravenous injection and oral administration of glucose were given to 11 New Zealand white rabbits to compare the differences of liver  $T_2^*$ , aortic flow (AF), portal vein flow (PVF), aortic area (AA) and portal vein area (PVA) before and at 5 min, 10 min, 20 min, 30 min after administrations. AF and PVF were acquired by two dimensional (2D) Phase Contrast MR (2D-PCMR). The impacts of AF and PVF upon BOLD fMRI were analyzed.

**Results:** AF and PVF declined at 5 min after oxygen inhalation and were significantly different from baseline, then reverted to baseline. No significant difference was observed in liver  $T_2^*$ , AA and PVA before and after oxygen inhalation. AF, PVF, AA and PVA showed no significant difference before and after glucose intravenous injection, while liver  $T_2^*$  increased gradually with significant difference. AF and liver  $T_2^*$  were significantly different before and after glucose oral administration and increased gradually, AA was significantly different before and after glucose administration at 10 min and 20 min. PVF and PVA started to be different from baseline at 10 min. Greatest variation of  $T_2^*$  (19.6%) was induced by glucose oral administration after 30 min.

**Conclusion:** Rabbit liver  $T_2^*$  increasing by glucose intravenous injection is possibly associated with glycogen synthesis, provides the possibility to evaluate liver function. Glucose oral administration demonstrated an optimal comparative effect of raising  $T_2^*$ , however, resulted from the superposition of increased glycogen synthesis and blood flow. Inhalation of pure oxygen didn't alter the rabbit liver  $T_2^*$ , which may possibly result from an offset between the increased concentration of oxyhemoglobin and decreased blood flow.

**Keywords:** rabbit liver, blood oxygen level dependent, stimulation agent, pure oxygen, glucose

### Introduction

Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) remains the only non-invasive imaging technique capable of reflecting *in vivo* blood oxygen level. Its imaging mechanisms are as follows: the iron ions of deoxyhemoglobin contain unpaired electrons and have a paramagnetic property that shortens the transverse relaxation time of nearby protons, i.e., tissue  $T_2^*$  value is negatively

correlated with the deoxyhemoglobin concentration; therefore, *in vivo* oxygen level can be measured, according to this theory.<sup>1</sup>

BOLD fMRI was originally applied to brain imaging and extended to other organs such as liver, kidney, and muscle.<sup>2-4</sup> As the biggest digestive organ of the human body, the liver has two sets of blood supply systems: the hepatic artery and the portal vein, as well as a dense capillary network. Therefore, it is a highly perfused organ that can serve as a good platform for the application of BOLD fMRI. Under diseased conditions such as inflammation, fibrosis, and cancer, the micro-environmental blood oxygen level of the liver becomes different from that of the normal liver parenchyma.<sup>5-7</sup> Moreover, blood oxygen consumption of the liver tissue also differs under different physiological and pathological conditions.<sup>6,8,9</sup> Therefore, measurement of micro-environmental blood oxygen level via BOLD fMRI may facilitate the

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Department of Radiology, West China Hospital of Sichuan University, No. 37 Guoxue Lane, Chengdu 610041, Sichuan, China

\*Corresponding author, Phone: +86-13881918066, Fax: +86-21-64085875, E-mail: songlab\_radiology@163.com

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assessment of liver functions under normal and diseased conditions.

At present, there are few reports on the application of BOLD fMRI to liver imaging and repeatability of results of these reports is not strong. The major reason is that there are too many factors affecting the results of BOLD fMRI—tissue  $T_2$  relaxation time, spin density, blood volume fraction, iron deposition, and body movement can all be the influencing factors.<sup>10</sup> As a result, direct measurement of the BOLD fMRI signal cannot genuinely reflect *in vivo* blood oxygen level. How to avoid the influencing factors and correlate measurements with the blood oxygen level are difficulties yet to be dealt with. Some scholars have investigated the factors contributing to BOLD fMRI.<sup>11</sup> However, complex mathematical models, additional contrast agent, and introduction of additional MR sequences in the study significantly increased the difficulty of applying BOLD fMRI.

Use of stimulation agent may be a simple and precise solution. Using a stimulating agent with a specificity of altering tissue blood oxygen level to calculate the  $T_2^*$  variation before and after the administration by subtraction calculation, multiple influencing factors can be excluded within a short time window by self-comparison. Although the real tissue blood oxygen concentration cannot be obtained via this strategy, calculation of  $T_2^*$  variation still genuinely reflects the changes of blood oxygen, which provides the possibility of evaluating tissue bioactivity.

As oxygen and glucose may lead to changes of blood oxygen levels in liver tissues,<sup>12,13</sup> which are based on the mechanism of oxyhemoglobin generating and oxygen consumption concomitant with glycogen synthesis, this study adopted oxygen and glucose as stimulating agents to explore the feasibility of their application in the BOLD fMRI of the liver. Since oxygen and glucose may alter tissue blood flow, this study also analyzed their impacts upon blood flow.

## Materials and Methods

### *Model for experiment animal*

The eleven healthy New Zealand white rabbits (with a weight of  $1.8 \pm 0.4$  kg) were provided by the Experimental Animal Centre of West China, Sichuan University. All the rabbits were handled according to the Regulations for Administration of Laboratory Animals by the Ministry of Health of People's Republic of China and the Regulations for Administration of Laboratory Animals by the West China Hospital of Sichuan University. Animals were fasted for 8 h and deprived of water for 8 h prior to the scanning. Inhaled induction of anesthesia with 4% isoflurane was applied to all the rabbits and then the anesthesia was maintained using 3% phenobarbital (30 mg/kg) injected via the ear vein; after injection of the muscle relaxant succinylcholine chloride (1 mg/kg), animals were intubated and connected to the animal ventilator; respiratory rate was 30/min and the tidal volume was 10 ml/kg, Ti/Te was 1:2. Filtered air was used in

the ventilator. After that, gastric intubation was performed, preparing for the injection of glucose solution. Finally, an MR electrocardiogram (ECG)-gated electrode was implanted after skin preparation of the rabbits' chest and abdomen.

### *MRI scanning*

The Siemens Trio Tim 3.0T MRI scanner was used and the 6-channel phased array coil was adopted as the scanning coil.

True Fast Image with Steady-state Precession (TrueFISP) sequence was used to assist the precise positioning for the two dimensional-Phase Contrast MR sequence (2D-PCMR). The general scanning parameters were TR 320 ms, TE 1.4 ms, 50° Flip Angle, and Slice Thickness 3 mm. Transverse, sagittal, and coronal scans were performed respectively to include the aorta and portal vein. Matrix and FOV were set according to the position.

2D-PCMR sequence was used to measure rabbit portal vein flow (PVF) and aortic flow (AF). PC velocity was set to 20 cm/s, 20° Flip Angle, TR and TE were automatically set by the ECG-gated system (the electrodes were located on the rabbit's chest and abdomen, distributed triangularly); Matrix  $256 \times 208$ , FOV  $18 \text{ cm} \times 15 \text{ cm}$ , bandwidth 540 Hz, 3 segments; 16 scan phases per cardiac cycle. The breath-holding scanning was facilitated by the use of a ventilator. The scanning plane was determined by the TrueFISP sequence images to ensure that the scanning plane was perpendicular to the direction of the PVF and AF.

Multiple Gradient Recalled Echo (mGRE) sequence was used in BOLD fMRI to generate  $T_2^*$  map. The specific scanning parameters were TR 90 ms, TE 3.29–40.88 ms, TE gap 5.37 ms, 8 echos, 60° Flip Angle, Matrix  $256 \times 208$ , FOV  $18 \text{ cm} \times 15 \text{ cm}$ , and Slice Thickness 3 mm. Breath-holding scanning was facilitated by the use of a ventilator.

### *Procedures for the stimulation of MRI scanning*

A: All MR sequences were performed to obtain baseline values. Animals breathed pure oxygen (100%  $O_2$ ) for 10 min and then switched to breathing air. Data with PCMR and mGRE sequences were obtained at 5 min, 10 min, 20 min, and 30 min after initiating oxygen breathing. After scanning, rabbits were left undisturbed for 30 min.

B: Baseline values were re-obtained by repeating PCMR and mGRE sequences. Intravenous injection of saline solution containing 15% glucose (0.4 ml/min for 15 min) was given via the rabbit ear vein, while PCMR and mGRE sequences were rescanned at 5 min, 10 min, 20 min and 30 min after injection. After the scanning, rabbits were left undisturbed for 5 h. Additional anesthetics were administered 2–3 h after scanning depending on the depth of anesthesia observed.

C: Baseline values were re-obtained by repeating PCMR and mGRE sequences. Rapid injection of glucose solution (10 g glucose was diffused in 80 ml pure water) was administered via the gastric tube, while PCMR and mGRE sequences were rescanned at 5 min, 10 min, 20 min and 30 min after initiating administration.

### Image processing and analysis

#### $T_2^*$ Measurement in rabbit liver parenchyma

The Siemens Syngo-Imaging software was adopted by a radiologist with 5-year experience in MRI diagnosis to select the slice of  $T_2^*$  map containing a maximal liver area of the right lateral lobe, which is smaller than the left lateral lobe but thicker and away from the diaphragm to minimize the impacts of diaphragm motion. The region of interest was placed at the center of the right lateral lobe.

#### Measurement of the rabbit AF and PVF

Two radiologists (one with 5 years of experience in MRI diagnosis and the other with 6) utilized the Argus software (Argus, Siemens Medical Solution, Erlangen, Germany) to outline the ROIs at the portal vein origin as well as those at the abdominal aorta from the same level in transverse 2D-PCMR images. If the vessel was morphologically regular, a circular ROI was drawn under the premise that the boundary of the ROI did not exceed the vessel wall; if the vessel was morphologically irregular, the ROI was drawn manually. The abdominal aorta area (AA) and portal vein area (PVA) were calculated automatically.

#### Statistical analysis

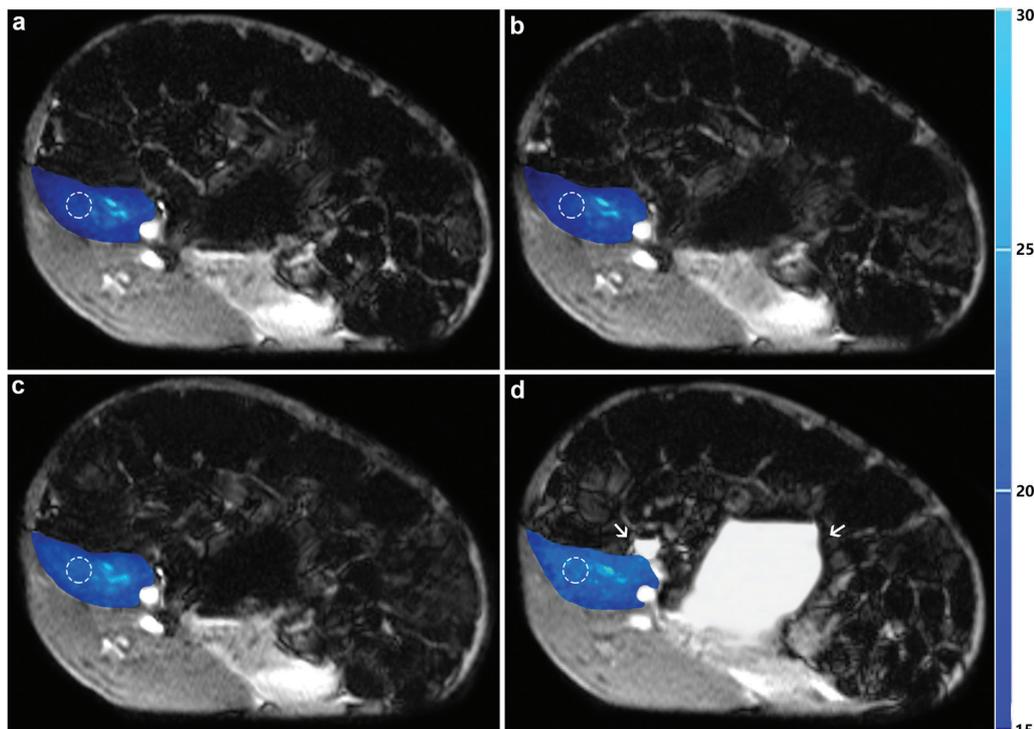
All the statistical analyses were performed using the SPSS for windows, version 16.0 (SPSS Inc, Chicago, IL, USA). All the measurement data were expressed as mean  $\pm$  standard deviation (mean  $\pm$  standard deviation [SD]) and were accurate to 1 decimal place. Univariate Repeated measures ANOVA was used to compare AF, PVF,  $T_2^*$ , AA,

and PVA before and at 5 min, 10 min, 20 min and 30 min after administrations of pure oxygen and glucose, and the consistency of baseline of AF, PVF,  $T_2^*$ , AA, and PVA among the 3 groups before administrations were also validated. Greenhouse-Geisser and Huynh-Feldt (when Greenhouse-Geisser  $\geq 0.7$ ) corrections were performed when Mauchly spherical test was significant. Bonferroni pairwise comparison was performed before and after administrations at each time point. For all the analyses, the confidence interval was 95% and values were considered as statistically different at the level of  $P < 0.05$ .

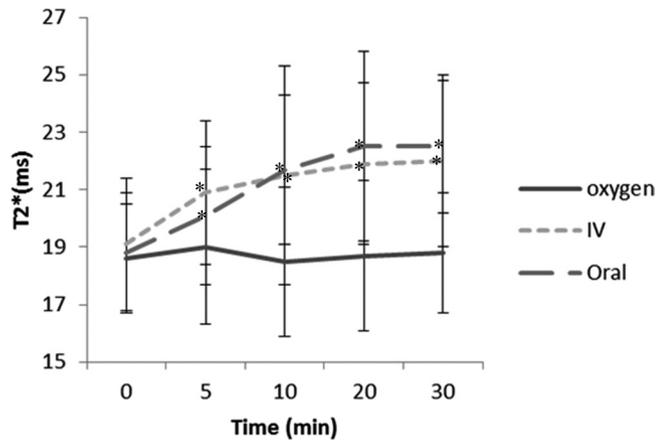
### Results

No significant difference was observed in baseline data before initiating the three stimulation agents. Figure 1–6 shows the variations and statistical results regarding the  $T_2^*$  value, AF, PVF, AA and PVA before and after the use of three stimulation agents.

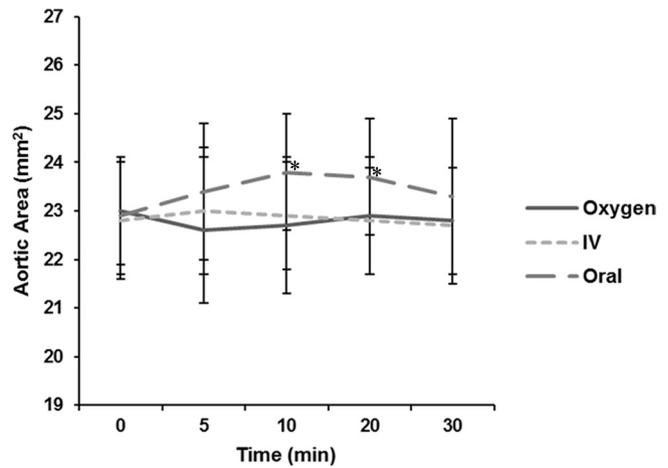
In the pure oxygen inhalation group, AF and PVF decreased at 5 min after inhalation (from  $41.2 \pm 5.1$  and  $8.3 \pm 0.8$  ml/min to  $39.1 \pm 4.3$  and  $7.0 \pm 1.4$  ml/min, respectively) and both were significantly different from pre-inhalation levels. 5 min later, both AF and PVF rapidly reverted to baseline levels and demonstrated no statistically significant differences when compared to pre-inhalation levels.  $T_2^*$  value showed no statistically significant difference before and after inhalation. AA and PVA showed slight decreasing at 5 min after inhalation but no significant difference was observed in all time points of post-inhalation compared to pre-inhalation levels.



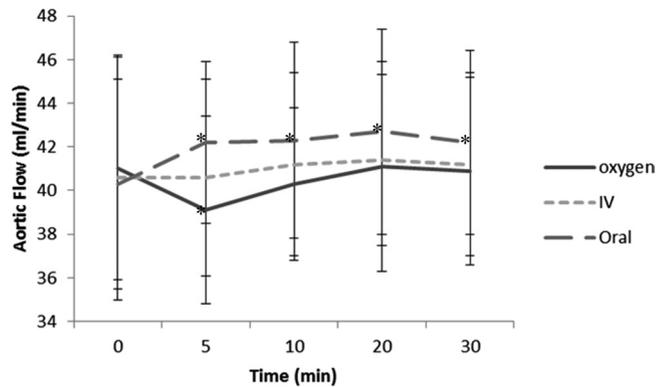
**Fig. 1**  $T_2^*$  maps of the same New Zealand white rabbit using different stimulation strategies; The blue areas indicate the right lateral lobe of liver. The white circles indicate ROIs; (a) First baseline  $T_2^*$  map, (b)  $T_2^*$  map 20 min after oxygen inhalation, (c)  $T_2^*$  map 20 min after glucose injection, (d)  $T_2^*$  map 20 min after oral administration of glucose. The white areas (arrow pointing) indicate glucose solution in the stomach and intestine.



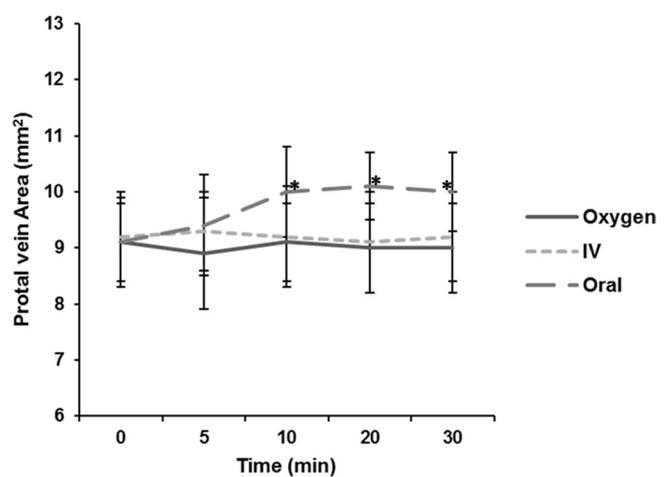
**Fig. 2** Rabbit liver  $T_2^*$  values vary with time after pure oxygen inhalation, intravenous injection of glucose, and oral administration of glucose. \*indicates statistical difference when compared to the level prior to the use of stimulation agents ( $P < 0.05$ ).



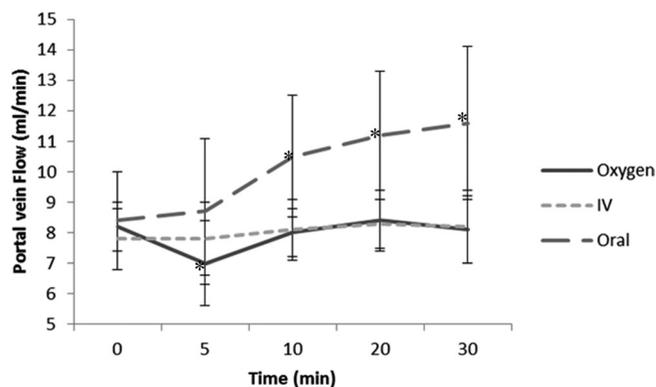
**Fig. 5** Rabbit aortic areas (AAs) vary with time after pure oxygen inhalation, intravenous injection of glucose, and oral administration of glucose. \*indicates statistical difference when compared to the level prior to the use of stimulation agents ( $P < 0.05$ ).



**Fig. 3** Rabbit aortic flows (AFs) vary with time after pure oxygen inhalation, intravenous injection of glucose, and oral administration of glucose. \*indicates statistical difference when compared to the level prior to the use of stimulation agents ( $P < 0.05$ ).



**Fig. 6** Rabbit portal vein areas (PVAs) vary with time after pure oxygen inhalation, intravenous injection of glucose, and oral administration of glucose. \*indicates statistical difference when compared to the level prior to the use of stimulation agents ( $P < 0.05$ ).



**Fig. 4** Rabbit portal vein flows (PVFs) vary with time after pure oxygen inhalation, intravenous injection of glucose, and oral administration of glucose. \*indicates statistical difference when compared to the level prior to the use of stimulation agents ( $P < 0.05$ ).

In the glucose injection group, AF, PVF, AA and PVA showed no statistically significant differences before and after the injection of glucose.  $T_2^*$  value was significantly different before and after the injection of glucose at each time point and increased gradually.

In the oral glucose administration group, AF and  $T_2^*$  value were significantly different before and after orally administering the glucose and increased gradually with time. AA was significantly different before administration and after administration at 10 min and 20 min. PVF and PVA started to increase after administration and was significantly different from the pre-administration level after 10 min. Greatest variation of  $T_2^*$  (19.6%) was induced by glucose oral administration after 30 min compared to baseline.

## Discussion

Due to the invasiveness of intravenous injection, potential organ injury by high concentration glucose and pure oxygen inhalation, as well as management of breathing and movement conveniently,<sup>14,15</sup> the animal experiment was chosen in our study. New Zealand white rabbit is the appropriate animal model which has left and right liver lobes and its portal vein and bile duct are accompanied by the hepatic artery, which demonstrates an anatomy similar to that of the human liver.<sup>16</sup> The liver volume of the New Zealand rabbit is relatively large, which is suitable for MR post processing and can reduce measurement difficulty while increasing the signal-to-noise ratio by more and bigger voxels.

Before the experiment, we believed that oxygen inhalation could alter the rabbit liver  $T_2^*$  value since rapid pure oxygen intake could quickly raise the concentration of oxyhemoglobin in the blood, which may theoretically reduce the deoxyhemoglobin concentration in liver tissue and thus increase the  $T_2^*$  value. However, interestingly, our results proved that pure oxygen inhalation could not elevate the rabbit liver  $T_2^*$  value, while the results of BOLD fMRI studies on healthy human liver also reported similar results, but not in cirrhosis.<sup>17</sup> Our study found that at 5 min after pure oxygen inhalation, both AF and PVF were significantly reduced, which may theoretically result from vasoconstriction of pure oxygen,<sup>2</sup> however, AA and PVA showed the insufficient evidence (no significant difference at 5 min compared to baseline even with slight decreasing). Barbiturates potentiate the biosynthesis of Nitric Oxide (NO) and decreased  $Ca^{2+}$ -induced contractile responses of vascular smooth muscle which cause vascular depressant effect,<sup>18,19</sup> thus, maintained using phenobarbital for anesthesia in our study may contribute to the insignificant change of AA and PVA. Consequently, significant decreasing of AF and PVF should result from heart rate reduction primarily by pure oxygen intake.

In a study conducted by Fan et al.<sup>2</sup> PVF was also reduced by 17% after human ventilation with alternately used pure oxygen and medical air, which was similar to our results. However, Hughes et al.<sup>20</sup> found that after oxygen inhalation the PVF in mice was actually increased, which, we infer, is possibly correlated with the inhalation method of pure oxygen and species (and of course, sample size and measurement methods will also affect the results). In conclusion, our results showed that pure oxygen inhalation did not alter the rabbit liver  $T_2^*$  value, which, we believe, may be the result of offset among the increased relative concentration of oxyhemoglobin in the blood, the decreased AF, and the reduced PVF.

Our study found that intravenous injection of glucose did not change PVF, which was consistent with the results of Brundin et al.<sup>21</sup> Also, it did not alter AF and AA, PVA in our study. Therefore, after excluding the interference caused by

blood flow, we believed that the constant increasing of liver  $T_2^*$  value after intravenous injection of glucose was closely associated with the physiological activities of the liver. Since intravenous injection of glucose can increase the blood glucose concentration and liver is an important energy reservoir organ in the body, the liver cell will transfer blood glucose into liver glycogen storage; the process of which will reduce the oxygen consumption in liver cells,<sup>13,22</sup> lower the liver deoxyhemoglobin level, and thus raise the  $T_2^*$  value. In a study led by Haque et al.<sup>13</sup> the swine liver  $T_2^*$  value was also significantly increased after intravenous injection of glucose; however, they did not measure the blood flow. In this study, our conclusion was based on the analysis of AF and PVF and therefore was more convincing.

We found that PVF and AF following oral administration of glucose were gradually increased with the increase of AF being slightly earlier than that of PVF (AF vs. PVF, 5 min vs. 10 min), and a similar trend was observed in AA and PVA. We believe this is associated with the fact that glucose solution would stimulate the functions of the digestive system and increase gastrointestinal blood flow: when the digestive system starts to operate, arterial blood flow will increase so as to provide sufficient energy and meanwhile gastrointestinal blood circulation will also increase so as to absorb nutritious materials; therefore, increased gastrointestinal blood will flow into the portal vein and increase the PVF. In this study, we found that liver  $T_2^*$  value following oral administration of glucose increased with time and statistical difference was observed between each of the time points. Combined with the aforementioned results of intravenous injection of glucose in the present study, we believe that the co-effect of the three factors of elevated AF and PVF, and increased liver glycogen synthesis resulted in the increased  $T_2^*$  value. Besides, there was the certain effect of glycogen synthesis on  $T_2^*$ . However, since the effect of arterial blood flow on liver  $T_2^*$  mainly depends on the hepatic artery and not the aortic artery, and taken into account that decreased blood flow of the hepatic artery after a meal was observed in a human study,<sup>23</sup> which was contrary to AF in our study, the effect of elevated AF and PVF on  $T_2^*$  is worthy of future study. Haque et al.<sup>13</sup> found that variation of the swine liver  $R_2^*$  following intravenous injection of glucose (from  $110.41 \pm 14.1 \text{ s}^{-1}$  to  $72.22 \pm 5.7 \text{ s}^{-1}$ ) was greater than that of the human with oral administration (from  $55.84 \pm 3.8 \text{ s}^{-1}$  to  $50.6 \pm 0.5 \text{ s}^{-1}$ ), while our study indicated that variation rate of  $T_2^*$  value (19.6%) induced by oral administration of glucose 30 min later was the greatest among the three groups, which may be associated with the dose of glucose and the species adopted.

## Conclusions

In conclusion, this study analyzes the impacts of pure oxygen inhalation, intravenous injection of glucose, and oral

administration of glucose upon the liver blood oxygen level; among the three strategies, both intravenous injection and oral administration of glucose could lead to changes in the liver blood oxygen level, indicating that these two strategies have the potential of becoming the stimulation agents for BOLD fMRI of the liver. In addition, intravenous injection of glucose did not alter the blood flow and the  $T_2^*$  variation induced from this strategy might directly associate with the hepatic activities of glycogen synthesis, providing a possibility for the evaluation of liver functions; however, as an invasive method that significantly elevates the level of blood sugar, this strategy may impose burden on organs. Oral administration of glucose is easy to perform and risk-free, also producing the greatest variation of  $T_2^*$  value (i.e., providing the optimal comparative effect) in the present study; however, under this strategy, the liver blood oxygen level was affected by the mutual interaction of changes in AF and PVF, as well as the hepatic synthesis of glycogen. Further investigations are required on how to analyze these effects quantitatively. Meanwhile, use of glucose as a stimulating agent may be highly risky for diabetic patients, limiting its clinical application.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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