# Hypoglycemia activation of cerebral blood flow is mediated by glucose transporter isoform 2: an in vivo CASL study

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## PURPOSE

Recent studies suggested that glucose transporter isoform 2 (GLUT2) could facilitate hypoglycemia-induced hormonal secretions [1] and might play an important role in other hypoglycemia-related responses. For example, the underneath mechanism of hypoglycemia activation of cerebral blood flow (CBF) remained not well understood[2]. Thus, we aimed to study the roles of GLUT2 in hypoglycemia-induced CBF increase. In this study, we applied the continuous arterial spin labeling MRI technique on GLUT2$^{-/-}$ mice and their counterparts at euglycemia and insulin-induced hypoglycemia.

## METHODS

**Animals:** GLUT2$^{-/-}$ mice were derived along with transgenic re-expression of GLUT1 in the pancreatic β–cells as previously described (RIPGLUT1GLUT2$^{-/-}$) [1]. At age of 16-20$^{th}$ weeks, animals were prepared and well maintained under 1-2% isoflurance thereafter to maintain breathing rates >100bpm, which had been shown capable of maintaining mice under physiological conditions, such as $PaCO_2$ in the range of 35-45mmHg. Tail bleeds were sampled and measured for glucose levels (Breeze glucose meter) immediately before and right after MR measurements, which were approximately one hour. An adjusted insulin infusion protocol, i.e. a bolus followed by a continuous rate, induced hypoglycemia in all mice. Once tail bleed glucose levels were reached less than 2mM, as hypoglycemic condition, the animals were scanned again. **MR measurements:** All MR measurements were performed at 9.4T (26cm diameter). CBF was measured using a well-established continuous arterial spin labeling (CASL) technique in combination with a home-built actively-detuned system. Four segmented semi-adiabatic EPI sequence was adopted with a labeling module to implement the CASL sequence (3 consecutive 2mm-thick slices, 23×15mm$^2$, 128×64 data matrix). Cerebral blood flow (CBF) was assessed at euglycemia and hypoglycemia. **Data Analysis:** CBF maps were calculated from 16 paired labeled and controlled images with a labeling efficiency 0.8 [3]. Significant difference was when $p< 0.05$.

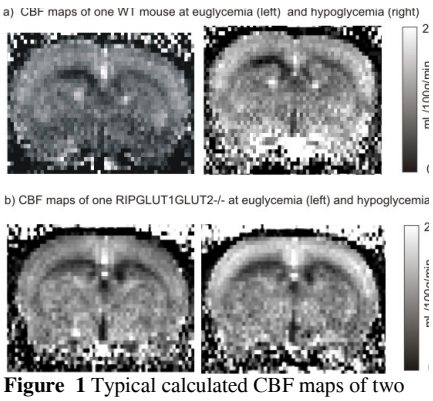


**Figure 1** Typical calculated CBF maps of two mouse brains at 9.4T.

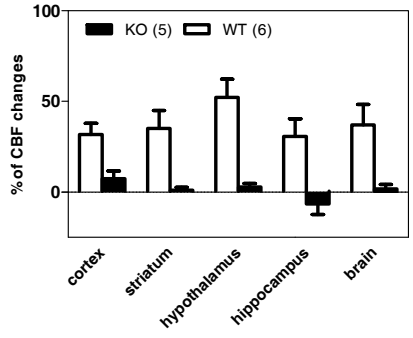


**Figure 2.** Summary of percent of CBF changes under insulin induced acute hypoglycemia from RIPGLUT1GLUT2-/- mice and their counter types when compared to those under euglycemia. The percent CBF changes of WT mice were significantly higher than those of RIPGLUT1GLUT2-/- mice ($p<0.05$).

**Table1** Summary of CASL on regional blood flow of mice at euglycemia

| Region | WT (ml/100g/min) | KO (ml/100g/min) |
|---|---|---|
| hippocampus | 111.7±14.0 | 122.4±7.4* |
| hypothalamus | 91.3±9.9 | 99.0±16.1 |
| cortex | 119.7±15.6 | 123.9±11.9 |
| brain | 102.7±6.3 | 106.4±6.6 |

"*" indicated $p<0.01$ using unpaired student t-test.

## RESULTS AND DISCUSSION

Glucose levels before insulin infusion were 10.1±1.4mM (mean±SDs, 8.6-12.4mM) in WT mice and 7.8±2.9mM (mean±SDs, 4.3-12.4mM) in RIPGLUT1GLUT2-/- mice, both of which were beyond hypoglycemia. While at euglycemia, CBF was globally higher in RIPGLUT1GLUT2$^{-/-}$ mice and significant in hippocampus (Figure 1, Table 1).

When administrating 25.2±5.9UI/kg insulin in the WT mice and 15.1±1.6UI/kg insulin in those RIPGLUT1GLUT2$^{-/-}$ mice, hypoglycemia condition was reached, i.e. 1.6±0.2mM (mean±SDs, 1.2-2.3mM) in the WT mice and 1.2±0.1mM (0.9-1.6mM) in the knockout mice, respectively. While at hypoglycemia, CBF changes of WT mice were beyond 30% and however CBF changes in RIPGLUT1GLUT2$^{-/-}$ mice were diminished (Figure 2).

The observed CBF increases upon hypoglycemia in the WT mice were consistent, but with a less degree, with previous study in other rodents [2, 4], which might due to the effects of isoflurane [5].

## CONCLUSION

In conclusion, our results in the RIPGLUT1GLUT2-/- mice suggested that hypoglycemia activation of cerebral blood flow could be mediated by GLUT-2 glucose transporter.

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