INTRODUCTION

Hypoglycemia remains a significant barrier to achieving adequate glycemic control in patients with diabetes mellitus. Repeated exposure to hypoglycemia results in defective glucose counterregulation leading to impaired hypoglycemia awareness. Incidences of both severe and asymptomatic hypoglycemia are reported frequently in type 1 and type 2 diabetes mellitus (T1DM and T2DM) patients. An earlier study from Boland et al, that monitored T1DM patients using a continuous glucose monitoring system, reported that T1DM patients frequently experience prolonged and asymptomatic hypoglycemia (glucose < 60 mg/dl). A similar study from Gehlaut et al in T2DM patients also reported exposure to frequent asymptomatic hypoglycemia (glucose < 70 mg/dl).
Neuronal damage and death occur in the hippocampus following exposure to severe hypoglycemia, and in the prefrontal, orbital, and piriform cortex following moderate hypoglycemia. In rat models, exposure to RH increases oxidative damage to hippocampal neurons, as well as microglial activation leading to chronic cognitive impairment. Previous animal studies examining spatial working memory in hippocampal function reported that RH enhances spatial working memory in rats in euglycemia but impairs it during subsequent hypoglycemic episodes. Reports also demonstrate impaired spatial working memory in rats in euglycemia but impairs it during further hypoglycemic conditions. Human studies are controversial; difficulties in controlling for diabetic history and related disease processes have produced mixed results. The Diabetes Control and Complications Trial (DCCT), and later the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study, concluded that the frequency of severe hypoglycemia was not related to subsequent cognitive impairment. Another recent study however observed that exposure to hypoglycemia correlates with worsening cognitive deficits, including impairments of learning and memory. In addition, the level of cognitive impairment, as a result of acute hypoglycemia in potentially hippocampus-mediated complex tasks (eg, driving), has been related to the severity of prior hypoglycemic episodes.

During normal glycemic periods, the human brain mainly depends on the systemic supply of glucose as the principal metabolic substrate. Exposure to hypoglycemia leads to several metabolic adaptations such as increased levels of glucose transporters, subsequently enhanced uptake of glucose, increase in brain glucose levels, increased levels of hexokinase I, increased glucose phosphorylation, and increased glycolytic flux in vitro. Besides, exposure to hypoglycemia and/or RH also affects the tricarboxylic acid (TCA) cycle in multiple ways. Therefore, the literature shows differential effects of hypoglycemia on various pathways of cellular metabolism. However, the global effect of RH exposure on the brain metabolome is not known. Understanding the effects of hypoglycemia on global brain metabolism may also help us understand the pathological consequences of hypoglycemia on important brain structures involved in cognitive functions such as the hippocampus. The primary objective of this study is to investigate the effects of mild-to-moderate hypoglycemia on hippocampal metabolism. We hypothesize that exposure to RH leads to metabolomic alterations in the hippocampus of insulin-treated streptozotocin-diabetic rats.

2 | MATERIALS AND METHODS

2.1 | Induction of diabetes, insulin treatment, and induction of recurrent hypoglycemia

All experimental procedures were carried out as per the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and in accordance with the protocols approved by the Animal Care and Use Committee of the University of Miami. Results are reported according to the ARRIVE guidelines to the best of our ability. Rats were made diabetic by injecting the pancreatic β-cell toxin streptozotocin. The rats having blood glucose values ≥250 mg/dl were included in the diabetic group. Insulin pellets were implanted subcutaneously (sc) after 2-3 weeks of diabetes induction. This group of animals was considered as insulin-treated diabetic (ITD) rats. ITD group rats were randomly divided into ITD + RH (n = 10) and ITD + RH + glucose (n = 10) groups. Rats belonging to ITD + RH or ITD + RH + glucose groups were exposed to five episodes of RH (hyperinsulinemic hypoglycemia) or RH + glucose (hyperinsulinemic euglycemia) over five consecutive days (1 episode/day), respectively (see Appendix S1 for details). One day after the last hypoglycemic exposure, the rats were euthanized by decapitation under isoflurane anesthesia (30% O₂/70% N₂O); brains were quickly excised; hippocampi were harvested, snap-frozen in liquid nitrogen, and stored at −80°C until use (Figure 1A).

2.2 | Metabolomic and enzyme studies

Samples were analyzed in a blinded manner. The sequence of analysis was randomized using a random number generator to select the run order. Metabolomic data were analyzed using MetaboAnalyst 3.0 software. Connections between related metabolites were generated using MetaMapR in order to build a network that displays structural similarity based on a metabolite’s PubChem substructure fingerprints. The hippocampus was thawed on ice and homogenized, and its supernatant was collected and enzymatic assays were performed following the manufacturer’s instructions. Refer Appendix S1 for details.

2.3 | Statistical analysis

Comparison of the two groups was performed using the Student’s t test for all parameters. Significant outlier data points, if any, as identified by Grubbs’ test were excluded from further analysis. The results are presented as mean ± SD. A P-value of <0.05 was considered statistically significant. Statistical methods used for analyzing metabolomics results are provided in the metabolomics methods section above.

3 | RESULTS

3.1 | Glucose levels following induction of diabetes, during insulin treatment, and hypoglycemia

The experimental procedure is presented in Figure 1A. The results show that the glucose levels prior to insulin treatment in RH-exposed ITD animals and ITD + RH + glucose rats were not statistically different. Similarly, there was no significant difference between the blood glucose levels measured after insulin treatment in ITD + RH and ITD + RH + glucose groups (Figure 1B). To avoid unwanted hypoglycemia, blood glucose levels during...
insulin treatment were kept slightly above euglycemic levels. The mean blood glucose levels during induction of hypoglycemia (during hyperinsulinemic hypoglycemia) in the ITD + RH group were 53 ± 14 mg/dl. These values were significantly lower than the blood glucose levels observed in ITD + RH + glucose (during hyperinsulinemic euglycemia) (236 ± 42 mg/dl) (Figure 1C).

3.2 Exposure to recurrent hypoglycemia alters hippocampal metabolic pathways

Here, we evaluated the effect of RH exposure in ITD rats on hippocampal metabolism by nontargeted, global metabolite profiling. In total, 386 peaks were captured, 115 of which are structurally annotated metabolites. Basal metabolic differences and putative RH-regulated pathways were identified by comparing ITD + RH (n = 9) and the control group (ITD + RH + glucose, n = 10). The principal component analysis revealed some separation between the groups along principal component 1 (PC1, 58.9% of total variance), indicating a small overall difference between metabolite profiles of ITD + RH and ITD + RH + glucose groups (Figure 2A). Comparison of individual metabolites found 65 significantly altered metabolites in the ITD + RH group (n = 9) compared with the ITD + RH + glucose group (n = 10) (Student’s t-test, P < 0.05, false discovery rate: FDR < 0.1, Figure 2B and Table S1). Of these 65 metabolites, the majority were increased (55 metabolites), while only a small fraction was decreased in abundance (10 metabolites).

Pathway analysis using the KEGG pathway database identified six significantly altered pathways in the hippocampus of RH-exposed ITD rats, when compared to the ITD + RH + glucose group: (a) alanine, aspartate, and glutamate metabolism (aspartic acid, alanine, fumaric acid, glucosamine 6-phosphate); (b) glycine, serine, and threonine metabolism (glyceric acid, betanin, sarcosine, threonine); (c) β-Alanine metabolism (β-alanine, aspartic acid, ureidopropionic acid); (d) glyoxylate and dicarboxylate metabolism (glyceric acid and malic acid); (e) glycerolipid metabolism (glyceric acid dihydroxyacetone phosphate); and (f) citrate cycle (malic acid, fumaric acid) (≥2 hits, impact > 0.05, P ≤ 0.01, Figure 2C). Enrichment analysis also identified several other significantly altered pathways (≥2 hits, fold enrichment ≥ 2.0, P ≤ 0.01, Figure 2D). Ratios presented next to each bar in Figure 2D denote the number of metabolites identified vs the total number of metabolites in that pathway. These include the urea cycle (fumaric acid, alanine, aspartic acid, ADP); the mitochondrial electron transport chain (fumaric acid, ADP, dihydroxyacetone phosphate); protein biosynthesis (alanine, threonine, aspartic acid); malate-aspartate shuttle (aspartic acid, fumaric acid, ADP); and protein folding (ADP, dihydroxyacetone phosphate, fumaric acid).
DEWAN ET AL.

3.3 | Substrate kinetics of key glycolytic enzymes

After observing significant changes in the metabolites that belong to key cellular pathways, we examined whether these changes were due to altered substrate kinetic properties of the enzymes involved in each respective pathway. Since it was practically and technically challenging to evaluate substrate kinetic properties of all enzymes of metabolic pathways affected by the exposure to RH, we evaluated substrate kinetic properties of key glycolytic enzymes (hexokinase, phosphofructokinase, and pyruvate kinase) as this pathway was significantly affected by exposure to RH. For hexokinase, we evaluated substrate kinetics for two of its substrates (ie, glucose and ATP). We did not observe any significant differences in $K_m$ value for either substrate in both experimental groups (Figure 5B). However, the $V_{max}$ for glucose was significantly lower (42%, $P < 0.005$) for ITD + RH animals ($0.18 \pm 0.05 \text{ mmol/L}$) when compared to animals belonging to ITD + RH + glucose group ($0.30 \pm 0.07 \text{ mmol/L}$) (Figure 5A). In contrast, the $V_{max}$ for ATP was significantly higher (77%, $P < 0.05$) for ITD + RH animals ($0.49 \pm 0.21 \text{ mmol/L}$) when compared to animals belonging to the ITD + RH + glucose group ($0.28 \pm 0.06 \text{ mmol/L}$) (Figure 5B).
For phosphofructokinase, we evaluated substrate kinetics for two of its substrates (ie, fructose-6-phosphate and ATP). We did not observe any significant differences in $K_m$ value for fructose 6-phosphate in either experimental group (Figure 5D). However, the $K_m$ value for ATP was significantly higher ($292\%$, $P < 0.001$) for ITD + RH animals (41 ± 15 µM) when compared to animals belonging to the ITD + RH + glucose group (10 ± 3 µM) (Figure 5D). This significant increase in $K_m$ value for ATP indicates that RH exposure decreases affinity of phosphofructokinase for ATP. The $V_{max}$ for fructose 6-phosphate was significantly lower ($75\%$, $P < 0.001$) for ITD + RH animals (3.4 ± 2.6 mmol/L) when compared to animals belonging to the ITD + RH + glucose group (14 ± 3 mmol/L) (Figure 5C). The opposite was observed for the $V_{max}$ value of ATP. The $V_{max}$ for ATP was significantly higher ($40\%$, $P < 0.05$) for ITD + RH animals (12.4 ± 1.4 mmol/L) when compared to animals belonging to the ITD + RH + glucose group (8.8 ± 2.3 mmol/L) (Figure 5C).

For pyruvate kinase, we evaluated substrate kinetics for two of its substrates (ie, phosphoenolpyruvate and ADP). The $K_m$ value for phosphoenolpyruvate was significantly higher ($994\%$, $P < 0.005$) for ITD + RH animals (580 ± 280 µM) when compared to animals belonging to ITD + RH + glucose group (53 ± 5 µM) (Figure 5F). These results indicate lower affinity of pyruvate kinase for phosphoenolpyruvate and thus lower enzyme efficiency in converting phosphoenolpyruvate into pyruvate. These results are supported by metabolomics data where we observed significantly higher levels of an upstream metabolite, 2-phosphoglycerate ($226\%$), in ITD + RH rats compared to ITD + RH + glucose animals. This effect may further be exacerbated during subsequent hypoglycemia exposure due to expected lower levels of substrates for glycolysis. The opposite was observed for the $K_m$ value for ADP.

The $K_m$ value for ADP was significantly lower ($84\%$, $P < 0.05$) for ITD + RH animals (0.39 ± 0.09 mmol/L) when compared to animals belonging to the ITD + RH + glucose group (2.4 ± 2.2 mmol/L) (Figure 5F). The $V_{max}$ for phosphoenolpyruvate was significantly higher ($44\%$, $P < 0.005$) for ITD + RH animals (17 ± 3 mmol/L) when compared to animals belonging to the ITD + RH + glucose group (12 ± 1 mmol/L) (Figure 5E). The opposite was observed for the $V_{max}$ value of ADP. The $V_{max}$ for ADP was lower ($41\%$) for ITD + RH animals (14 ± 3 mmol/L) when compared to animals belonging to the ITD + RH + glucose group (23 ± 19 mmol/L) (Figure 5E). However, this difference was not statistically significant. Overall, these results demonstrate a severe impact of RH exposure on the substrate kinetic properties of glycolytic enzymes.

4 | DISCUSSION

Both T1D and T2D patients experience frequent mild-to-moderate hypoglycemia throughout their life, and current research has implicated this effect on several important brain processes. Since the brain relies mainly on glucose as a source of energy, these recurrent events of hypoglycemia are likely to affect brain metabolism leading to metabolic alterations. While earlier studies demonstrated that hypoglycemia affects several metabolomic pathways in the brain, they did not address the effect of recurrent hypoglycemia on hippocampal metabolism. Here, we employed a metabolomic approach to further elucidate the contribution of RH to hippocampal metabolism. We first evaluated the global metabolic profile of the hippocampus in RH-exposed ITD rats as compared to euglycemic controls.
We chose to evaluate the impact of 5 episodes of hypoglycemia, per earlier studies. Available techniques to study metabolomics utilize analytical chemistry technologies such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) to provide a comprehensive profile of the metabolites present in a biological sample. MS is more commonly used owing to its superior sensitivity, mass accuracy, and mass resolution. Metabolomics has other discernable advantages over other technologies used to study metabolism. Compared with the 100 000 transcripts and 1 000 000 proteins found in humans, there is a relatively smaller number of metabolites (~25 000) to examine. These downstream metabolites integrate genomic, transcriptomic, and proteomic variability. Precise measurement of these metabolites in disease and control conditions provides comprehensive insight into mechanistic abnormalities. Our present study and earlier studies by others reported large percentages of altered metabolites in various experimental conditions. Based on this, it appears that metabolomic studies are more sensitive to detect the impact of various conditions on cellular functioning. We observed that prior exposure to RH leads to several differences in 65 metabolites belonging to major metabolic pathways. Our results demonstrate that RH exposure leads to metabolomic alterations in the hippocampus of ITD rats. Stress can affect glucose homeostasis and thus may also affect overall tissue metabolism. It is possible that stress produced by procedures employed in our experiments, such as insulin/glucose injections and tail puncture for blood glucose measurements, may also affect glucose homeostasis. We do not expect the impact of such stress on our conclusion, as animals belonging to both RH and RH + glucose groups were exposed to similar experimental procedures, and tissues were harvested overnight after exposure to RH or RH + glucose.

The phosphocreatine (Pcr)/creatine kinase system manages high-energy demands of the brain. During periods of ischemia, or glutamate toxicity, Pcr converts to creatine and ATP to provide a protective effect to surrounding tissues. An association between depletion of these metabolites and severity of neurocognitive performance was shown earlier. Neuroprotective effects of creatine supplementation have been shown in rat models. We observed an increase in creatine levels in the hippocampus of RH-exposed rats as compared to the euglycemic controls. This observed increase in creatine levels may be due to a compensatory response to RH exposure.
Higher creatine levels may help maintain hippocampal energy status during the initial phase of hypoglycemia.

An earlier study evaluated the potential RH-induced adaptation in glucose phosphorylation that may preserve glucose flux during subsequent hypoglycemia. They reported that the exposure to RH leads to increased hypothalamic glucose phosphorylation. In the present study, we did not observe increased glucose phosphorylation, indicating that prior RH exposure may not preserve glucose flux during subsequent hypoglycemia in the hippocampus. These differences may be due to a brain region-specific effect of RH, and further studies may help identify the observed differential effects of RH on various brain regions.

Although we did not observe any statistically significant differences in levels of glucose-6-phosphate among both experimental groups, we did observe changes in substrate kinetic properties of hexokinase. These results indicate that although substrate kinetic properties of hexokinase were altered by the exposure to RH, these changes did not have any effect on glycolysis, at least during euglycemia. Our results also indicate that since $K_m$ values for both glucose and ATP were not altered by RH exposure, changes in substrate kinetic properties of hexokinase would not have any significant impact on glycolysis during subsequent hypoglycemia.

We observed a robust decrease in the $V_{\text{max}}$ for fructose-6-phosphate in ITD + RH animals (Figure 5C). This robust decrease in $V_{\text{max}}$ indicates that phosphofructokinase may not be efficiently converting fructose 6-phosphate into fructose 1,6-bisphosphate. Although our metabolomics analysis did not detect levels of fructose 1,6-bisphosphate, we did observe a nonsignificant decrease in levels of glyceraldehyde 3-phosphate and a significant decrease in levels of dihydroxyacetone phosphate in the ITD + RH group (Figure 4A). These results indicate that the observed altered metabolome profile of glycolysis intermediates may be explained partly due to higher $K_m$ of ATP and lower $V_{\text{max}}$ of phosphofructokinase for fructose 6-phosphate. The observation of decreased $K_m$ of ADP and increased $V_{\text{max}}$ for phosphoenolpyruvate may in fact be compensatory changes from an increased $K_m$ for phosphoenolpyruvate and decreased $V_{\text{max}}$ for ADP. It is important to note however that the data suggest that these compensatory mechanisms are not strong enough to alleviate the effects of RH on glycolysis. Considering the differential regulation of phosphofructokinase in neurons and astrocytes and the expression of a different splice variant of pyruvate kinase in neurons and astrocytes, it is possible that some of the changes we observed may be specific to astrocytes and not neurons. However, further studies dissecting the impact of hypoglycemia exposure on neuronal...
and astrocytic metabolism may help determine the potential differential impact of hypoglycemia on these two cell types.

We observed a significant decrease in the levels of succinate and an increase in fumarate. These results indicate that within the hippocampus of RH-exposed rats, succinate is more efficiently converted into fumarate or it is used for other roles such as protein succinylation. These results also indicate that mitochondrial electron transport chains may receive more electrons from FAD-linked substrates. However, this hypothesis remains to be tested.

The levels of two of urea cycle metabolites (aspartate and citrulline) were higher in the ITD + RH group (Figure 4C). An earlier study evaluating the effect of anesthesia exposure-induced cognitive dysfunction observed a significant increase in the levels of aspartic acid in the hippocampus of isoflurane-treated rats, and this increase was positively correlated with the degree of cognitive dysfunction. T-maze training of rats increases citrulline levels in the dentate gyrus and prefrontal cortex and oral administration of citrulline alleviates cerebral ischemia-induced memory deficits. Our results indicate that the increased citrulline levels in the hippocampus of RH-exposed rats may be a compensatory mechanism to preserve cognitive functions in the setting of the hippocampus of RH-exposed rats, succinate is more efficiently converted into fumarate and it is used for other roles such as protein succinylation. These results also indicate that mitochondrial electron transport chains may receive more electrons from FAD-linked substrates. However, this hypothesis remains to be tested.

Our experimental conditions suggest that the exposure to RH leads to the activation of calcium-dependent phospholipase A2 (PLA2). Activation of PLA2 leads to the breakdown of membrane phospholipids including phosphatidylcholine resulting in increased levels of phosphatidylcholine metabolites such as phosphocholine. An earlier study demonstrated increased levels of phosphatidylcholine metabolites such as phosphocholine in cerebrospinal fluid of Alzheimer’s disease patients. Increased phosphocholine levels in our experimental conditions suggest that the exposure to RH leads to PLA2 activation via increased intracellular calcium levels resulting in breakdown of membrane phospholipids leading to cellular damage in the hippocampus, and this damage may lead to RH exposure-induced hippocampal dysfunction.

In conclusion, our results demonstrate that the exposure to RH has a profound impact on hippocampal metabolism. Our analysis also demonstrated that the changes in levels of these metabolites severely impact several metabolic pathways. These metabolic changes may be responsible for impaired hippocampal function observed following exposure to RH.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health grant NS073779, the University of Florida’s Southeast Center for Integrated Metabolomics (NIH grant U24DK097209), and the Evelyn F. McKnight Brain Institute. We thank Dr. Brant Watson for critical reading of this manuscript. The funding agencies were not involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Kunjn R. Dave https://orcid.org/0000-0002-0173-5338

REFERENCES

1. Seaquist ER, Anderson J, Childs B, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care. 2013;36(5):1384-1395.
2. Cryer PE. Diverse causes of hypoglycemia-associated autonomic failure in diabetes. N Engl J Med. 2004;350(22):2272-2279.
3. Davis SN, Shavers C, Mosqueda-Garcia R, Costa F. Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. Diabetes. 1997;46(8):1328-1335.
4. Calles-Escandón J, Koch KL, Hasler WL, et al. Glucose sensor-augmented continuous subcutaneous insulin infusion in patients with diabetic gastroparesis: An open-label pilot prospective study. PLoS ONE. 2018;13(4):e0194759.
5. Choudhary P, Amiel SA. Hypoglycaemia: current management and controversies. Postgrad Med J. 2011;87(1026):298-306.
6. Gehlaut RR, Dogbey GY, Schwartz FL, Marling CR, Shubrook JH. Hypoglycemia in type 2 diabetes—more common than you think: a continuous glucose monitoring study. J Diabetes Sci Technol. 2015;9(5):999-1005.
7. Gómez AM, Muñoz OM, Marin A, et al. Different indexes of glycemic variability as identifiers of patients with risk of hypoglycemia in type 2 diabetes mellitus. J Diabetes Sci Technol. 2018;12(5):1007-1015. https://doi.org/10.1177/1932296818758105
8. Henriksen MM, Andersen HU, Thorsteinsson B, Pedersen-Bjergaard U. Hypoglycemic exposure and risk of asymptomatic hypoglycemia in type 1 diabetes assessed by continuous glucose monitoring. J Clin Endocrinol Metab. 2018;103(6):2329-2335.
9. Little SA, Speight J, Leelarathna L, et al. Sustained reduction in severe hypoglycemia in adults with type 1 diabetes complicated by impaired awareness of hypoglycemia: two-year follow-up in the HypoCOMPASS randomized clinical trial. Diabetes Care. 2018;41(8):1600-1607.
10. Ölofsson AF, Polonsky WS, Bolinder J, et al. A randomized clinical trial of the effect of continuous glucose monitoring on nocturnal hypoglycemia, daytime hypoglycemia, Glycemic variability, and hypoglycemia confidence in persons with type 1 diabetes treated with multiple daily insulin injections (GOLD-3). Diabetes Technol Ther. 2018;20(4):274-284.
11. Wood A, O’Neal D, Furler J, Ekinci EI. Continuous glucose monitoring: a review of the evidence, opportunities for future use and ongoing challenges. Intern Med J. 2018;48(5):499-508.
12. Boland E, Monsod T, Delucia M, Brandt CA, Fernando S, Tamborlane WV. Limitations of conventional methods of self-monitoring of blood glucose: lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes. Diabetes Care. 2001;24(11):1858-1862.

13. Bree AJ, Puente EC, Daphna-Iken D, Fisher SJ. Diabetes increases brain damage caused by severe hypoglycemia. Am J Physiol Endocrinol Metab. 2009;297(1):E194-201.

14. Tkacs NC, Pan Y, Raghupathi R, Dunn-Meynell AA, Levin BE. Cortical Fluoro-Jade staining and blunted adrenomedullary response to hypoglycemia after noncompliant hypoglycemia in rats. J Cereb Blood Flow Metab. 2005;25(12):1645-1655.

15. Won SJ, Yoo BH, Kauppinen TM, et al. Recurrent/moderate hypoglycemia induces hippocampal dendritic injury, microglial activation, and cognitive impairment in diabetic rats. J Neuroinflammation. 2012;9:182.

16. McNay EC, Sherwin RS. Effect of recurrent hypoglycemia on spatial cognition and cognitive metabolism in normal and diabetic rats. Diabetes. 2004;53(2):418-425.

17. McNay EC, Williamson A, McCrimmon RJ, Sherwin RS. Cognitive and neural hippocampal effects of long-term moderate recurrent hypoglycemia. Diabetes. 2006;55(4):1088-1095.

18. McNay E. Recurrent hypoglycemia increases anxiety and amygdala norepinephrine release during subsequent hypoglycemia. Front Endocrinol (Lausanne). 2015;6:175.

19. Diabetes C, Complications Trial, Epidemiology of Diabetes I, Complications Study Research G, et al. Long-term effect of diabetes and its treatment on cognitive function. N Engl J Med. 2007;356(18):1842-1852.

20. Hansen TI, Olsen SE, Haferstrom E, et al. Cognitive deficits associated with impaired awareness of hypoglycaemia in type 1 diabetes. Diabetologia. 2017;60(6):971-979.

21. Cox DJ, Penberthy JK, Zrebiec J, et al. Diabetes and driving mishaps: frequency and correlations from a multinational survey. Diabetes Care. 2003;26(8):2329-2334.

22. Lubow JM, Piñón IG, Avogaro A, et al. Brain oxygen utilization is maintained by the differential expression of glycolytic isoforms. Diabetes. 1995;44(12):1399-1404.

23. Wahren J, Ekberg K, Fernqvist-Forbes E, Nair S. Brain substrate utilization during acute hypoglycaemia. Diabetologia. 1999;42(7):812-818.

24. Boyle PJ, Nagy RJ, O’Connor AM, Kempers SF, Yeo RA, Qualls C. Adaptation in brain glucose uptake following recurrent hypoglycemia. Proc Natl Acad Sci USA. 1994;91(20):9352-9356.

25. Koranyi L, Bourey RE, James D, Mueckler M, Fiedorek FT Jr, Permutt MA. Glucose transporter gene expression in rat brain: Pretranslational changes associated with chronic insulin-induced hypoglycemia, fast- ing, and diabetes. Mol Cell Neurosci. 1991;2(3):244-252.

26. Kumagai AK, Kang YS, Boaro JRD, Partridge WM. Uptregulation of blood-brain barrier GLUT1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. Diabetes. 1995;44(12):1399-1404.

27. Marin-Hernández A, López-Ramírez SY, Del Mazo-Monsalvo I, et al. Modeling cancer glycolysis under hypoglycemia, and the role played by the differential expression of glycolytic isoforms. FEBS J. 2014;281(15):3325-3345.

28. Mastaitis JW, Wurmbach E, Cheng H, Sealfon SC, Mobbs CV. Acute induction of gene expression in brain and liver by insulin-induced hypoglycemia. Diabetes. 2005;54(4):952-958.

29. McCall AL, Fixman LB, Fleming N, Tornheim K, Chick W, Ruderman NB. Chronic hypoglycemia increases brain glucose transport. Am J Physiol. 1986;251(4 Pt 1):E442-447.

30. Osundji MA, Hurst P, Moore SP, et al. Recurrent hypoglycemia increases hypothalamic glucose phosphorylation activity in rats. Metabolism. 2011;60(4):550-556.

31. Pelligrino DA, Segil LJ, Albrecht RF. Brain glucose utilization and transport and cortical function in chronic vs. acute hypoglycemia. Am J Physiol. 1990;259(5 Pt 1):E729-735.

32. Jiang L, Herzog RI, Mason GF, et al. Recurrent antecedent hypoglycemia alters neuronal oxidative metabolism in vivo. Diabetes. 2009;58(6):1266-1274.

33. Criego AB, Tkac I, Kumar A, Thomas W, Gruetter R, Seaquist ER. Brain glucose concentrations in patients with type 1 diabetes and hypoglycemia unawareness. J Neurosci Res. 2005;79(1-2):42-47.

34. Amoral AI, Teixeira AP, Sonnewald U, Alves PM. Estimation of intracellular fluxes in cerebellar neurons after hypoglycemia: importance of the pyruvate recycling pathway and glutamine oxidation. J Neurosci Res. 2011;89(5):700-710.

35. Telushkin PK, Nozdraчev AD, Potapov PP, Medvedeva NB, Stel’makh AY. Glycolysis and oxidation enzyme activity in rat brain during insulin-induced hypoglycemia against the background of alloxa-induced diabetes mellitus. Bull Exp Biol Med. 2005;140(6):695-697.

36. Dave KR, Tamariz J, Desai KM, et al. Recurrent hypoglycemia exacerbates cerebral ischemic damage in streptozotocin-induced diabetic rats. Stroke. 2011;42(5):1404-1411.

37. Xia J, Wishart DS. Using metaAnalyst 3.0 for comprehensive metabolomics data analysis. Curr Protoc Bioinformatics. 2016;55:14.10.11-14.10.91.

38. Grapov D, Wanichthanarak K, Fiehn O, MapR: pathway independent metabolomic network analysis incorporating unknowns. Bioinformatics. 2015;31(16):2757-2760.

39. Rehni AK, Dave KR. Impact of hypoglycemia on brain metabolism during diabetes. Mol Neurobiol. 2018;55(12):9075-9088.

40. Shukla V, Fuchs P, Liu A, et al. Recurrent hypoglycemia exacerbates cerebral ischemic damage in diabetic rats via enhanced post-ischemic mitochondrial dysfunction. Transl Stroke Res. 2019;10(1):78-90.

41. Newgard CB. Metabolomics and metabolic diseases: Where do we stand? Cell Metab. 2017;25(1):43-56.

42. Prosser GA, Larrouy-Maumus G, de Carvalho LP. Metabolomic strategies for the identification of new enzyme functions and metabolic pathways. EMBO Rep. 2014;15(6):657-669.

43. Avanesov AS, Ma S, Pierce KA, et al. Age- and diet-associated metabolome remodeling characterizes the aging process driven by damage accumulation. Elife. 2014;3:e02077.

44. Denihan NM, Walsh BH, Reinke SN, et al. The effect of haemolysis on the metabolomic profile of umbilical cord blood. Clin Biochem. 2015;48(7-8):534-537.

45. Esko T, Hirschhorn JN, Feldman HA, et al. Metabolomic profiles as reliable biomarkers of dietary composition. Am J Clin Nutr. 2017;105(3):547-554.

46. Karl JP, Margolis LM, Murphy NE, et al. Military training elicits marked increases in plasma metabolomic signatures of energy metabolism, lipolysis, fatty acid oxidation, and ketogenesis. Physiol Rep. 2017;5(17):e14307 https://doi.org/10.14814/phy2.14307.

47. Lee S, Jiang WJ, Choi B, Joo SH, Jeong CH. Comparative metabolomic analysis of HPAC cells following the acquisition of erlotinib resistance. OncoLett. 2017;13(5):3437-3444.

48. Showalter MR, Nonnecke EB, Linderholm AL, et al. Obesogenic diets alter metabolism in mice. PLoS ONE. 2018;13(1):e0190632.

49. Kuo T, McQueen A, Chen TC, Wang JC. Regulation of glucose homeostasis by glucocorticoids. Adv Exp Med Biol. 2015;872:99-126.

50. Bartlett D, Rae C, Thompson C, et al. Hippocampal area metabolites relate to severity and cognitive function in obstructive sleep apnea. Sleep Med. 2004;5(6):593-596.

51. Brewer GJ, Wallmann TW. Protective effect of the energy precursor creatine against toxicity of glatamate and beta-amyloid in rat hippocampal neurons. J Neurochem. 2000;74(5):1968-1978.

52. Mills E, O’Neill LA. Succinate: a metabolic signal in inflammation. Trends Cell Biol. 2014;24(5):313-320.
53. Trettier L, Patocs A, Chinopoulos C. Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. Biochim Biophys Acta. 2016;1857(8):1086-1101.
54. Hu R, Huang D, Tong J, Liao Q, Hu Z, Ouyang W. Aspartic acid in the hippocampus: a biomarker for postoperative cognitive dysfunction. Neural Regen Res. 2014;9(2):143-152.
55. Liu P, Jing Y, Collie ND, Chary S, Zhang H. Memory-related changes in L-citrulline and agmatine in the rat brain. Hippocampus. 2009;19(7):597-602.
56. Yabuki Y, Shioda N, Yamamoto Y, et al. Oral L-citrulline administration improves memory deficits following transient brain ischemia through cerebrovascular protection. Brain Res. 2013;1520:157-167.
57. Liu P, Smith PF, Appleton I, Darlington CL, Bilkey DK. Regional variations and age-related changes in nitric oxide synthase and arginase in the sub-regions of the hippocampus. Neuroscience. 2003;119(3):679-687.
58. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998;336(Pt 1):1-17.
59. Auer RN. Hypoglycemic brain damage. Metab Brain Dis. 2004;19(3–4):169-175.
60. Peng TI, Jou MJ. Oxidative stress caused by mitochondrial calcium overload. Ann N Y Acad Sci. 2010;1201:183-188.
61. Cheng B, McMahon DG, Mattson MP. Modulation of calcium current, intracellular calcium levels and cell survival by glucose deprivation and growth factors in hippocampal neurons. Brain Res. 1993;607(1-2):275-285.
62. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res. 2009;50(Suppl):S237-242.
63. Walter A, Korth U, Hilgert M, et al. Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients. Neurobiol Aging. 2004;25(10):1299-1303.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Dewan N, Shukla V, Rehni AK, et al. Exposure to recurrent hypoglycemia alters hippocampal metabolism in treated streptozotocin-induced diabetic rats. CNS Neurosci Ther. 2020;26:126–135. https://doi.org/10.1111/cns.13186