Published in final edited form as:

Neurocrit Care. 2017 April ; 26(2): 239–246. doi:10.1007/s12028-016-0313-3.

Influence of glycemic control on endogenous circulating ketone concentrations in adults following traumatic brain injury

Stephanie M. Wolahan, PhD, Mayumi L. Prins, PhD, David L. McArthur, PhD, MPH, Courtney R. Real, BSN, RN, David A. Hovda, PhD, Neil A. Martin, MD, Paul M. Vespa, MD, FACS, and Thomas C. Glenn, PhD

UCLA Brain Injury Research Center and Department of Neurosurgery, David Geffen School of Medicine at UCLA, University of California – Los Angeles, Los Angeles, CA

Abstract

Introduction—The objective was to investigate the impact of targeting tight glycemic control (4.4–6.1 mM) on endogenous ketogenesis in severely head injured adults.

Methods—The data were prospectively collected during a randomized, within-patient crossover study comparing tight to loose glycemic control, defined as 6.7–8.3 mM. Blood was collected periodically during both tight and loose glycemic control epochs. Post hoc analysis of insulin dose and total nutritional provision was performed.

Results—Fifteen patients completed the crossover study. Total ketones were increased 81 μ M ([38 135], $p < 0.001$) when blood glucose was targeted to tight (4.4–6.1 mM) compared with loose glycemic control (6.7–8.3 mM), corresponding to a 60% increase. There was a significant decrease in total nutritional provisions ($p = 0.006$) and a significant increase in insulin dose ($p = 0.008$).

Conclusions—Permissive underfeeding was tolerated when targeting tight glycemic control but total nutritional support is an important factor when treating hyperglycemia.

Keywords

Glycemic control; ketone bodies; glucose; insulin

INTRODUCTION

High admission glucose readings as well as persistent hyperglycemia have been correlated to poor neurologic outcomes following traumatic brain injury (TBI) (1, 2) resulting from the toxic effects of high glucose on cells. Intensive insulin therapy (IIT) to keep glucose at normoglycemic levels was shown to improve mortality and infections in ICU patients and was attributed to avoiding hyperglycemic episodes better than conventional insulin therapy (3, 4). Further studies suggested targeting tight glycemic control with IIT (4.4–6.1 mM) resulted in unacceptable risks of hypoglycemic episodes for routine ICU application (5, 6). Following TBI, cerebral glucose uptake was shown to be impaired at normoglycemic levels when concomitant with cerebral metabolic crisis (7). Altogether, cerebral metabolic dysfunction acutely following TBI is not an issue of glucose supply to cerebral tissue yet tight glycemic control was repeatedly associated with cerebral metabolic distress (8, 9).

The consensus on glycemic control acutely following TBI is to avoid hypoglycemic episodes (blood glucose less than 3.9 mM) and hyperglycemic episodes (greater than 11.1 mM) as standard of care (10–12). The focus of research on tight glycemic control is IIT but less widely discussed is the role of nutrition in these studies. TBI patients are commonly hyperglycemic yet undernourished. This led us to question the balance between nutritional provisions and insulin infusion in a within-patient randomized crossover trial of tight versus loose glycemic control (9). We performed a post hoc analysis of total nutritional provisions and insulin dose in fifteen adult TBI patients.

Because there is evidence of hypocaloric supplementation in ICU studies, we were also interested to know if reduced cerebral glucose uptake could be augmented by the ketone bodies, acetoacetate (AcAc) and β -hydroxybutyrate (BHB). Ketone bodies are important cerebral fuels during fasting, are generated through lipid catabolism, and can be metabolized by the brain to sustain cerebral tissue through periods of low glucose. In animal models of TBI, ketones have been shown to generate cytosolic acetyl-CoA (13)), increase mitochondrial oxidative phosphorylation in contusional tissue (14), and prevent oxidative stress-mediated mitochondrial dysfunction (15).

We set out to study the influence of glycemic control on endogenous ketogenesis. Following consent, patients were randomly assigned to target tight glycemic control (4.4–6.1 mM) or loose glycemic control (6.7–8.3 mM) for 24 hours at which point the glycemic target would switch to the other range for 24 hours to complete the randomized within-patient controlled study. We collected blood from severe TBI patients during both glycemic control epochs, quantified arterial ketone concentrations, and modeled the ketone concentrations with linear mixed effects models, using time since injury, insulin dose, and nutritional provisions as predictors in the model. Our hypothesis is tight glycemic control enhances ketogenesis that leads to increased serum levels of ketones.

MATERIALS AND METHODS

Cohort criteria

Fifteen patients consented to the University of California at Los Angeles (UCLA) Brain Injury Research Center (BIRC) study of moderate to severe head injuries, approved by the UCLA Medical Institutional Review Board for human research, were included. Eligible patients for UCLA BIRC studies include all mechanically ventilated head-injured patients, aged 16 years and older, admitted to the UCLA Medical Center within 24 hours of injury. Eligible patients have admission Glasgow Coma Scale (GCS) scores ≥ 8 or, if higher, deteriorate to ≥ 8 prior to emergency department discharge or present with positive gross CT findings. Exclusion criteria include pre-existing neurologic illness, prior head injuries, and any history of diabetes mellitus. Details of the patient cohort are presented in Table 1.

The general management protocol of TBI patients includes maintenance of ICP <20 mmHg and CPP >60 mmHg (16). Patients were initially treated with a standard approach to control blood glucose, including subcutaneous regular insulin (Humulin; Eli Lilly, Indianapolis, IN) for admission blood glucose above 8.3 mM and a standard insulin sliding scale protocol to keep blood glucose between 5.6 and 8.3 mM. Nutritive support was begun by enteral feeding

routes as soon as possible. Enteral nutrition (Osmolite 1.2, Abbott Nutrition) was provided via nasogastric tube with feedings adjusted for caloric goals individually, targeting 25–30 kcal/kg with 1.3–1.5 g_{protein}/kg within a 24 hour period. Eight of 15 patients were monitored with cerebral microdialysis at the time of biofluid collection, as previously described (17) using the CMA70 probe (10-cm flexible shaft, 10-mm membrane length, 20-kD cutoff, CMA, Stockholm, Sweden).

Study design

Following consent, patients were randomly assigned to target tight or loose glycemic control first, switching to the other arm after 24 hours. Fifteen patients completed the crossover (Table 1). Tight glycemic control was achieved by an intensive insulin infusion protocol to target blood glucose concentrations of 4.4–6.1 mM, which is a normal glucose concentration in a healthy, fasted adult. Serial blood glucose measurements were obtained with a point-of-care testing device (Accucheck) hourly to titrate insulin infusion rate. Loose glycemic control, defined here as targeting blood glucose to 6.7–8.3 mM (8), was achieved by reducing insulin administration, either lowering insulin infusion rate or switching to a sliding scale protocol, and/or supplemental intravenous dextrose administration.

At scheduled times during the glycemic control epoch, blood was drawn for NMR analysis subject to constraints including catheter removal or declining health status. Blood was collected in heparin-coated tubes and placed on ice. After centrifugation, samples were stored at –80°C until the time of analysis. In addition to the hourly blood samples required for point-of-care glucose testing, blood was drawn during both glycemic control epoch for hospital laboratory blood gas analysis.

Nutritional therapy, intravenous medications prepared in dextrose solutions, and propofol were documented and included to quantify the total daily caloric provision. Medication information, nutritional support, bedside glucose readings (point-of-care glucose, or POC glucose), insulin dose, and clinical blood gas laboratory results were obtained from the UCLA BIRC database. Nutritional provision at the time of blood draw is defined as the total kilocalories delivered in the preceding 24 hours by both enteral and parenteral means. Enteral nutrition provision at the time of blood draw accounts only for kilocalories delivered enterally in the last 24 hours. Both metrics are presented in units of kilocalories per kilogram per day. Average insulin dose is defined as the total insulin units (IU) infused in the preceding 24 hours, presented in units of IU/hour (4). Mean amplitude glycemic excursions (MAGE) were determined for each glycemic control assignment epoch (18, 19) and was calculated with the following equation: $\sum \lambda / n$, where $\lambda > \sigma$ and σ is the standard deviation of all n glucose measurements within a 24 hour period.

Ketone measurements

Ketone concentrations were determined by ¹H NMR spectroscopy. The NMR sample preparation protocol for plasma was developed from the methods described by Gowda et. al. (20). Following solvent removal, plasma samples were reconstituted in 650 μ L of 0.1 M phosphate buffered solution in deuterium oxide at pH 7.4, prepared with 0.1 mM ¹³C-

labeled sodium formate (isotopic enrichment 99%, Cambridge Isotope Laboratories, Andover, MA) as an NMR internal standard.

All NMR spectra were collected using standard Bruker *cpmgpr1d* pulse program on a Bruker AVANCE 600 MHz NMR Spectrometer equipped with an inverse broadband probe (parameter settings NS 32, SW 8 kHz, RG 128, O1 4.70 ppm, D1 5s, 90° pulse 13 μ s, and a 200 ms CPMG decay period). Processed spectra were imported into R, version 3.1.3 (21), using the *rNMR* package (rNMR version 1.1.8) (22). Ketones of interest were quantified by integration of a particular chemical shift range (23, 24). We normalized plasma metabolite concentrations by the glucose concentrations determined with the Analox glucose analyzer (Analox GM7, Analox Instruments, London, UK) similar to the method described by Rasmussen and colleagues (25). There was a strong correlation between linearly interpolated POC glucose at the time of blood draw and glucose concentrations measured with a laboratory benchtop analyzer at the time of NMR analysis ($r_{pb}=0.89$, [0.83 0.93], $p<0.001$).

Statistical analysis

Due to nonnormalities in data distributions, we used robust statistical analytic methods (26). Cohort statistics are presented with Harrell-Davis estimates of medians and interquartile ranges (IQR). To summarize characteristics of the glycemic control assignment epochs, we use 20% trimmed means and the dependent Yuen test. Correlation coefficients are a robust analogue of Pearson's correlation, the percentage bend correlation (r_{pb}), followed by the 95% confidence interval and p-value. The Williams test was used to test the significance of the difference between two correlations, as implemented in the *r.test()* function from the *psych* package (27).

Due to the repeated measures design and unbalanced number of ketone measurements between epochs, we used mixed effects models as implemented in the *nlme* package (28, 29). To predict arterial ketone concentrations, we began with a full candidate mixed effects model including post-injury day (PID = post-injury hour/24), average insulin dose, total nutritional provision, glycemic control assignment, and all possible interactions. The random effect included was subject. Inclusion of random slopes in the full candidate model were rejected because there was no improvement to the fit. We modeled the logarithmic-transform of arterial ketone concentration to control heteroscedasticity. The best approximating model (Table 3) was selected by multimodel inference, an information-theoretic approach, such that inferences about model precision are made across the entire set of models with the Akaike information criteria (AIC) (30, 31). Model results are back-transformed into units of μ M.

RESULTS

Glycemic control epoch characterization

There was no difference in the average PID of the initiation of the tight and loose glycemic control studies nor in the duration of the glycemic control epochs (Table 2). No periods of hypoglycemia were observed during the loose glycemic control epochs. Five patients experienced periods of hypoglycemia, defined as POC glucose below 3.9 mM, ranging from

2% to 13% of the assigned tight glycemic control epoch. Average point-of-care (POC) blood glucose readings were significantly lower when patients were assigned to tight glycemic control. There was no difference between the average mean amplitude glycemic excursions (MAGE) values ($p=0.90$). No seizures were observed during any glycemic control epoch.

We compared nutritional and insulin therapy during tight and loose glycemic conditions to assess the characteristics of glycemic control. On average, patients received nutritional therapy at a reduced rate under tight glycemic control. The total nutritional provision was decreased 8.3 kcal/kg ([3.0 13.6], $p=0.006$) from loose to tight glycemic control (Figure 1A). Enteral nutritional provision was decreased 5.5 kcal/kg ([−0.5 11.4], $p=0.066$) under tight glycemic control (Figure 1B). Non-enteral nutrition calories include propofol and dextrose-containing medications and there is a great deal of variability between patients. Insulin infusions were ordered for all patients under tight glycemic control. Nine of 15 patients also received insulin infusions under loose glycemic control. Average insulin dose was significantly higher under tight glycemic control, increasing 1.3 IU/hr ([0.4 2.1], $p=0.008$) (Figure 1C).

Blood plasma ketones

The correlation between arterial BHB and AcAc was significantly larger ($p<0.001$) under tight glycemic control ($r_{pb}=0.90$ [0.82 0.95], $p<0.001$) than loose glycemic control ($r_{pb}=0.27$ [−0.04 0.53], $p=0.084$). Total ketones were not correlated to insulin dose ($p=0.995$) and glucose concentration ($p=0.20$). There was a weak correlation to PID ($r_{pb}=0.22$ [−0.002 0.41], $p=0.053$) and a moderate negative correlation to total nutritional provision ($r_{pb}=−0.48$ [−0.63 −0.29], $p<0.001$). There were no significant correlations between arterial glucose, insulin dose, and nutrition provision within the full data set or within the tight and loose control subsets. There was no discernable change between arterial and jugular venous concentrations of BHB or AcAc and arterial and jugular concentrations were strongly correlated ($r_{pb}=0.98$ [0.97 0.99], $p<0.001$).

Arterial ketone concentrations were modeled as the log-transform of total ketone, the sum of BHB and AcAc, concentrations quantified with NMR spectroscopy. The mixed effects model results presented in Table 3 reveal arterial total ketone concentrations increased, on average, 81 μM ([38 135], $p<0.001$) between loose and tight glycemic control. Increased total nutritional provision significantly decreased arterial ketones 4 μM ([1 7], $p<0.001$) for a 1 kcal/kg increase in nutritional provision. To put this in context, assuming glycemic control, insulin, and PID are constant, arterial ketones would decrease approximately 25 μM for a 6.2 kcal/kg increase in total nutritional provision, the average change in total nutritional provisions described above (Figure 1A).

Interaction terms in the best approximating model revealed that the ketogenic response associated with tight glycemic control was muted over time. Average insulin dose and PID had nonsignificant effects as individual predictors but model selection indicated the following two interaction terms important to the best approximating model: PID by glycemic control and PID by average insulin dose. The model estimates that, assuming nutrition and insulin are constant, the increase in arterial ketone concentration from loose to tight glycemic control (i.e. 81 μM) is significantly decreased one day later by 80 μM ([−100

–57], $p < 0.001$). Alternatively, this interaction term corresponds to an additional increase in arterial ketones one day before. The second interaction term, PID by average insulin dose, indicates very similar trends in the data to the first because switching from loose to tight glycemic control is often achieved by increasing insulin dose. Assuming all else is constant, arterial ketones decrease 19 μM ([12 27], $p < 0.001$) for a 1 IU/hr increase in insulin dose one day later and similarly increase one day before.

Microdialysis

Previous research showed tight glycemic control was associated with critically low microdialysis glucose values. For eight patients monitored with microdialysis, tight glycemic control was associated with a significant decrease in all CMD metabolites (Table 4). Arterial plasma ketone concentrations were negatively correlated to CMD glucose under tight glycemic control ($r_{pb} = -0.56$ [–0.79 –0.20], $p = 0.004$) and were not correlated to other CMD measures. Although the change in arterial ketone to CMD glucose correlation coefficients did not reach significance at the 0.05 level ($p = 0.080$), there was a weaker, nonsignificant correlation between plasma ketones and CMD glucose under loose glycemic control ($r_{pb} = -0.11$ [–0.47 0.28], $p = 0.58$).

DISCUSSION

Tight glycemic control enhanced ketogenesis in the severely head injured cohort included in this randomized, within-patient crossover study. Post hoc analysis shows a significant decrease in total nutritional provisions and a significant increase in insulin dose during tight glycemic control compared with loose glycemic control. Holding insulin, nutrition, and PID constant, total arterial ketone concentrations increased 81 μM ([38 135], $p < 0.001$) when blood glucose was targeted to tight (4.4–6.1 mM) compared with loose glycemic control (6.7–8.3 mM). These results show that adult TBI patients have the ability to produce ketones even when receiving caloric supplementation.

As the brain recovers from injury, cerebral energy demands are high at the same time that cerebral glucose metabolism is depressed and systemic metabolism is elevated. The healthy brain supplements its primary energy substrate, glucose, with lactate and ketones under various conditions (32–36). Following TBI, ketones may be an efficient way to fuel the recovery process. Prins and colleagues studied cerebral ketone metabolism and showed exogenous infusion of BHB resulted in cerebral BHB oxidation (13). Both juvenile and adult head injured rats were found to have increased MCT protein expression compared with controls (37) and the ketogenic diet was shown to prevent mitochondrial dysfunction (15).

In both healthy and critically ill adults, cerebral ketone uptake increases as arterial ketone concentrations increase (38, 39) but augmentation of ketones does not necessarily increase cerebral ketone flux in ICU patients (40). A limitation of this observational study is we were unable to measure cerebral ketone flux. Further research using isotope-based tracer infusion studies is required to determine if the endogenous circulating arterial ketones observed in this cohort are being oxidized by the injured brain. The patients included in this study reached total ketone levels expected in a healthy adult after a moderate fast (>24 hours) even when receiving continuous nutritional support. Our data suggest a varied level of ketogenesis

during critical care that is not simply the result of starvation, insulin resistance, or hypoglycemia. Limitations of this study include the lack of a washout period between study days and the collection of blood samples to measure ketones at irregular times within the glycemic control epochs.

Tight glycemic control was associated with significant decreases in all CMD metabolites measured (Table 4). In general, low CMD glucose can be associated with poor outcomes, particularly when CMD LPR is high (8, 9, 16). For the eight patients monitored with CMD included in this cohort, the corrected estimates of CMD metabolites and microdialysis marker burdens during tight and loose glycemic control are consistent with the results presented by Vespa et. al. (2012). If the injured brain supplements cerebral energy production with ketone oxidation in response to increased plasma ketone levels, this could support the recovering cerebral tissue when glucose metabolism is dysfunctional or glucose availability is reduced, a risk associated with IIT targeting normoglycemia. There is high variability in plasma ketones and, in light of a reduced ketogenic response in some patients, a prospectively designed study will be necessary to fully assess the relationship between ketones, cerebral oxidation of this supplemental substrate, and energy crisis.

Knowing that controlling blood glucose in the NeuroICU improves outcomes, understanding the relationship between the systemic responses to dysfunctional cerebral glucose metabolism after trauma could reveal potential therapeutics. Endogenous ketogenesis was reported in severely head injured adults studying the impact of providing glucose acutely (41, 42). Our study shows that endogenous ketogenesis can occur when receiving standard enteral nutrition combined with intravenous insulin infusion. The reality in intensive care is that nutritional targets are routinely missed, glycemic control guidelines remain inconclusive, and clinical trials have not been able to decisively answer these questions (3, 6, 43, 44).

The increase in endogenous ketogenesis was concurrent with reduced caloric intake and increased insulin dose under tight glycemic control. This confounding limits our conclusions but, as the only example of a within-patient comparison of tight and loose glycemic control protocols, could have serious implications for future research on IIT in TBI patients and all critically ill patients. The benefits of tight glycemic control are likely moderated by glycemic variability, time since injury, and the patient's tolerance of enteral nutrition. Patient-tailored nutritional support, glycemic control, and insulin therapy primarily relies on monitoring blood glucose and assumptions about systemic resting metabolic rates based on the sex, age, height, and weight of healthy adults. The presence of increased endogenous ketogenesis under tight glycemic control shows the body responds by supplementing glucose with other cerebral energy sources. Exogenous infusion of supplemental substrates may be a key component of a potentially powerful therapy to support systemic and cerebral recovery in addition to glucose.

CONCLUSIONS

Ketogenesis was significantly increased in adult TBI patients when assigned to tight glycemic control, targeting 4.4–6.1 mM by intensive insulin therapy, compared to loose

glycemic control, targeting 6.7–8.3 mM by reduced insulin infusion. Post hoc analysis revealed there was a significant decrease in total nutritional provision under tight glycemic control. Tight glycemic control was associated with a 60% increase in total ketone concentration, increasing 81 μ M from loose to tight glycemic control. A prospectively designed study will be necessary to fully assess the relationship between ketones, cerebral oxidation of this supplemental substrate, and energy crisis.

Acknowledgments

The authors would like to thank the nursing and research staff in the Ronald Reagan UCLA Medical Center neurological intensive care unit. This material is based upon work supported by the UCLA Brain Injury Research Center. The Bruker AV500 NMR spectrometer used was supported by the National Science Foundation under equipment grant no. CHE-1048804.

FUNDING SUPPORT

NIH P01NS058489; grant recipient David Hovda, Ph.D.

NIH R21NS093136-01; grant recipient Thomas Glenn, Ph.D.

REFERENCES

1. Lam A, Winn H, Cullen B, Sundling N. Hyperglycemia and neurological outcome in patients with head injury. *J Neurosurg.* 1991; 75:545–551. [PubMed: 1885972]
2. Liu-DeRyke X, Collingridge DS, Orme J, Roller D, Zurasky J, Rhoney DH. Clinical Impact of Early Hyperglycemia During Acute Phase of Traumatic Brain Injury. *Neurocrit Care.* 2009; 11:151–157. [PubMed: 19479209]
3. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in critically ill patients. *N Engl J Med.* 2001; 345:1359–1367. [PubMed: 11794168]
4. Van den Berghe G, Wouters P, Bouillon R, Weekers F, Verwaest C, Schetz M, et al. Outcome benefit of intensive insulin therapy in the critically ill: Insulin dose versus glycemic control. *Crit Care Med.* 2003; 31:359–366. [PubMed: 12576937]
5. Oddo M, Schmidt J, Carrera E, Badjatia N, Connolly E, Presciutti M, et al. Impact of tight glycemic control on cerebral glucose metabolism after severe brain injury: A microdialysis study. *Crit Care Med.* 2008; 36:3233–3238. [PubMed: 18936695]
6. Finfer S, Chittock D, Su S, Blair D, Foster D, Dhingra V, et al. Intensive versus Conventional Glucose Control in Critically Ill Patients. *N Engl J Med.* 2009; 360:1283–1297. [PubMed: 19318384]
7. Magnoni S, Tedesco C, Carbonara M, Pluderi M, Colombo A, Stocchetti N. Relationship between systemic glucose and cerebral glucose is preserved in patients with severe traumatic brain injury, but glucose delivery to the brain may become limited when oxidative metabolism is impaired: implications for glycemic control. *Crit Care Med.* 2012; 40:1785–1791. [PubMed: 22610183]
8. Vespa P, Boonyaputthikul R, McArthur D, Miller C, Etchepare M, Bergsneider M, et al. Intensive insulin therapy reduces microdialysis glucose values without altering glucose utilization or improving the lactate/pyruvate ratio after traumatic brain injury. *Crit Care Med.* 2006; 34:850–856. [PubMed: 16505665]
9. Vespa P, McArthur D, Stein N, Huang SC, Shao W, Filippou M, et al. Tight glycemic control increases metabolic distress in traumatic brain injury: A randomized controlled within-subjects trial. *Crit Care Med.* 2012; 40:1923–1929. [PubMed: 22610193]
10. Le Roux P, Menon DK, Citerio G, Vespa P, Bader MK, Brophy GM, et al. Consensus Summary statement of the International Multidisciplinary Consensus Conference on multimodality Monitoring in Neurocritical Care. *Neurocrit Care.* 2014 Published online 11 September 2014.

11. Badjatia N, Vespa P. the Participants of the International Multi-disciplinary Consensus Conference on Multimodality Monitoring. Monitoring nutrition and glucose in acute brain injury. *Neurocrit Care*. 2014 Epub ahead of print.
12. Oddo M, Schmidt J, Mayer S, Chiolerio R. Glucose control after severe brain injury. *Curr Opin Clin Nutr Metab Care*. 2008; 11:134–139. [PubMed: 18301088]
13. Prins ML, Lee SM, Fujima L, Hovda DA. Increased cerebral uptake and oxidation of exogenous betaHB improves ATP following traumatic brain injury in adult rats. *J Neurochem*. 2004; 90:666–672. [PubMed: 15255945]
14. Davis LM, Pauly JR, Readnower RD, Rho JM, Sullivan PG. Fasting is neuroprotective following traumatic brain injury. *J Neurosci Res*. 2008; 86:1812–1822. [PubMed: 18241053]
15. Greco T, Glenn TC, Hovda DA, Prins ML. Ketogenic diet decreases oxidative stress and improves mitochondrial respiratory complex activity. *J Cerebr Blood Flow Metab*. 2015 Oct. Pii: 0271678X15610584. [Epub ahead of print].
16. Vespa P, Bergsneider M, Hattori N, Wu HM, Huang SC, Martin N, et al. Metabolic crisis without brain ischemia is common after traumatic brain injury: A combined microdialysis and positron emission tomography study. *J Cerebr Blood Flow Metab*. 2005; 25:763–774.
17. Vespa P, McArthur D, O’Phelan K, Glenn T, Etchepare M, Kelly D, et al. Persistently low extracellular glucose correlates with poor outcome 6 months after human traumatic brain injury despite a lack of increased lactate: A microdialysis study. *J Cerebr Blood Flow Metab*. 2003; 23:865–877.
18. Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes*. 1970; 19:644–655. [PubMed: 5469118]
19. Siegelar SE, Holleman F, Hoekstra JBL, DeVries JH. Glucose variability: does it matter? *Endocrine Reviews*. 2010; 31:171–182. [PubMed: 19966012]
20. Gowda G, Tayyari F, Ye T, Suryani Y, Wei S, Shanaiah N, et al. Quantitative Analysis of Blood Plasma Metabolites Using Isotope Enhanced NMR Methods. *Anal Chem*. 2010; 82:8983–8990. [PubMed: 20879716]
21. R Core Team. R: A Language and environment for statistical computing. Vienna, Austria: 2013. ISBN 3-900051-07-00. Available from: <http://www.R-project.org/>
22. Lewis I, Schommer S, Markley J. rNMR: open source software for identifying and quantifying metabolites in NMR spectra. *Magn Reson Chem*. 2009; 47:S123–S126. [PubMed: 19821464]
23. Wishart D, Lewis M, Morrissey J, Flegel M, Jeroncic K, Xiong Y, et al. The human cerebrospinal fluid metabolome. *J Chromatogr B*. 2008; 871:164–173.
24. Ulrich E, Akutsu H, Doreleijers J, Harano Y, Ioannidis Y, Lin J, et al. BioMagResBank. *Nucleic Acids Res*. 2008; 36:D402–D408. [PubMed: 17984079]
25. Rasmussen P, Nyberg N, Jaroszewsk J, Krogh-Madsen R, Secher N, Quistorff B. Brain nonoxidative carbohydrate consumption is not explained by export of an unknown carbon source: evaluation of the arterial and jugular venous metabolome. *J Cerebr Blood Flow Metab*. 2010; 30:1240–1246.
26. Wilcox, R. Introduction to robust estimation and hypothesis testing. Academic Press; 1997.
27. Revelle W. Package ‘psych’; 2015. R package version 1.5.8. Available from: <http://CRAN.R-project.org/package=psych>.
28. Pinheiro, J., Bates, D. Mixed-Effects Models in S and S-PLUS. Springer; 2000.
29. Pinheiro J, Bates D, DebRoy S, Sarkar D. R Core Team. nlme: Linear and Nonlinear Mixed Effects Models; 2013. R package version 3.1-108. Available from: <https://cran.r-project.org/web/packages/nlme/index.html>.
30. Burnham, KP., Anderson, DR. Model selection and multimodel inference: A practical information-theoretic approach. 2nd. Springer; 2002.
31. Barton K. MuMIn: Multi-model inference; 2013. R package version 1.9.5. Available from: <http://CRAN.R-project.org/package=MuMIn>.
32. Herzog RI, Jiang L, Herman P, Zhao C, Sanganahalli BG, Mason GF, et al. Lactate preserves neuronal metabolism and function following antecedent recurrent hypoglycemia. *J Clin Invest*. 2013; 123:1988–1998. [PubMed: 23543056]

33. Dalsgaard M. Fuelling cerebral activity in exercising man. *J Cerebr Blood Flow Metab.* 2006; 26:731–750.
34. Glenn TC, Martin NA, Horning MA, McArthur DL, Hovda DA, Vespa PM, et al. Lactate: Brain fuel in human traumatic brain injury. A comparison to normal healthy control subjects. *J Neurotrauma.* 2015 Epub ahead of print.
35. Prins ML. Cerebral metabolic adaptation and ketone metabolism after brain injury. *J Cerebr Blood Flow Metab.* 2008; 28:1–16.
36. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids.* 2004; 70:309–319.
37. Prins M, Giza C. Induction of Monocarboxylate Transporter 2 Expression and Ketone Transport following Traumatic Brain Injury in Juvenile and Adult Rats. *Dev Neurosci.* 2006; 28:447–456. [PubMed: 16943667]
38. Moore TJ, Lione AP, Sugden MC, Regen DM. Hydroxybutyrate transport in rat brain: developmental and dietary modulations. *Am J Physiol Endocrinol Metab.* 1976; 230:619–630.
39. Pan JW, Telang FW, Lee JH, de Graaf RA, Rothman DL, Stein DT, et al. Measurement of β -hydroxybutyrate in acute hyperketonemia in human brain. *J Neurochem.* 2001; 79:539–544. [PubMed: 11701757]
40. Beylot M, Guiraud M, Grau G, Bouletreau P. Regulation of ketone body flux in septic patients. *Am J Physiol Endocrinol Metab.* 1989; 257:E665–E674.
41. Robertson C, Goodman J, Narayan R, Contant C, Grossman R. The effect of glucose administration on carbohydrate metabolism after head injury. *J Neurosurg.* 1991; 74:43–50. [PubMed: 1984505]
42. Ritter A, Robertson C, Goodman J, Contant C, Grossman R. Evaluation of a carbohydrate-free diet for patients with severe head injury. *J Neurotrauma.* 1996; 13:473–485. [PubMed: 8880611]
43. Singer P, Anbar R, Cohen J, Shapiro H, Shalita-Chesner M, Lev S, et al. The tight calorie control study (TICACOS): a prospective, randomized, controlled pilot study of nutritional support in critically ill patients. *Intensive Care Med.* 2011; 37:601–609. [PubMed: 21340655]
44. Bilotta F, Caramia R, Cernak I, Paoloni F, Doronzio A, Cuzzone V, et al. Intensive Insulin Therapy After Severe Traumatic Brain Injury: A Randomized Clinical Trial. *Neurocrit Care.* 2008; 9:159–166. [PubMed: 18373223]

Figure 1.

Nutritional therapy was reduced and insulin therapy was increased under tight glycemic control. **A.** Total nutritional provision in the preceding 24 hours decreased 8.3 kcal/kg ([3.0 13.6], $p=0.006$) between loose and tight. **B.** Enteral nutritional provision decreased 5.5 kcal/kg ([−0.5 11.4], $p=0.066$) between loose and tight. **C.** Insulin dose increased 1.3 IU/hr ([0.4 2.1], $p=0.008$) between loose and tight glycemic control. Data are presented as violin plots where the outside border estimates the shape of the data distribution; the horizontal black line plots the mixed effects model corrected estimate; the top and bottom of the

rectangles the upper and lower 95% confidence intervals for the estimate, respectively; and the grey horizontal lines the Harrell-Davis estimates of first, second, and third quartiles from lowest to highest.

Table 1

Patient cohort

Number of patients	15 (4 Females)
Age	38 (IQR 26–52)
Glasgow Coma Scale (GCS) score	4 (IQR 3–8)
Glasgow Outcome Scale extended (GOSe) score at discharge	2.4 (IQR 2.0–3.1)
GOSe at 6 months post-injury [†]	3.3 (IQR 2.0–5.0)
Blood ketone measurements per glycemic control epoch	3, range 1–5
Number patients first assigned tight glycemic control	6 (40%)

[†]3 subjects lost to follow up.

Table 2

Descriptives of glycemic control epochs, including post-injury day of initiation of glycemic control epochs, duration of glycemic control epochs, average point-of-care (POC) glucose, mean amplitude glycemic excursions (MAGE) during the glycemic control assignments, and hematocrit test results.

	Tight glycemic control	Loose glycemic control	t value (DF)	p value
<i>Descriptives of glycemic control epochs</i>				
PID of start of glycemic control epoch (day)	4.3 [3.2 5.5]	4.0 [3.1 4.9]	0.6 (8)	0.58
Duration of glycemic control epochs (hour)	26.8 [21.5 32.0]	25.9 [23.1 28.8]	0.4 (8)	0.70
Average POC glucose (mM)	5.9 [5.6 6.2]	7.4 [7.0 7.8]	-7.8 (8)	<0.001
MAGE (mM)	0.22 [0.13 0.31]	0.21 [0.11 0.31]	0.1 (8)	0.90
Hematocrit (%) [†]	28.5 [26.9 30.0]	28.6 [26.8 30.4]	-0.2 (9)	0.84

[†] 14 of fifteen patients had clinical blood labs ordered under both glycemic control conditions.

Table 3

Summary of mixed effects models of arterial blood ketone concentrations (μM) including the following factors and all interactions: glycemic control, total nutritional provision, average insulin dose, and post-injury day (PID). The intercept estimate represents the model fit for arterial total ketones under loose glycemic control, at 0 IU/hr insulin dose, at 17 kcal/kg total nutritional provision, and at PID 5.24 (nutritional provision and PID were mean centered).

	Full candidate model		Best approximating model	
	Estimate [95%CI]	t-value (DF=52)	Estimate [95%CI]	t-value (DF=61) p-value
Intercept	129 [80 207]	18.3	137 [93 200]	24.7
Tight glycemic control	90 [6 226]	2.0	81 [38 135]	4.1 <0.001
Total nutritional provision	-2 [-6 2]	-1.1	-4 [-7 1]	-2.8 0.007
Average insulin dose	9 [-30 64]	0.4	-3 [-14 8]	-0.5 0.60
PID	-10 [-38 27]	-0.5	-15 [-31 4]	-1.5 0.14
<i>Interaction terms</i>				
Glycemic:nutrition	-4 [-11 3]	-1.0		
Glycemic:insulin	-42 [-90 22]	-1.2		
Glycemic:PID	-91 [-127 -37]	-2.7	-80 [-100 -57]	-5.4 <0.001
Nutrition:insulin	-5 [-15 6]	-0.9		
Nutrition:PID	-1 [-4 2]	-0.8		
Insulin:PID	9 [-13 36]	0.7	19 [12 27]	5.2 <0.001
Glycemic:Nutrition:Insulin	5 [-9 20]	0.6		
Glycemic:Nutrition:PID	1 [-2 5]	0.7		
Glycemic:Insulin:PID	13 [-3 31]	1.5		
Nutrition:Insulin:PID	4 [-5 13]	0.8		
Glycemic:Nutrition:Insulin:PID	-3 [-10 5]	-0.7		

Table 4

Cerebral microdialysis glucose, lactate, pyruvate and Lactate:Pyruvate ratio (LPR) (N=8).

At the time of blood collection				
	Tight glycemic control	Tight glycemic control	t value (DF=42)	p value
Glucose (mM)	1.0 [0.4 1.5]	1.5 [0.9 2.1]	5.3	<0.001
Lactate (mM)	3.5 [2.6 4.3]	4.4 [3.6 5.3]	4.8	<0.001
Pyruvate (μM)	142 [112 171]	170 [141 199]	3.8	<0.001
LPR	24 [23 26]	26 [24 28]	2.0	0.048
Microdialysis marker burden (% time)				
	Tight glycemic control	Tight glycemic control	t value (DF=42)	p value
Glucose 0.6mM	47 [11 83]	39 [NA 84]	1.4	0.20
LPR 28	26 [NA 58]	10 [0 20]	1.3	0.22