Serial changes in plasma ketone concentrations in patients with acute brain injury

White, Hayden; Venkatesh, Balasubramanian; Jones, Mark; Fuentes, Hesly

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Introduction

Acute brain injury (ABI) including, traumatic brain injury (TBI), cerebrovascular accident (CVA) and subarachnoid haemorrhage (SAH) are common and catastrophic events resulting in significant morbidity and mortality. Much of the pathophysiology leading to the ongoing injury relates to disruption of the normal metabolic pathways of the brain and the subsequent inability to adequately meet cerebral energy requirements. While glucose under normal circumstances provides the major substrate for cerebral metabolism, hyperglycemia clearly leads to worse outcomes following ABI[1]. Ketones may represent an alternative source of fuel in these instances [2].

Ketogenesis is the process by which ketone bodies (beta-hydroxybutyrate (BHB) and acetoacetate), during times of starvation or catecholaminergic stress, are produced via fatty acid metabolism. In health, glucose is the brain's main metabolic substrate for fulfilling energy requirements [3]. During times of oxidative stress however, the brain may alter its ability to use glucose as a substrate for metabolic activity[4]. During these periods, ketones are capable of supplying up to 70% of basal cerebral energy requirements [5]. There is evidence that after acute brain injury, ketone utilization may be preferred to glucose, that it improves cerebral energy production and decreases cerebral injury [6]. Animal models simulating both rapidly developing pathologies (glutamate excitotoxicity, hypoxia/ischemia) and neurodegenerative conditions (Parkinson's disease, Alzheimer's disease) and more recently TBI have found ketosis produces a number of beneficial effects including; improved cerebral energetics, decrease cerebral injury and improved cerebral blood flow [7, 8].

There is a paucity of research examining ketones in critically ill patients with ABI and the role of ketone supplementation. Existing research has demonstrated a number of potential benefits to exogenous ketone administration. These include; that ketones can be given enterally or intravenously with minimal side effects, utilization of ketone bodies increase with rising blood concentrations, hyperketosis is associated with improved glucose control, increased plasma levels lead to increase in cerebral uptake and improvement in cognitive function in patients with chronic neurodegenerative disorders. However, well designed outcome studies are lacking [9-12].

Many unanswered questions remain including optimum route of administration and target plasma concentration. Generally, plasma ketone concentrations can range from <0.1 mmol/l in the postprandial state to over 6 mmol/l during prolonged fasting/starvation states and may reach 25 mmol/l in uncontrolled diabetes. However, little is known about ketone physiology during critical illness and ABI.

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Tamaki et al investigated the physiologic changes to ketone bodies in patients with SAH [13]. They noted a strong correlation between ketone body concentrations and catecholamine levels. Although the pathogenesis differs, many regulatory hormones are abnormally raised post ischemic stroke, TBI or SAH. As such, catecholamine, cortisol and glucocorticoid levels are universally increased acutely while there is evidence that the production of counteregulatory hormones such as insulin may be impaired [14-16].

The principle drivers for ketogenesis include fasting (glucopenia) and catecholaminergic stress[2]. We hypothesised that ketone concentrations would remain low in patients with ABI although, the impact of critical illness with its associated catecholaminergic stress is unpredictable. Furthermore, the unpredictability of enteral absorption during the critically ill period may lead to altered glucose and insulin metabolism. Prior to undertaking studies on ketone supplementation in these patients, it is important to quantify baseline concentrations. The purpose of this study is to examine the physiological changes in ketone concentrations following ABI.

Materials and Methods

This dual-site, prospective, observational study was conducted over a 12 month period to define baseline ketone levels in plasma and cerebrospinal fluid (CSF) following acute brain injury. The study setting was two metropolitan Intensive Care Units (ICU). Ethics approval was granted by the Princess Alexandra Hospital Human Research Ethics Committee. The requirement for written informed consent was waived by the institutional review board.

Patients were enrolled as soon as possible following admission to ICU or following diagnosis of CVA. All patients > 18 years old with an ICP monitor admitted to ICU following TBI, SAH or acute CVA were included. (CVA patients did not require ICP monitor to be included). Exclusion criteria included type I diabetes and pregnancy.

Patients' fulfilling the inclusion criteria had blood samples collected immediately following enrolment and then daily from the morning specimens. All clinical observations were taken on admission and then from 8 am daily. Patients remained on study for 7 days or until death or discharge from ICU. Samples were obtained simultaneously for ketones (both Beta-hydroxybutyrate (BHB) and acetoacetate), glucose and arterial blood gases: (pH, CO_2, HCO_3, base excess, anion gap, lactate). If an extraventricular device was present (SAH), CSF was tested for BHB and Acetoacetate.

Acetoacetate concentrations were determined using an automated modification of the original endpoint procedure described by Williamson et al on a Cobas Bio centrifugal analyser[17] (Roche, Basel, Switzerland). Specimens were collected in lithium heparin tubes (#456083, Greiner Bio-One, Kremsmunster, Austria).

Beta Hydroxybutyrate (D-3-Hydroxybutyrate) was measured quantitatively using the Stanbio Laboratory β-Hydroxybutyrate LiquiColor Assay Kit (Boerne, TX, USA) on the Beckman DxC800 Unicell (Brea, CA, USA) analyser using a spectrophotometric endpoint assay. Specimens were collected in lithium heparin tubes (#456083, Greiner Bio-One, Kremsmunster, Austria).

Patients were fed a weight based diet with a caloric goal of achieving 25 – 30 kcal/kg/d and a protein intake of 1 – 1.2 g/kg/d.

Demographic data was collected including participant age, sex and primary diagnosis. Further clinical data including intracranial pressure (ICP), (Glascow Coma Scale) GCS and nutrition (number of days of enteral nutritition) were also collected.

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With 33 included patients we had 80% power to detect a half standard deviation change in Ketone level over the 7 days of follow up assuming a 5% level of significance. A linear mixed model was used to estimate the change in Ketone level over the 7 days of follow up. Within patient correlations were accounted for by the inclusion of random effects for each patient.

Results

38 patients with ABI were recruited into the study (22 F and 16 M) and followed for up to 7 days (table1). The admission diagnoses included 19 CVA, 8 SAH and 11 with TBI. 22 patients completed the full 7 days observations, 7 CVA, 7 SAH and 8 TBI. The overall mortality rate was 23%. During this period the mean highest ICP varied between 18 mmHg and 31 mmHg and CPP from 66 mmHg to 82 mmHg (table 2). There was no evidence of an association between BHB and ICP based on Spearman correlation (p=0.46 for ICP lowest and p=0.96 for ICP highest)

During the study period, plasma BHB levels were increased initially but normalized by day 3 and acetoacetate levels remained within the normal range (normal BHB < 0.20 mmol/l, acetoacetate < 0.30 mmol/l). The change in BHB was significant (table 3). There was no statistically significant difference in BHB concentrations between genders a gender difference in BHB (P>0.05). More specifically there was insufficient evidence of a difference at baseline (P=0.24) or over time (P=0.085). Plasma lactate concentrations peaked at 1.69 mmol/l and glucose varied between 7.87 mmol/l and 11.21 mmol/l. There were 30 observations in 10 patients where BHB could be measured in both blood and CSF (in patients with SAH). There is no evidence of differences in blood BHB by diagnostic group (P=0.39) or evidence of interaction between trend over time and diagnostic group (P=0.19).When the data are averaged over patients there is weak evidence of correlation (Spearman's rho = 0.62, P=0.054)

These results as shown in table 3 suggest BHB and PaO_2 decreased over the 7 days; pH and bicarbonate increased; but there was insufficient evidence to show changes over time for any other variable.

The results in the table 4 were based on decomposing the time-dependent covariates (pH, bicarbonate, glucose) into components based on the mean values for each patient; and the differences within patients[18]. There was no evidence of association between the time-dependent covariates and acetoacetate and also between glucose and BHB but there was evidence of associations between the differences in pH and bicarbonate within patients and BHB, however there was no evidence of association between the mean values of each patient for pH and bicarbonate, and BHB. There is therefore evidence that both pH and bicarbonate are associated with BHB but that this association is due to differences within patients and not differences between patients.

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Note that there were only 6 (3%) occasions when patients did not received nutrition. This occurred for 5 patients at day 1 and for 1 patient on day 2. Due to the small number of patients not fed over the follow up period this variable was not included in the analysis.

Discussion

The purpose of this study was to examine baseline ketone levels in blood and CSF following ABI. We noted a reduction in BHB over the course of the study period with levels peaking at 0.32 ±0.58 mmol/l on day 2 and subsequently decreased to 0.08±0.03 mmol/l by day 7. This reduction was seen across all diagnostic groups. This finding may reflect a number of physiological changes during the acute phase of illness. Certainly catecholamine levels are raised following ABI. Termed "autonomic dysfunction syndrome" elevated catecholamine levels were initially described in SAH and stroke but also occur in patients with TBI. Raised catecholamine levels are associated with poor outcome and the use of B-blockers to limit secondary injury is associated with improved outcomes post TBI [19] This was confirmed by Tamaki et al who demonstrated significantly raised epinephrine and norepinephrine concentrations post SAH and the corresponding increase in ketones[13]. Despite only collecting data on admission and day 30, they noted significant decrease in ketone body levels which correlated with a decrease in plasma catecholamine concentrations.

Catecholaminergic stress is a major trigger for ketogenesis and leads to partial degradation of long-chain fatty acids liberated from white adipose tissue and subsequent generation of ketone bodies. The other potential trigger for ketone production is glucopenia brought about by fasting[2]. It is not uncommon for delays to occur in the establishment of adequate nutrition in the critically ill population. The reasons are numerous but include delayed initiation of feeds and a tendency to initial absorption delays in the ABI population[20]. We postulated that the combination of catecholaminergic stress and inadvertent fasting was responsible for the initial rise in BHB.

Ketone bodies can provide energy to the brain at times of glucose shortage and may displace glucose as preferred energy source in cerebral tissue following ABI. In fact, Owen et al demonstrated that ketone body uptake was sufficient to supply 70% of cerebral energy requirements[21]. The optimal level of BHB is unknown although laboratory experiments suggest plasma levels of approximately 4.0 mmol/l or greater are required to protect against cerebral oxidative stress[22]. Although controversial, a similar, plasma concentration is thought to be required to inhibit seizures in children with poorly controlled epilepsy[23]. Our results suggest that post ABI, intrinsic levels of BHB are insufficient to provide significant cerebral energy augmentation. Exogenous BHB supplementation would be required to raise BHB levels to clinically significant concentrations.

The blood brain barrier is relatively impermeable to ketones and BHB is transported into the CSF by a carrier protein which regulates the rate of uptake. Cerebral BHB concentrations are therefore affected by overall plasma concentrations and time. Previous animal research from our group noted that a continuous IV infusion of saline/BHB leads to a dose dependent increase in plasma and CSF BHB concentrations in healthy rats and that increases in brain

levels of BHB are dependent on plasma concentrations[24]. Furthermore, we found a correlation between the final plasma, CSF and brain concentrations of BHB, suggesting that cerebral BHB concentrations are dependent on plasma and CSF. Of the current SAH patients we investigated, we were able to collect 30 matching plasma/CSF specimens noting a correlation coefficient of 0.62. The mean CSF level was 0.09 mmol/l. Lamers et al found a similar relationship between blood and CSF BHB in 58 children following a period of fasting (r=0.71). Similarly, Owens et al noted that the CSF ketone concentration was directly proportional to the blood ketone-body concentration in 8 obese patients undergoing a 21 day fast[25,26]. Owen noted relatively low blood (0.070 mmol/l) and CSF (0.042 mmol/l) ketone concentrations initially. However, after a 21 day fast, there was a significant increase in blood BHB (4.95 mmol/l) which was similarly reflected in the CSF (2.09 mmol/l). These and other studies confirm the relationship between blood and CSF BHB. Although starvation is an effective means of increasing blood ketones, the evidence suggests that early feeding improves outcome in critically ill patients[27]. Therefore, in order to produce a sufficiently raised plasma concentration an external source of ketones will be required.

Plasma (and subsequently CSF) ketones may potentially be increased by several means. Traditionally ketogenic diets, which consist of high fat, low carbohydrates in varying concentrations, have been utilized for this purpose. Most experience has been gained from using ketogenic feeds to control seizures in children with poorly controlled epilepsy[28]. Although generally unpalatable, ketogenic diets are fairly effective at increasing plasma ketone levels. There is far less evidence in the adult population, where these diets have proven less ketogenic. Ritter et al examined several ketogenic diets in an animal model of ischemia, one of which was subsequently evaluated in adult patients with TBI[12]. Although the difference between the ketogenic diet and control groups was significant, plasma BHB levels remained low, at less than 0.6 mmol/l, well below what may be considered a therapeutic level. Another option is to provide ketones intravenously as a salt. Pan and Blomqvist have demonstrated an increase in plasma and brain levels of BHB following an intravenous infusion[11,29]. Hiraide et al were able to increase ketone levels to 1.5 mmol/l with a 3 hr infusion of BHB at 25 umol/kg/l[9]. However, the high cost of producing intravenous ketone formulations makes the IV route uneconomical. More recently Kieran et al have demonstrated significant increase in plasma ketones following the oral administration of a ketone ester, with BHB reaching plasma levels of up to 3.30 mmol/l[30]. In the future, this may provide an effective and affordable means for inducing ketosis.

This study has several limitations. Firstly, the high SD for BHB is due to 4 patients with particularly high values (>1 mmol/l). Three of those patients had cerebrovascular disease and the fourth patient had subarachnoid haemorrhage. It likely also reflects the relatively small sample size. Secondly, catecholamine levels were not measured. The only study of which we are aware comparing ketones and catecholamine levels in patients with SAH performed

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measurements on day 1 and 30 only. We therefore have no direct comparison over the shorter period. Certainly, it has been demonstrated catecholamine levels are initially high following TBI and then decrease steadily over the subsequent 7 days [14-16]. Our results are similar to Ritter et al where ketone levels steadily decrease in their control group until about day 4 and then remained low until day 14 [12]. Thirdly, we did not record the total calorie intake per patient per day. We did however; observe that there were only 6 (3%) occasions when a patient wasn't fed. The feeding regimens are fairly standard providing 25-30 kcal/kg/d of energy and 1.0-1.2 g/kg/d of protein. As noted, the main determinant of ketogenesis is low insulin concentrations and high catecholamine levels. Although the average glucose concentrations remained above 7.5 mmol/l, insulin was not measured and its effects are unpredictable in the acute setting. Lastly, exogenous catecholamine administration was not recorded. Studies in health adults have demonstrated a correlation between exogenous catecholamine administration and ketone concentrations [31]. It is therefore conceivable that this could play a role in ketogenesis in unstable ABI patients.

Conclusion

In this study, we have demonstrated that patients with ABI who are fed a standard diet fail to produce significant levels of ketosis intrinsically. Thus, to achieve elevated plasma concentrations of ketones, an external source of ketones is required. The results provide a basis to proceed to interventional studies of ketone supplementation in patients with brain injury.The challenge then is to design studies to demonstrate that; ketosis can be achieved using one of these formulations, and that the side effect profile is acceptable and finally, that outcomes can be improved.

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Table 1. Demographic data of patients with acute brain injury

	N	Age	Sex (m/f)	LOS (hours)	ICU mortality (%)
Total	38	60 ± 19	16/22	266 ± 250	23.7
CVA	19	61 ± 19		179 ± 147	15.8
SAH	8	64 ± 13		456 ± 418	12.5
TBI	11	55 ± 21		277 ± 161	45.5

All data were expressed as mean± standard deviation or number (percentage).

CVA: cerebraovascular accident; SAH: subarachnoid haemorrhage; TBI: traumatic brain injury

Table 2. Clinical and biochemical observations measured daily over trial period

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
ICP (mmHg)	20±10	18±6	22±14	24±13	25±11	31±17	25±13
CPP (mmHg)	66±11		73±10		80±24		82±22
BHB(plasma) (mmol/l)	0.29 ±0.33	0.32±0.58	0.19±0.24	0.2 ±0.23	0.22±0.41	0.1 ±0.06	0.08±0.03
Acetoacetate (plasma) (mmol/l)	0.19 ±0.16	0.16±0.29	0.09 0.05	0.12±0.10	0.13±0.15	0.09±0.08	0.05±0.04
Glucose (mmol/l)	8.26 ±5.52	8.25±2.31	8.3 ±2.04	7.87±1.61	8.18±2.04	8.10±15.9	8.17±2.36
Lactate (mmol/l)	1.69 ±1.09	1.46±0.63	1.49±0.65	1.32±0.55	1.27±0.58	1.62 ±1.8	1.25±0.49
pH	7.41 ±0.05	7.43±0.06	7.44±0.07	7.45±0.07	7.45±0.05	7.44±0.07	7.44±0.05
HCO_3 (mmol/l)	22.67±3.24	23.7±3.45	24.9±6.14	25.3±3.76	25.8 ±3.8	25.8±4.08	25.8±3.58
Na (mmol/l)	140±4.8	141±4.9	141±6.1	143±5.9	142±6.9	142±8.04	142±7.28
Ag	6.5 ±3.0	5.4±2.45	5.6±3.15	10.6±2.05	6.5 ±2.7	5.9 ±3.30	6.5 ±2.7

CPP: cerebral perfusion pressure; ICP: intracranial pressure; BHB: beta-hydroxybutyrate; Ag: anion gap

Table 3. Trends over time based on mixed models analysis

	Estimate	SE	P-value
BHB	**-0.033**	**0.011**	**0.004***
Acetoacetate	-0.010	0.007	0.14
pH	**0.0076**	**0.0017**	**<0.0001***
Lactate	-0.044	0.032	0.16
Glucose	0.21	0.21	0.32
$PaCO_2$	0.51	0.32	0.11
PaO_2	**-3.7**	**0.91**	**<0.0001***
Bicarbonate	**0.62**	**0.081**	**<0.0001***
Sodium	0.15	0.13	0.25
Anion gap	0.11	0.34	0.76

BHB: beta-hydroxybutyrate

*$P<0.05$

Table 4. Correlations between parameters based on mixed models

Outcome	Predictor	Estimate	SE	P-value
BHB	pH (mean)	-0.99	0.86	0.25
	pH (difference)	**-1.27**	**0.53**	**0.018***
BHB	Bicarbonate (mean)	-0.0071	0.013	0.58
	Bicarbonate (difference)	**-0.033**	**0.0091**	**0.0004***
BHB	Glucose (mean)	-0.014	0.015	0.34
	Glucose (difference)	0.00066	0.0038	0.87
Acetoacetate	pH (mean)	-0.21	0.58	0.71
	pH (difference)	-0.13	0.32	0.68
Acetoacetate	Bicarbonate (mean)	-0.0041	0.0083	0.62
	Bicarbonate (difference)	-0.0054	0.0052	0.31
Acetoacetate	Glucose (mean)	0.0074	0.011	0.50
	Glucose (difference)	0.011	0.0066	0.10

BHB: beta-hydroxybutyrate

*$P<0.05$