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Infusion of sodium DL-3-ß-hydroxybutyrate decreases cerebral injury biomarkers after resuscitation in experimental cardiac arrest

Filippo Annoni[1,2*], Fuhong Su[1,2], Lorenzo Peluso[1,3,4], Ilaria Lisi[5], Enrico Caruso[5], Francesca Pischiutta[5], Elisa Gouvea Bogossian[1], Bruno Garcia[1,2], Hassane Njimi[1], Jean-Louis Vincent[1], Nicolas Gaspard[6,7], Lorenzo Ferlini[6], Jacques Creteur[1], Elisa R. Zanier[5] and Fabio Silvio Taccone[1,2]

Abstract

Aims Cerebral complications after cardiac arrest (CA) remain a major problem worldwide. The aim was to test the effects of sodium-ß-hydroxybutyrate (SBHB) infusion on brain injury in a clinically relevant swine model of CA.

Results CA was electrically induced in 20 adult swine. After 10 min, cardiopulmonary resuscitation was performed for 5 min. After return of spontaneous circulation (ROSC), the animals were randomly assigned to receive an infusion of balanced crystalloid (controls, n = 11) or SBHB (theoretical osmolarity 1189 mOsm/l, n = 8) for 12 h. Multimodal neurological and cardiovascular monitoring were implemented in all animals. Nineteen of the 20 animals achieved ROSC. Blood sodium concentrations, osmolarity and circulating KBs were higher in the treated animals than in the controls. SBHB infusion was associated with significantly lower plasma biomarkers of brain injury at 6 (glial fibrillary acid protein, GFAP and neuron specific enolase, NSE) and 12 h (neurofilament light chain, NFL, GFAP and NSE) compared to controls. The amplitude of the stereoelectroencephalograph (sEEG) increased in treated animals after ROSC compared to controls. Cerebral glucose uptake was lower in treated animals.

Conclusions In this experimental model, SBHB infusion after resuscitated CA was associated with reduced circulating markers of cerebral injury and increased sEEG amplitude.

Keywords Cardiac arrest, Beta hydroxybutyrate, Anoxic injury, Ischemia–reperfusion, Ketone bodies

*Correspondence:
Filippo Annoni
filippo.annoni@hubruxelles.be
[1] Department of Intensive Care, Erasme Hospital, Université Libre de Bruxelles, Lennik Road 808, 1070 Brussels, Belgium
[2] Experimental Laboratory of Intensive Care, Free University of Brussels, Brussels, Belgium
[3] Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy
[4] Department of Anesthesiology and Intensive Care, Humanitas Gavazzeni, Via M Gavazzeni 21, 24125 Bergamo, Italy
[5] Laboratory of Traumatic Brain Injury and Neuroprotection, Department of Acute Brain and Cardiovascular Injury, Istituto Di Ricerche Farmacologiche Mario Negri IRCCS, Via Mario Negri 2, 20156 Milan, Italy
[6] Department of Neurology, Erasme Hospital, Lennik Road 808, 1070 Brussels, Belgium
[7] Neurology Department, School of Medicine, Yale University, New Haven, CT, USA

Introduction

Cardiac arrest (CA) is a dramatic clinical condition associated with an abrupt loss of cerebral perfusion [1]. The overall rate of survival with good neurological outcome varies around the globe but remains < 10% among patients suffering from out-of-hospital CA (OHCA) [2, 3]. Post-anoxic brain damage accounts for most of the deaths [4, 5]. There is no specific recommended treatment to reduce the burden of brain injury after CA, so research into possible new therapies is of critical importance.

The brain has limited energy storage capacities, so is particularly vulnerable to a sudden interruption in the perfusion of metabolic substrates. Ketone bodies (KBs) are a family of adipose tissue-derived biochemical compounds normally produced by the body in situations of reduced energy substrate availability, such as fasting, prolonged starvation, or exercise [6]. In such conditions, the brain becomes an active consumer and its capacity to metabolize KBs increases to adapt to glucose shortage [7]. In addition to the physiological production of KBs, a state of ketosis (i.e., increased plasma KB concentration) can be induced either by a specific ketogenic diet or by exogenous supplementation with KB salts or esters.

KBs are considered as possible therapeutic agents in a vast range of neurological conditions, including traumatic brain injury, stroke, refractory epilepsy, and neurodegenerative disorders [8]. Potential mechanisms of action may include the modulation of inflammation via hydrocarboxylic acid receptors (HCA) [9], reduced monocyte recruitment [10], and decreased oxidative stress, apoptosis, and neuronal hyperexcitability, while promoting brain repair [8, 11–13].

The extensive neuroprotective properties of KBs have recently been extended by reports of possible cardioprotective properties after ischemia–reperfusion and myocardial infarction in both humans, large and small animal models [14–16], suggesting that KBs could represent a therapeutic strategy in the post-resuscitation phase of CA. Increased ketosis provided some neuroprotective effects in an experimental CA rodent model using a ketogenic diet [13, 17–20] and in another rodent study using an intraperitoneal injection of ß-hydroxybutyrate (ßOHB) [20]. However, the use of ketogenic diet and intraperitoneal administration are not applicable to acute clinical practice.

The aim of this study was therefore to assess whether infusion of KBs could decrease cerebral injury after resuscitation in an established, clinically relevant swine model of CA.

Methods

The results published within this manuscript are part of a larger series of experiments that tested the effects of alternative energy substrates in the context of CA (protocol number 704N), and data from the control group have therefore been reported previously [22], in accordance with the 3Rs principle (Replacement, Reduction and Refinement) to reduce the total number of animals included. All experimental procedures were approved by the Institutional Review Board for Animal Care of the Free University of Brussels (Belgium) (number of Ethical Committee approval: 704 N) and followed ARRIVE (Animal Research: Reporting in Vivo Experiments, see Supplementary material online, Table S1) guidelines. Care and handling of the animals were in accord with National Institutes of Health guidelines (Institute of Laboratory Animal Resources).

Surgical preparation and CA model

A detailed description of the model has been published previously [21, 22]. On the day of the experiment, the selected adult swine was sedated in the cage with a combined intramuscular injection of midazolam (1 mg/kg) and ketamine (10 mg/kg), in the neck and placed on the operating table. After obtaining peripheral venous access, a continuous infusion of sufentanyl citrate (0.2–1 µg/kg/h) was started, and a catheter was inserted in the femoral artery for invasive arterial pressure monitoring, orotracheal intubation was performed and ventilation started in controlled volume mode with a tidal volume of 8 ml/kg, 5 cmH_2O of positive end-expiratory pressure (PEEP), fraction of inspired oxygen (FiO_2) of 1, and inspiratory to expiratory ratio of 2, with a square wave flow pattern. A 1% mixture of inspired sevoflurane was started, and ventilation parameters were progressively adjusted to ensure a $PaCO_2$ between 35 and 45 mmHg and a PaO_2 > 70 mmHg, with the minimally required FiO_2. A continuous infusion of rocuronium (1- 4 mg/kg/h) and sufentanyl citrate (3.5 µg/kg/h) was maintained for the entire experiment and 2 g of amoxicillin-clavulanate (Sandoz, Basel, Switzerland) administered as slow intravenous bolus. A Foley catheter was then surgically placed in the bladder, followed by a three-lumen central venous catheter in the external jugular vein, a pulmonary artery catheter, and an upward internal jugular single-lumen catheter to sample venous cerebral outflow. The animal was then proned and multimodal neuromonitoring placed. Neuromonitoring consisted of two cerebral microdialysis (CMD) catheters, a multifunctional probe measuring intracranial pressure (ICP), cerebral temperature, and brain tissue oxygen tension ($PbtO_2$), a laser Doppler

probe, and two stereoelectroencephalography (sEEG) wires (one for each parietal lobe).

The animal was returned to the supine position and ventricular fibrillation induced electrically using a pacemaker wire. No treatment was given for 10 min, and cardiopulmonary resuscitation (CPR) was then started, using an automated chest compressor at the rate of 100/minute, and continued for 5 min; ventilation was resumed. After one minute of CPR, an intravenous dose of epinephrine was administered, and at the end of the 5 min CPR period, a biphasic electric countershock was delivered. If there was no return of spontaneous circulation (ROSC), an additional minute of CPR was given followed by an electric countershock, and an additional dose of epinephrine was given after 7 min of CPR. ROSC was defined as the presence of a cardiac rhythm along with a mean arterial pressure (MAP) > 65 mmHg for more than 20 min.

After ROSC, the animal was proned and observed for 12 h. All animals were treated with targeted temperature management at 34 °C during the observation phase, at the end of which the animals were sacrificed with potassium chloride injection and brain tissue samples harvested. Death was confirmed by ventricular fibrillation waves on the EKG trace and a concomitant decrease in arterial blood pressure.

Randomization and study interventions

On the day of the experiment, animals were randomly assigned (simple randomization, following a predetermined schedule) to receive either a bolus of 20 mL of NaCl 0.9% at the start of CPR followed by a continuous infusion of 0.18 g/Kg/h of sodium DL-3-ß-hydroxybutyrate (SBHB, GoldBio, St Louis, MO, US) during the observation period (Ketone Body group), or a bolus of 20 mL of NaCl 0.9% at CPR initiation followed by a continuous infusion of balanced crystalloids during the observation period (Control group) (Fig. 1). The SBHB solution had an expected osmolarity of 1189 mOsm/l and a concentration of 0.075 g/ml. Because of the expected associated changes in plasma sodium concentrations, blinding was not feasible. In the KB animals, specific safety limits were pre-established to standardize the administration of SBHB and reproduce conditions translatable to clinical practice: every hour the infusion rate was reduced by 20% if the arterial Na^+ exceeded 155 mEq/l or plasma osmolarity was ≥ 320 mOsm/l.

Throughout the experiment, a MAP target of > 65 mmHg was used in both groups and achieved with norepinephrine administration whenever necessary. All animals received a continuous infusion of balanced crystalloids (5–10 ml/Kg/h), and any additional fluid administration was titrated to keep the pulse pressure variation (PPV) < 14%, without surpassing the baseline pulmonary capillary wedge pressure. Hyperglycemia was left uncorrected if blood glucose concentration was < 300 mg/dL within the first hour after ROSC or < 250 mg/dL later during the study period and otherwise corrected with an infusion of 10 Units of insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark).

Monitoring and sampling

Arterial blood samples were obtained prior to CA induction (T0), 20 min after ROSC (T1), and 6 h (T2) and 12 (T3) hours later. Jugular vein blood gas analyses were carried out before and after endotracheal intubation, at T0 and T1, and then hourly. The arterial-jugular venous differences in glucose and lactate at each time point were

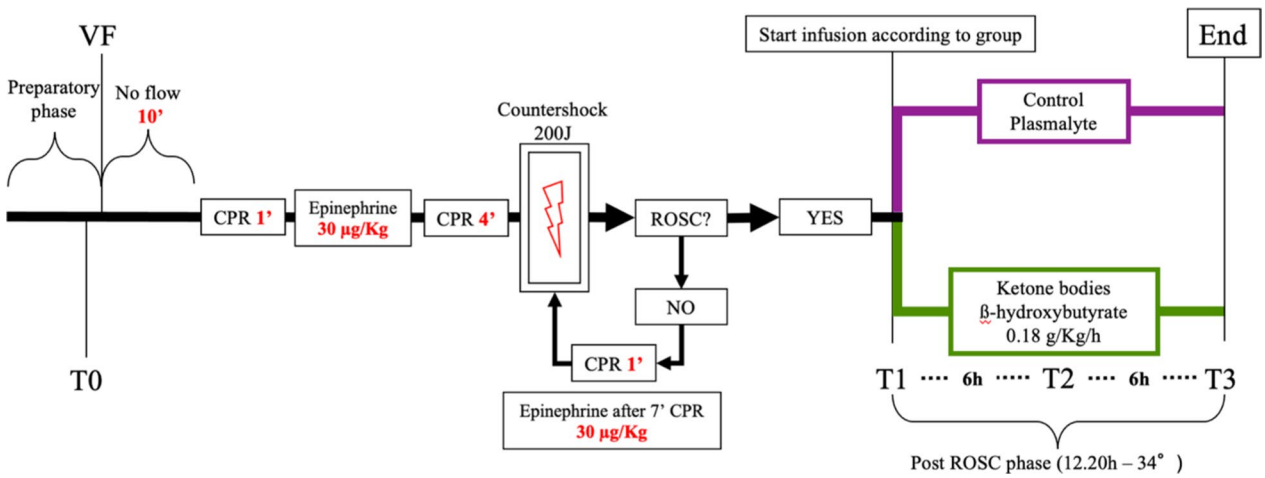

Fig. 1 Timeline of the experiment. The Control group is represented in mauve, and the Ketone Body group in green. VF: ventricular fibrillation; CPR: cardiopulmonary resuscitation; ROSC: return of spontaneous circulation; T0-3: blood sampling timepoints

used as proxies for cerebral uptake of glucose and lactate, respectively. Central venous mixed blood was collected at T0, T1, and then every three hours to allow instrument calibration. CMD samples were taken from the same catheter at T0 and then every hour.

ß-hydroxybutyrate concentrations were measured in treated animals and in a representative group of controls (n = 4) using a point-of-care approach with a dedicated veterinary tool (Nova Vet, Nova Medical, Waltham, MA, US) at T0, T1, T2, and T3. Plasma levels of the brain injury biomarkers, glial fibrillary acid protein (GFAP, #102336), neurofilament light chain (NFL, #103400), and neuron specific enolase (NSE, #102475), were measured on an SR-X analyzer according to the manufacturer's instructions (Quanterix, Billerica, MA). A single batch of reagents was used for each analyte.

After animal sacrifice, two cortical sections of about 0.5 cm^3 of brain parenchyma were harvested from each parietal lobe and immediately frozen in liquid nitrogen. Gene expression analysis was subsequently carried out on a selected number of genes representative of different injury-response related pathways (i.e., inflammation, structural integrity, apoptosis, endothelial function, and oxidative stress) [21, 22].

Offline analysis of the filtered EEG signal (1–15 Hz; 4th order Butterworth bandpass filter, filtfilt function in Matlab) enabled extraction of the signal amplitude (Hilbert function in Matlab). All computing was carried out using a sliding 1 min window with 50% overlap. Two independent neurophysiologist experts in reading EEGs who were blinded to the group assignment assessed the presence of suppressed background or burst suppression at T3 [21, 22].

Statistical analysis

Given the absence of similar protocols in the literature, the relatively small sample size in other protocols investigating the impact of ketosis in CA, and an expected survival rate of > 90% [21], an a priori sample size of 8 animals in the KB group was considered adequate for study purposes. Most recorded variables (i.e., blood pressure, heart rate, ICP, $PbtO_2$, brain temperature and cerebral blood flow [CBF]) were recorded continuously with a sampling frequency between 1 and 100 Hz. Data were extracted as means over periods of 60 s and successively reduced to means over 10 min.

Continuous variables are expressed as means with standard deviation or medians with interquartile range, and discrete variables as percentages with 95% confidence intervals. Categorical variables were compared using Fisher's exact test or a Chi-square test, as appropriate. For multiple group comparisons, ANOVA, Welch's, or Mann–Whitney tests were used as appropriate. For the evolution of continuous variables over time, a linear mixed-effect model fitted for restricted maximum likelihood estimation (REML) was used. EEG data were analyzed as aggregates over the observation period; outliers were detected and eliminated using the ROUT method with a Q = 1% (where Q is the maximum desired false discovery rate). For variation in gene expression compared to the Control group, a one sample t-test or Wilcoxon test was used, as appropriate. A value of $p < 0.05$ was considered statistically significant.

Data analyses were performed using GraphPad Prism (version 10.1.1 for Macintosh, GraphPad Software, La Jolla, CA, US) and Matlab (version 9.7, R2019b update 9, The MathWorks Inc., Natick, MA, US).

Results

Twenty animals were included in the study (9 in the KB group and 11 controls), of which 19 achieved ROSC and were included in the analyses. During the post-resuscitation phase, one animal in the KB group died of refractory distributive shock 7 h after ROSC. The baseline characteristics of the two groups are given in Table 1.

The duration of resuscitation was 300 [300–360] seconds in the control group and 360 [315–420] seconds in the KB group ($p = 0.14$). There were no differences between groups in the number of epinephrine doses, maximal end-tidal CO_2 during CPR, or the incidence of arrythmia during the 30 min following ROSC.

Because the animals were fasted for the 12 h prior to the experiment, circulating levels of ß-hydroxybutyrate were measured in a representative group of animals to ensure the absence of ketosis in the control group. At T2 (1.0 [IQR0.9–1.3 vs 0.1 [0.1–0.17]) and T3 (0.9 [IQR 0.8–1.10] vs. 0.1 [IQR 0.1–0.25]), animals in the KB group had significantly higher plasma ß-hydroxybutyrate levels than control animals (both $p < 0.001$; Fig. 2). No adaptation in KB infusion rate was necessary during the study period, respective to the pre-determined safety thresholds.

Hemodynamic and physiological parameters

The MAP was similar between groups throughout the experiment ($p = 0.007$ for interaction, $p = 0.48$ for group difference; Figure S1A). There was no difference in norepinephrine requirements between groups during the observation period, even when the last two hours of the animal that died of distributive shock were excluded ($p > 0.99$ for interaction in both cases, Figure S1B and S1C). There were no statistically significant differences in the evolution of other measured hemodynamic variables over time between groups (Figure S2).

During the experiment, there were no differences in pH, PCO_2, lactate, or glucose between groups (Fig. 3A–D). The KB group had a significantly higher plasma

Table 1 Baseline characteristics of the study groups

Variable	Group	
	Control (n = 11)	KB (n = 8)
Male, n (%)	8 (72)	4 (50)
Weight, Kg	47.9 (5.8)	47.6 (3.2)
Temperature, °C	38.0 (0.8)	37.4 (0.9)
Arterial pH	7.51 (0.03)	7.50 (0.01)
Arterial lactate, mmol/L	1.3 (0.3)	1.5 (0.45)
Arterial glucose, g/dl	112.5 (31.7)	94 (11.5)
P/F	435.7 (57.3)	470 (50.9)
$PaCO_2$, mmHg	41.6 (3.1)	40.9 (2.1)
Na^+, mmol/L	133.4 (2.37)	132.7 (2.02)
K^+, mmol/L	3.8 (0.3)	3.7 (1.9)
Cl^-, mmol/L	100.7 (1.5)	101.3 (1.4)
Osmolarity, osm/L	268.5 (3.2)	266 (4)
MAP, mmHg	83 (12)	89 (13)
HR, bpm	87 (14)	86 (14)
CVP, mmHg	7.3 (3.2)	10.5 (2.3)
SvO_2, %	60.8 (7.9)	61.3 (7.9)
CO, L/min	5.8 (1.1)	4.8 (1)
PPV, %	11.4 (1.8)	11 (1.9)
PCWP, mmHg	10.5 (2.9)	13.7 (1.9)
CPO, Watt	0.8 (0.3)	0.8 (0.2)
DO_2, ml/min	624.5 (180.5)	530.2 (125.8)
VO_2, ml/min	245.7 (93.5)	231.8 (60.6)
OER, %	39.4 (6.3)	44.4 (6.9)
Diuresis, ml*	600 (256–825)	400 (157–1075)
ICP, mmHg*	8.0 (6.1–10.3)	11.8 (7.9–14.3)
$PbtO_2$, mmHg*	33.2 (31.9—38.3)	43.2 (40.1—47.9)
Hemoglobin, g/dl	8.9 (0.7)	8.0 (0.4)

Data are reported as mean (SD) or median (IQR). P/F = ratio between partial arterial oxygen pressure and fraction of inspired oxygen; $PaCO_2$ = arterial partial pressure of carbon dioxide; MAP = mean arterial pressure; HR, heart rate; CVP = central venous pressure; SvO_2 = mixed venous oxygen saturation; CO = cardiac output; PPV = pulse pressure variation; PCWP = pulmonary capillary wedge pressure; CPO = cardiac power output; DO_2 = oxygen delivery; VO_2 = oxygen consumption; OER = oxygen extraction ratio; ICP = intracranial pressure; $PbtO_2$ = brain tissue oxygen pressure. * = $p < 0.05$

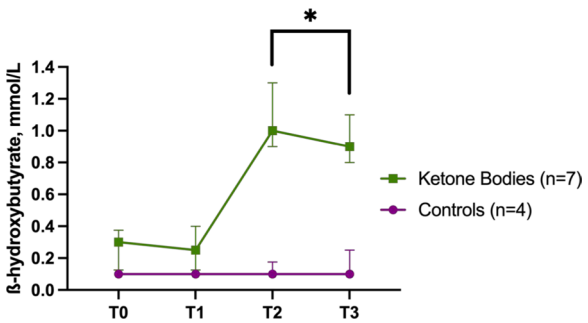

Fig. 2 Circulating ß-hydroxybutyrate concentrations (medians with interquartile ranges). Differences between groups over time were assessed using a linear mixed model. Fisher's LSD test was used for multiple comparisons. * = $p < 0.05$

sodium concentration ($p < 0.001$ for interaction), similar potassium concentration ($p = 0.09$ for interaction), and higher osmolarity ($p < 0.001$ for interaction) than the control group throughout the study period (Fig. 3E–G).

Diuresis was higher in the KB group in the last four hours of the study ($p < 0.001$ for interaction, $p = 0.13$ for group difference, Fig. 4A), but cumulative fluid balance was more positive in the KB than in the control group (median 6.2 [IQR 4.6–7.6]vs 8.9 L [IQR7.5–9], $p = 0.009$, Fig. 4B).

Multimodal neuromonitoring

ICP, cerebral perfusion pressure, $PbtO_2$, cerebral temperature, and regional perfusion pressure did not differ between groups. ICP and $PbtO_2$ remained within the normal ranges for most measurements in both groups (Figure S3). Cerebral microdialysis measurements of glucose, lactate, lactate-to-pyruvate ratio, lactate-to-glucose ratio, glutamate, and glycerol were also similar between groups (Figure S4). Using the arterial-jugular difference in glucose as a proxy, when all samples where considered, cerebral uptake of glucose was higher in the KB group (i.e., a lower proportion of positive results in the ketone bodies group, 62 vs. 83%, $p < 0.001$); there was no difference between groups in the cerebral uptake of lactate (14 vs. 7%, $p = 0.13$, Figure S5).

Circulating biomarkers

Circulating levels of GFAP and NSE were statistically significantly lower at T2 and T3 and levels of NFL at T3 in the KB group compared to controls (all $p \leq 0.01$—Fig. 5 **A–C**). Circulating levels of aspartate transaminase and alanine transaminase were statistically significantly lower at T2 in the KB group than in the controls. Plasma creatinine was lower in the KB group than in controls at T1 and T2, and urea was lower at T2 and T3. Troponin-I levels were similar in the two groups; lactate dehydrogenases, alkaline phosphatase, and creatinine phosphokinase were lower at T2 and T3 in the KB group than in controls (Figure S6).

sEEG

The mean sEEG amplitude was higher and followed a different trajectory in the KB group than in controls ($p < 0.001$ for interaction, $p = 0.007$ for group); the mean standard deviation, kurtosis, and skewness were not significantly different between groups. (Fig. 6A–D). At T3, the proportion of animals with suppressed background or suppression-burst patterns was lower in the KB group than in the controls (29% vs. 100; $p = 0.007$—Figure S7). No animal had status epilepticus at T3.

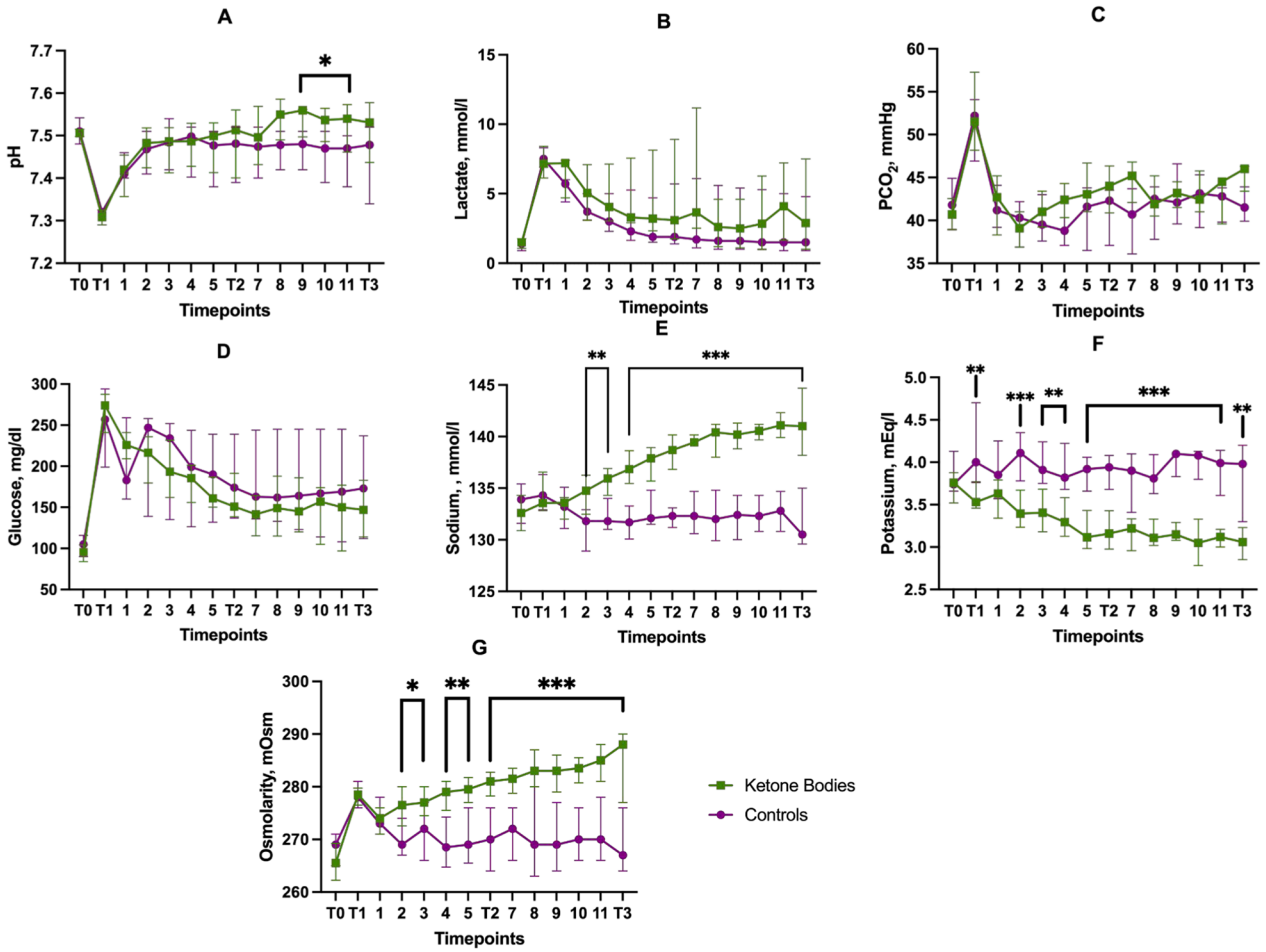

Fig. 3 Main physiological variables over time during the study period (median values with interquartile ranges). **A** pH; **B** lactate; **C** PCO_2; **D** glucose; **E** sodium; **F** potassium; **G** osmolarity. *$=p<0.05$, **$=p<0.01$; ***$=p<0.001$. Differences between groups over time were assessed using linear mixed models. Fisher's LSD test was used for multiple comparisons. For controls n = 11, for ketone bodies n = 8

Gene expression

The expression of Microtubule-Associated Protein 2 (MAP2); Glial Fibrillary Acid Protein (GFAP); Cluster of Differentiation molecule 11ß (CD11ß); Platelet and Endothelial Cell Adhesion Molecule 1 (PECAM1); Caspase 3 and 8 (CASP3 and CASP8) and Heme Oxygenase 1 (HO-1) was numerically lower in the KB group, but none of the differences was statistically significant. CD11ß expression was slightly higher in the treated group, but again without reaching statistical significance (Figure S8).

Discussion

Continuous infusion of SBHB in the post-resuscitation phase after CA reduced circulating levels of the brain injury biomarkers GFAP, NFL and NSE, and an early increase in the amplitude of the EEG signal. The induced ketosis was mild and, despite a significant increase in plasma osmolarity and sodium due to the formulation, there was no significant hemodynamic impact soon after a resuscitated cardiac arrest. There was a higher overall fluid balance by the end of the study in the KB group.

The existing evidence on the effects of KBs in CA is scant, and relies on a few studies in small animals, mostly treated with a ketogenic diet prior to CA. In a series of experiments, Tai et al. induced mechanical CA of 8 min in rodents that had been receiving a ketogenic diet for 25 days. Compared to controls, the ketogenic diet animals had no spontaneous seizures within 24 h after CA and had fewer myoclonic jerks in response to auditory stimuli [17]. In another study performed by the same authors, a 25 day ketogenic diet was associated with decreased cerebral neurodegeneration (i.e., the number of Fluoro-jade stained neurons) in multiple regions of the brain, including the hippocampus, thalamus, and cerebellum [18].

Those results were further reinforced by another series of experiments in rats, which showed that the

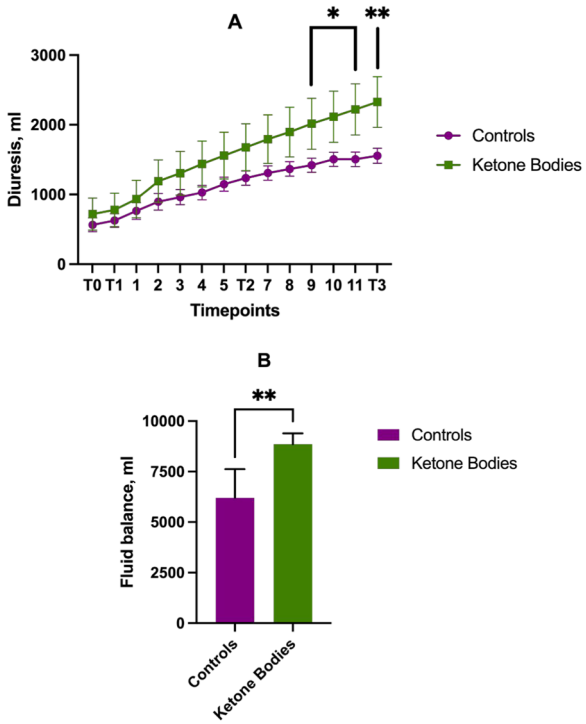

Fig. 4 Diuresis over time (**A**) and cumulative fluid balance (**B**) (mean values and standard error means). Differences between groups over time (**A**) were assessed using a linear mixed model. Fisher's LSD test was used for multiple comparisons. * = $p < 0.05$, ** = $p < 0.01$. Difference between groups (**B**) was assessed using the Mann–Whitney test. For controls n = 11, for ketone bodies n = 8

neuroprotective effects of KB in CA were mediated by K_{ATP} channel activation [19]. Similarly, in mice that underwent potassium-induced CA (8 min), 4 weeks of ketogenic diet prior to the event attenuated brain injury, with overall improved survival and better neurological functional scores and behavioral tests, while reducing brain glucose consumption and production of reactive oxygen species [13]. In rodents exposed to an 8 min-long CA that received an intraperitoneal injection of 200 mg/Kg of ßOHB after ROSC, treated animals had improved survival rates and neurological function at 72 h and reduced mitochondrial fission compared to controls [20].

Our findings are in line with these data, having found a reduction in the circulating levels of GFAP, a marker of blood brain barrier breakdown and astrocytic reactivity [23], NFL, a marker of axonal damage and neurodegeneration [24], and NSE, a marker of neuronal injury recommended in clinical practice as an outcome predictor after CA [25]. Our study has some key differences from the previous literature. First, we used a clinically relevant model of CA using the management strategies currently used in humans during and after CPR. Moreover, compared to the lissenchephalic brain structure of rodents, swine have a gyrencephalic structure, closely resembling the human's brain architecture. Second, we used a continuous infusion of a KB salt, rather than a ketogenic diet, enabling rapid administration after ROSC, thus improving the translatability of our findings to the acute clinical setting.

Despite the marked increase in plasma osmolarity due to sodium load during the infusion, we could not identify any impact on hemodynamic variables, including MAP and CO nor we could detect significant changes in ICP, cerebral oxygenation, or regional CBF. Nevertheless, we cannot exclude that the increased tonicity may have a significant impact on these variables in some settings, for example in the context of prolonged CA, normothermia, or prolonged observation. The impact of a hypertonic solution was associated with an increase in cerebral perfusion pressure during CPR in a swine model of CA [26], and, in another swine CA model, hypertonic-hyperoncotic fluid (starches) administration was associated with reduced astroglial injury as indicated using circulating levels of S110ß protein and troponin I [27].

Our group has previously investigated the impact of hypertonic sodium lactate in the same model [22] and found that it was associated with a significant reduction in vasopressor needs as well as a reduction in GFAP, but not NFL or NSE. We cannot conclude from the current results that KBs have a specific or more pronounced protective action on neurons compared to hypertonic lactate, but it is possible that these solutions, although similar in many aspects (both hypertonic with high Na content and energy substrates), could have different effects in specific cerebral cell populations. Importantly, the SBHB solution was administered exclusively after ROSC. In another experiment [20], KBs were given after ROSC using a single intraperitoneal dose of 200 mg/Kg, whereas we administered a much higher dose throughout the study period (i.e., a total of 2,160 mg/Kg), which may have elicited different biological activities. Moreover, we used a protocolized approach to ensure that no macroscopic hemodynamic imbalances among animals (e.g., different cerebral perfusion pressure or CO) could explain any difference in organ injury. Although KB have been reported to be able to decrease brain glucose consumption, we were not able to detect such macroscopic effect in extracellular glucose concentrations but only in the different proportions of positive delta glucose concentration between arterial and jugular districts.

sEEG was recorded continuously during the post-resuscitation phase and we detected a different mean amplitude between groups. In the KB group, the amplitude increased over time after ROSC and most of the animals regained a continuous EEG trace by T3. Nevertheless, those data must be interpreted with caution because an

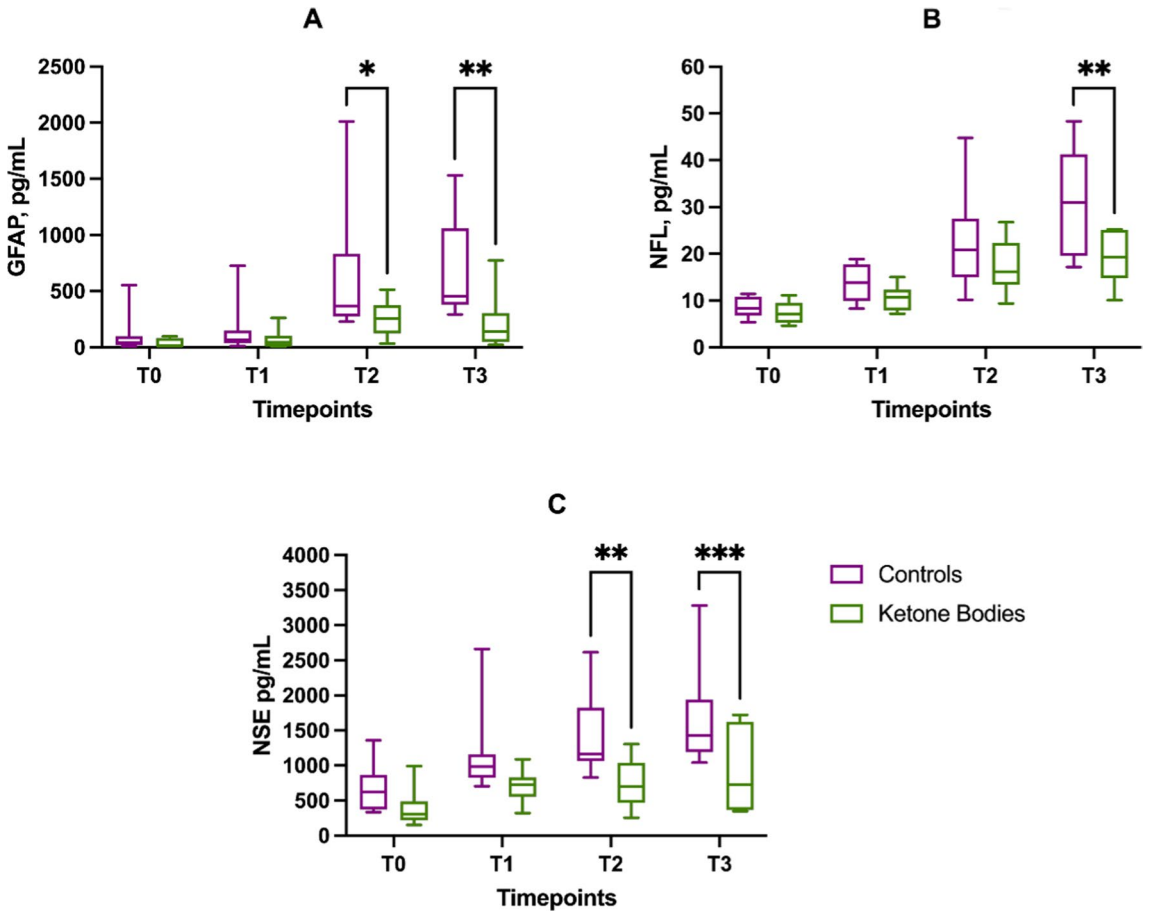

Fig. 5 Circulating biomarkers of brain injury. **A** glial fibrillary acid protein (GFAP), **B** neurofilament light chain (NFL), and **C** neuron specific enolase (NSE). Boxes represent median values and interquartile ranges (25th–75th), whiskers extend between minimum and maximum values. * = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.001$. Differences between groups over time were assessed using a linear mixed model. Fisher's LSD test was used for multiple comparisons. For controls n = 11, for ketone bodies n = 8

increase in amplitude does not necessarily mean a more favorable neurological outcome. To be noted, ketosis has also been linked with a delayed onset of inhalational anesthetics, and we could not completely rule out for an interaction between KB and anesthetic gas [28]. Our results should nevertheless encourage further research in this direction. By placing extensive neuromonitoring, we were able to assume that the effects of the treatment were not the result of different ICP values between groups, induced by the increased tonicity of the solution, and were able to describe and follow the evolution of brain activity (sEEG), cerebral O_2 delivery ($PbtO_2$), and cerebral metabolites (CMD).

Nevertheless, our model has several limitations, some of which have been previously reported [22]. First, we did not allow the animals to wake at the end of the experiment, so were not able to evaluate the neurological status. Second, we did not perform morphological or histological analyses, and collected only samples from the cerebral cortex in few animals, and not from deep brain structures particularly sensitive to hypoxia, such as the hippocampus. Third, multimodal neuromonitoring should be interpreted with caution when results are generalized to the whole brain. Nevertheless, in CA the whole brain is subjected to ischemia–reperfusion and therefore the nature of the lesions should be more homogeneous than after trauma or intracerebral hemorrhage. Fourth, we used an arbitrary dose of KB, which may not be optimal. We based our choice on a study in healthy humans in which the infusion of ß-hydroxybutyrate was associated with increased myocardial blood flow [29]. We were limited in the use of higher doses because that would have necessitated infusing even larger quantities of fluids. To this regard, it is important to note that no reduction in KB infusion rate was necessary, as the safety threshold were not surpassed during the observation period. Fifth, some analyses were carried on a reduced number of animals due to technical limitations.

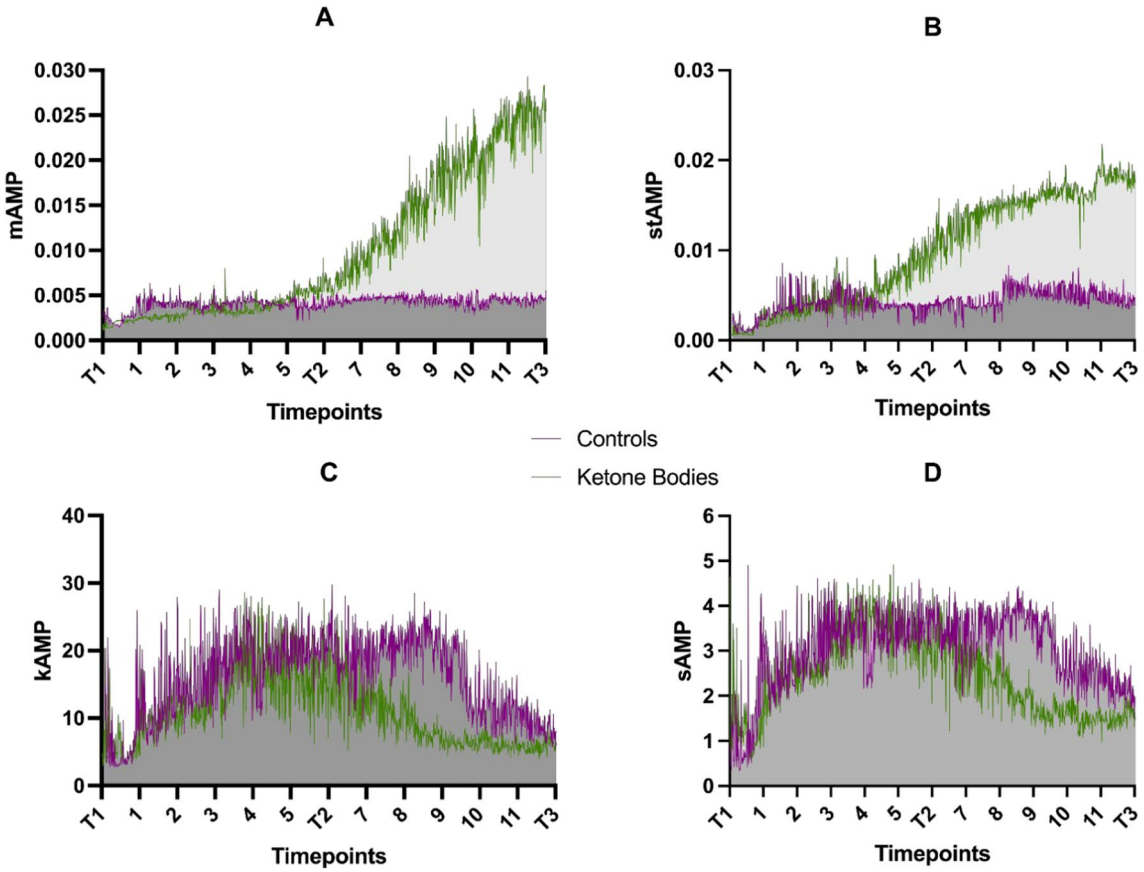

Fig. 6 EEG variables at different time points (median values, after removal of outliers); **A** = mean amplitude; **B** = mean standard deviation; **C** = mean kurtosis; **D** = mean skewness. Differences between groups over time were assessed using a linear mixed model. Fisher's LSD test was used for multiple comparisons. For controls n = 11, for ketone bodies n = 8

In particular, the circulating KB were tested on a random sample of four control animals and one animal in the KB group couldn't be measured. Similarly, the gene expression was only measured on a limited number of available samples, limiting the interpretability of the results. Lastly, we did not provide more extensive measurements of cardiac or vascular function (e.g., P–V loops, coronary sinus samples, etc.), and we did not use serial ultrasound or more advanced monitoring techniques. The extensive neuromonitoring required a prone position of the animal during the observation period and thus prevented use of cardiac ultrasound, and we wanted to minimize the risk of secondary arrythmia induced by catheter manipulation after ROSC. Many aspects remain to be elucidated, including the effect of an increased level of ketosis, perhaps through association with enteral administration of KB, as well as the specific effect of a solution containing a single enantiomer of BHB. In addition, our data are limited to a short period after ROSC, but sustained ketosis over a prolonged period could also have a role in recovery after cardiac arrest, which remains open to investigation.

Conclusions

In this clinically relevant experimental model of resuscitated CA, infusion of SBHB was associated with reduced circulating markers of cerebral injury and increased sEEG amplitude.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13054-024-05106-8.

Additional file1 (DOCX 3183 KB)

Author contributions

FA, FST, JC, and JLV conceived the protocol; FA, FS and LP conducted the experiment procedures; FA, IL, EC, FP and ERZ performed cerebral biomarker tests and gene expression analyses; FA, HN and EGB elaborated the statistical analysis of the data. NG and LF analyzed the EEG trace; FA wrote the first draft of the manuscript. All authors provided substantial intellectual contributions, participated in the modification of the first draft, and approved the final version of this manuscript.

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Availability of data and materials
The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval
The Institutional Review Board for Animal Care of the Free University of Brussels (Belgium) approved all experimental procedures (number of Ethical Committee approval: 704 N), which were also in compliance with ARRIVE (Animal Research: Reporting in Vivo Experiments, **Table S1**) guidelines. Care and handling of the animals were in accord with National Institutes of Health guidelines (Institute of Laboratory Animal Resources).

Competing interests
Prof. Jean-Louis Vincent is the journal editor. We would like to thank Raumedic, via its Belgian subsidiary Rembrant Medical, for the free loan of the MPR2 monitor and technical assistance; Jolife AB/Stryker Lund, Sweden for the free loan of the Lucas III device; Bard Medical for the free loan of the Arctic Sun device. None of these companies or their affiliates participated in the development of the experimental protocol, had access to any of the data, or contributed or corrected any part of this manuscript.

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