Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats

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D’Agostino DP, Pilla R, Held HE, Landon CS, Puchowicz M, Brunengraber H, Ari C, Arnold P, Dean JB. Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. Am J Physiol Regul Integr Comp Physiol 304: R829–R836, 2013. First published April 3, 2013; doi:10.1152/ajpregu.00506.2012.—Central nervous system oxygen toxicity (CNS-OT) seizures occur with little or no warning, and no effective mitigation strategy has been identified. Ketogenic diets (KD) elevate blood ketones and have successfully treated drug-resistant epilepsy. We hypothesized that a ketone ester given orally as 1,3-butanediol acetoacetate diester (BD-AcAc2) would delay CNS-OT seizures in rats breathing hyperbaric oxygen (HBO2). Adult male rats (n = 60) were implanted with radiotelemetry units to measure electroencephalogram (EEG). One week postsurgery, rats were administered a single oral dose of BD-AcAc2, 1,3-butanediol (BD), or water 30 min before being placed into a hyperbaric chamber and pressurized to 5 atmospheres absolute (ATA) O2. Latency to seizure (LS) was measured from the time maximum pressure was reached until the onset of increased EEG activity and tonic-clonic contractions. Blood was drawn at room pressure from an arterial catheter in an additional 18 animals that were administered the same compounds, and levels of glucose, pH, P02, Pco2, β-hydroxybutyrate (BHB), acetoacetate (AcAc), and acetone were analyzed. BD-AcAc2 caused a rapid (30 min) and sustained (>4 h) elevation of BHB (>3 mM) and AcAc (>3 mM), which exceeded values reported with a KD or starvation. BD-AcAc2 increased LS by 574 ± 116% compared with control (water) and was due to the effect of AcAc and acetone but not BHB. BD produced ketosis in rats by elevating BHB (>5 mM), but AcAc and acetone remained low or undetectable. BD did not increase LS. In conclusion, acute oral administration of BD-AcAc2 produced sustained ketosis and significantly delayed CNS-OT seizures by elevating AcAc and acetone.

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SEIZURES FROM HYPERBARIC OXYGEN (HBO2), also known as central nervous system oxygen toxicity (CNS-OT), compromise the safety of undersea divers using rebreathers and patients undergoing HBO2 therapy (HBOT) (13). Breathing 100% O2 at barometric pressure (Pa) > 2.4 atmospheres absolute (ATA) increases the likelihood of seizures in patients, and current applications of HBOT routinely use up to 3 ATA HBO2 (48). The potential for CNS-OT is the primary limiting factor in HBOT. HBO2 provides a unique, reversible, and reproducible stimulus for generalized tonic-clonic seizures in animal models and is thus an effective model for assessing anti-seizure strategies.

Previous studies in rats show that fasting delays the onset of CNS-OT (4), presumably by fundamentally shifting brain energy metabolism. Fasting (24–36 h) delays the latency to seizure from HBO2 by up to 300%, which is comparable to high doses of anti-epileptic drugs (AEDs) (3, 50) and experimental anticonvulsants that block excitatory glutamatergic neurotransmission (10). During periods of fasting or ketogenic diet (KD) use, the body utilizes energy obtained from free fatty acids (FFA) released from adipose tissue; however, the brain is unable to derive significant energy from FFA (8). Hepatic ketogenesis converts FFAs into the ketone bodies β-hydroxybutyrate (BHB) and acetoacetate (AcAc), and a small percentage of AcAc spontaneously decarboxylates to acetone. During prolonged fasting or KD, large quantities of ketone bodies accumulate in the blood (>5 mM) and are transported across the blood-brain barrier (BBB) by monocarboxylic acid transporters (MCT1–4) to fuel brain function, and this ketone transport is enhanced under oxidative stress or limited glucose availability (40). The brain derives >60% of its energy from ketones when glucose availability is limited (8). The metabolic adaptations associated with fasting-induced ketosis improve mitochondrial function, decrease reactive oxygen species (ROS) production, reduce inflammation, and increase the activity of neurotrophic factors (34).

KD mimics the metabolic state of fasting (i.e., therapeutic ketosis) and is efficacious in treating drug-resistant seizure disorders (22). This therapeutic method is well established in children and adults (30). The anticonvulsant effects of the KD correlate with an elevation of blood ketones, especially AcAc and acetone (6, 36). The KD requires extreme dietary carbohydrate restriction and only modestly increases blood ketones compared with levels associated with prolonged fasting (8). Elevating blood ketones with ketogenic medical foods or exogenous ketones is largely ineffective or problematic for a variety of reasons. Ketogenic fats, like medium chain triglyceride oil (MCT oil), are generally not well tolerated by the gastrointestinal system, and supplementation produces only low levels of ketones (<0.5 mM) (27). Oral administration of BHB and AcAc in their free acid form is expensive and ineffective at producing sustained ketosis. One idea has been to buffer the free acid form of BHB with sodium salts, but this is largely ineffective at preventing seizures in animal models and causes a potentially harmful sodium overload at therapeutic levels of ketosis (6). However, esters of BHB or AcAc can effectively induce a rapid and sustained ketosis (7, 21) that...
mimics the sustained ketosis achieved with a strict KD or prolonged fasting without dietary restriction. Recent studies have demonstrated that orally administered esters of BHB are safe and well tolerated in rats (14) and humans (15). Producing esters of BHB or AcAc is expensive and technically challenging but offers great therapeutic potential (52). Orally administered ketone esters have the potential to induce ketosis and circumvent the problems associated with fasting-induced or diet-induced ketosis (15). The ketone ester that we synthesized and tested [R,S-1,3-butanediol acetoacetate diester (BD-AcAc2)] has been shown to induce therapeutic ketosis in dogs (12, 41) and pigs (21) and was proposed as a metabolic therapy during the recovery phase in normobaric air.

In this study, we explored the potential of ketone ester-induced therapeutic ketosis as a mitigation strategy against CNS-OT seizures. We hypothesized that oral administration of BD-AcAc2 mimics the anticonvulsant effect of fasting-induced ketosis and delays the onset of CNS-OT.

MATERIALS AND METHODS

Animal surgeries and procedures. All animal procedures were done in accordance with the University of South Florida Institutional Animal Care and Use Committee (IACUC) guidelines. All protocols were previously approved by the University of South Florida Institutional Animal Care and Use Committee (PHS Assurance No. A4100-01; and fully accredited by AAALAC as Program No. 000434) and by the Director for Veterinary Affairs, Department of the Navy, Bureau of Medicine and Surgery. Adult male Sprague-Dawley rats (250–300 g, n = 60) were obtained from Harlan, anesthetized in 3–5% isoflurane (in O2), and implanted with a 4ET radiotransmitter (Data Sciences International, DSI) using sterile surgical technique. Two pairs of wires were embedded in the skull between Bregma and Lambda, with one lead on either side of midline for each pair [electroencephalogram (EEG) recordings]. Rats were weighed immediately before surgery and subsequently once every 7 days, just before the weekly exposures to HBO2. After surgery, every animal recovered for ≥1 wk. To determine the time course of ketosis, 18 adult male Sprague-Dawley rats (250–350 g), pathogen-free, were purchased from a vendor (Harlan) and shipped 7 days after being implanted with carotid catheters. Rats were fasted for 18 h while still on water before the start of the experiment to ensure complete gastric emptying of prior food before oral gavage.

Hyperbaric radiotelemetry. The radiotelemetry system consisted of an implantable 4ET radiotransmitter able to amplify and broadcast signals via a receiver (model RPC-2, DSI PhysioTel) connected to an acquisition interface unit (ACQ 7700 Ponemah) via electrical penetrations in the wall of the hyperbaric chamber. The acquisition interface unit was connected to a computer for real time data collection and storage. The same acquisition unit also recorded chamber pressure and chamber temperature, which were measured, respectively, by a thermocouple and pressure gauge directly connected to the acquisition system via BNC (Bayonet Neill-Concelman) cables. Each animal was continuously monitored via a video camera (AXIS 221 Network Camera). The video of each experiment was recorded to confirm the timing of tonic-clonic seizures with EEG activity.

Acquisition/analysis software. Raw data were collected using DSI Ponemah software (version 4.90, P3 Ponemah Physiology Platform). GraphPad PRISM (version 3.03) was used for all statistical analyses. All values in this work were reported as means ± SE of measurement. Analysis of latency to seizure (LS) was performed using two-tailed, unpaired t-tests among the three groups (see Fig. 4D). ANOVA and repeated measures ANOVA were used to assess changes in blood ketones and blood gases. Holm-Sidak method was used for pairwise comparisons. Values were considered significant at P < 0.05.

Hyperbaric chamber HBO2 protocol. The hyperbaric system consisted of two main elements: 1) a Plexiglas chamber (~3 liter capacity, model PLY3114, Diamond Box, Buxco, Electronics) that housed the rat during the experiment, and 2) a hyperbaric chamber (~7.8 ATA MWP, Reimers Systems) that contained the Plexiglas chamber (Fig. 1, A and B) and functioned as the pressure vessel. Both
chambers were connected to an air compressor (model DK6086, oil-less rotary scroll compressor, Powerex).

HB02 exposures (dive profile) and seizure detection. At the start of each experiment (hyperbaric hyperoxia), both the main chamber and the animal chamber were filled with air. Test substances of distilled water (control), BD (10 g/kg), or BD-AcAc2 (10 g/kg) were administered by 3 ml oral gavage, and rats were placed into the Plexiglas chamber and allowed 10 min to acclimate, at which time the Plexiglas chamber was flushed with 100% O2. The animal was then allowed 15 min to acclimate before both chambers were compressed to 5 ATA (58.8 PSIG) in parallel at a rate of 0.7 ATA/min. The outer chamber was pressurized using air (capacity ~205 liters) to minimize the risk of an electrical-induced fire. Each experiment was visually monitored via a live camera. LS was calculated from the moment at which the internal and the external chambers reached 5 ATA until the onset of seizures. CNS-OT produces very powerful tonic-clonic seizures, but these are quickly reversible, usually when O2 is replaced with air, even if hyperbaric pressure is maintained. Seizure detection was based on the evaluation of two main parameters: 1) neurological seizures, confirmed by an enhanced EEG signal; and 2) behavioral seizures, represented by repeated spasmodic tonic-clonic motions of forelimbs and head (11). Neurological seizures, which consisted of high-amplitude (> 0.5 mV), high-frequency spikes lasting 10 to 30 s, and followed by polyspikes and wave formation (Fig. 1C), always preceded behavioral seizures by 1–2 s. After the onset of seizures, the Plexiglas chamber was flushed with air to quickly terminate seizure, and both chambers decompressed to sea level. Decompression rate was 1 ATA/min. Rats were then allowed a 15-min recovery period in air at 1 ATA before being removed from the chamber.

Synthesis of ketone esters. R,S-1,3-butane diol and t-buty lactoacetate were purchased from Sigma (Milwaukee, WI). All commercial solvents and reagents used were high-purity reagent-grade materials. The ketone ester synthesized, R,S-1,3-butane diol acetate diester (BD-AcAc2), is a nonionic sodium-free and pH-neutral precursor of AcAc. BD-AcAc2 was synthesized by transesterification of t-buty lactoacetate with R,S-1,3-butane diol (Savind, Seymour, IL). The resultant product consisted of a mixture of monoesters and diester, the ratio of which could be adjusted by varying the stoichiometry of reagents. After synthesis the crude product was distilled under reduced pressure to remove all solvents and starting point, which was the resultant BD-AcAc2, was obtained and assessed for purity using gas chromatography-mass spectrometry (GC-MS).

Measurement and analysis of blood glucose, ketones, gases, and pH. Test substances of distilled water (control), BD (10 g/kg), or BD-AcAc2 (10 g/kg) were administered by 3 ml oral gavage (this was time 0). Whole blood samples (10 μl) were acquired for analysis of glucose at USF utilizing a commercially available glucose-monitoring system (NovaMax Plus) at times 0, 30, 60, 120, 180, and 240 min. Likewise, for measurement of ketones, heparinized blood samples (200 μl) were collected into Eppendorf tubes at the same times. Samples were processed for the detection and quantification of BHB, AcAc, and acetone at Case Western Reserve University, Mouse Metabolic Phenotyping Center. Briefly, blood samples were chilled on ice for 30 s and centrifuged in a microcentrifuge (13,000 g) for 3–5 min, and plasma (>100 μl), treated with reducing reagent of cold 0.2 M sodium borodeuteride (NaBD4; Sigma, 205591, CAS 15681-89-7) was dissolved in 0.1 M NaOH (8.4 mg NaBD4 in 1 ml of 0.1 M NaOH) and then immediately frozen on dry ice before being stored at −80°C. Acetone was analyzed by the 60-min time point, which was the predicted peak of blood AcAc levels (21). Whole blood (300 μl) was collected in addition to the above collections, stabilized with cold 0.2 M NaBD4, and then immediately frozen on dry ice. Samples were stored at −80°C until analyzed for ketones. Internal standards of [2H6]BHB or [2H8]isopropanol were added to the treated plasma or blood samples (50 or 15 μl) and the BHB, AcAc (as M+1 of BHB), or acetone (as 2-propanol) metabolites were analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 5973 mass spectrometer, linked to a 6890 gas chromatograph equipped with an autosampler. Briefly, GC-MS conditions were either electron ionization (EI) mode or chemical ionization (CI) mode; the samples were detected by selected ion monitoring as the BHB- and AcAc-trimethylsilyl derivatives (EI) or the derivative of acetone-pentafluorobenzoyl (CI).

In addition, a 60-μl blood sample was withdrawn at each time point and immediately analyzed with a blood gas analyzer (cat no. GD7013, OPTI CCA-TS Blood Gas Analyzer, Global Medical Instrumentation) for blood pH, PO2, and PCO2.

RESULTS

Ketone ester induces rapid and sustained elevation of BHB, AcAc, and acetone. BD-AcAc2 caused a significant (P < 0.001) increase in BHB and AcAc at 30 min, which remained elevated for >4 h after administration at time 0 (t = 0) with gavage (Fig. 2, A–C). BD administration caused similar elevation in BHB (two-way repeated measures ANOVA; P < 0.001) but only modest elevation in AcAc when compared with BD-AcAc2 (Fig. 2B). The breakdown product of AcAc, acetone, was significantly higher at 60 min following BD-AcAc2 (two-tailed t-test; P < 0.001) but not BD administration (Fig. 2C). Blood pH following BD-AcAc2 or BD decreased (two-way repeated measures ANOVA; P < 0.001) compared with the control (P < 7.5), by a mean of 0.05 after 30 min and 0.1 after 1 h. No significant difference in pH was found between BD-AcAc2 and BD treatment (Fig. 2D). Administration of BD and BD-AcAc2 caused no significant changes in blood glucose (data not shown) relative to control (water).

Ketone ester-induced changes in blood PO2 and PCO2. There were no differences in PO2 after initial administration (time 0; t = 0) of water or BD, but PO2 values were considerably higher in BD-AcAc2 group (two-way repeated measures ANOVA; P < 0.001) and remained relatively hyperoxic (PO2 > 120 mmHg) during the 4-h experiment (Fig. 3A). The PCO2 of control, BD, and BD-AcAc2 groups were normal and not significantly different, despite the trend for increased PCO2 with BD (two-way repeated measures ANOVA; P = 0.053) (Fig. 3B).

Ketone ester delays CNS-OT. Figure 4, A–C, show three examples of real-time EEG recordings after a single intragastric administration (time 0) of water, BD, and BD-AcAc2, respectively. Administration of test substances occurred 30 min before reaching maximum HBO2 exposure (5 ATA O2). CNS-OT seizures were similar to EEG trace in Fig. 1C and were confirmed with video acquisition of tonic-clonic activity. LS was calculated as the percentage increase compared with the control (Fig. 4D). After the intragastric administration of BD-AcAc2 in 16 rats, the LS was significantly longer (574 ± 115%, t-test; P < 0.001). In contrast, BD administration did not delay CNS-OT. Figure 4E demonstrates the high individual variability in CNS-OT among rats.

DISCUSSION

In the present study, we tested the potential of ketone ester-induced therapeutic ketosis as a mitigation strategy against CNS-OT seizures. The major findings of the study demonstrate that a single oral administration of the ketone ester BD-AcAc2 caused rapid and sustained elevations of BHB (>3 mM), AcAc (>3 mM), and acetone (~0.7 mM) and increased resistance to seizures (LS = 574 ± 115%, P < 0.001)
compared with control (water) or BD, even though BD caused a significant increase in BHB.

The mechanism of ketone ester-induced delay in CNS-OT remains unknown, but evidence from our studies and previous research suggests that multiple factors contribute to the anti-convulsant effect of BD-AcAc2. The present study supports previous work that demonstrates seizure resistance is conferred by induction of fasting-level ketosis (4) and the anticonvulsant effect of AcAc or acetone (23, 32, 42). Other mechanisms that require further study include antioxidant neuroprotection (29, 35, 37) and enhanced metabolic efficiency (52) from functioning as an alternative metabolic substrate.

Fig. 2. Blood ketones following intragastric administration (time 0) of water, R,S-1,3-butanediol acetoacetate diester (BD-AcAc2), and 1,3-butanediol (BD). A: β-hydroxybutyrate (BHB) level was elevated compared with control after either ketogenic compound (P < 0.001); B: acetoacetate (AcAc) level was increased significantly by BD-AcAc2 (P < 0.001) compared with water or BD; C: acetone level increased significantly more after treatment with BD-AcAc2 (P < 0.001); D: pH was decreased compared with control after administration of either BD-AcAc2 or BD (P < 0.001). Data are means ± SE of n = 6 rats/group.

*Significance (P < 0.05) of treatment group from control (water) treated animals; #Significant difference (P < 0.05) between BD-AcAc2 and BD-treated animals as determined by two-way repeated measures ANOVA with post hoc Holm-Sidak test (A, B, D); t-test was used for C.

Fig. 3. Blood gas values following intragastric administration (time 0) of water, BD-AcAc2, and BD. A: P O2 was elevated after administration of BD-AcAc2 (P < 0.001); B: no significant differences in PCO2 were observed in either group. Data are means ± SE of n = 6 rats/group. *Significance (P < 0.05) of treatment group from control (water) treated animals.
Ketone ester administration produces “fasting ketosis”. The anticonvulsant effect of fasting and KD is well documented in humans and animal models and correlates with a rise in blood ketones (6, 36). Dietary-induced hepatic ketogenesis is dependent on maintaining a low insulin-to-glucagon ratio, which quickly reverses with carbohydrate consumption. Seizure protection is also reversed upon ingestion of calories from carbohydrate or excess protein (>20%), as seen in animal models and humans. BD-AcAc2 has little or no effect on blood glucose, and thus makes it an attractive option for mitigating CNS-OT and may represent a sought-after strategy for epilepsy to circumvent issues with compliance associated with KD (43). Our data suggest that the anticonvulsant benefits of fasting ketosis are conferred with BD-AcAc2, even in rats eating a...
standard (carbohydrate-containing) diet ad libitum. Blood ketones following BD-AcAc₂ administration were higher than those typically reported in rats fasted 24–36 h (9) or rats eating a KD (2, 5). Total blood ketones (BHB, AcAc, and acetone) after 1 h averaged >6 mM, which is generally only achieved with prolonged fasting (>7 days) in humans (8). Acetone levels measured after BD-AcAc₂ administration were significantly elevated relative to water and BD but below the levels typically needed to prevent seizures (>2 mM) in rats when given exogenously (39). The acetone level elevated by BD-AcAc₂ in our study (0.7 mM) was similar to brain acetone levels in epilepsy patients that have achieved complete seizure control with adherence to a strict KD (45).

Ketone-induced neuroprotection. One explanation for the mechanism by which BD-AcAc₂ delays CNS-OT is ketone-induced neuroprotection from hyperoxia-induced oxidative stress. This mechanism is plausible if one accepts the “free radical theory of CNS-OT,” which posits that the body’s antioxidant defenses are overwhelmed by increased production of ROS (24). In support of this theory is the observation that brain and blood levels of ROS and reactive nitrogen species increase just before HBO₂-induced seizures (13, 20). Previous research in our laboratory has shown that superoxide production and neuronal excitability in the CA1 hippocampus is tightly coupled to tissue O₂ concentration ranging from 20 to 95% (19). In addition, exogenous ketones also have direct neuroprotective effects in models of neurodegenerative disease (35). For example, ketones may prevent synaptic dysfunction by preserving brain metabolism during metabolic stress or oxidative stress from excess ROS production (29, 52). These data are consistent with our previous in vitro experiments, which showed that ketones significantly decrease superoxide production in primary rat neuronal cultures exposed to hyperoxia (16).

An unexpected finding was that BD-AcAc₂ caused a significant and sustained increase in blood pO₂ levels of ~30%. Its conceivable that these changes in pO₂ result from BD-AcAc₂-induced alterations in the neural control of autonomic regulation, including cardiorespiratory function (38). Further studies are needed to determine the specific contribution of BD-AcAc₂ on brain O₂ consumption, ventilatory drive, systemic blood pressure, and brain blood flow preceding CNS-OT.

Ketone ester-induced metabolic therapy. We hypothesize that CNS-OT results from metabolic dysfunction, which occurs secondary to hyperoxia-induced oxidative stress and hyperexcitability (Ref. 19, no. 1652). In this view, therapeutic ketosis can be considered metabolic therapy, since ketones serve as an alternative fuel for brain metabolism. Metabolic-based therapies have been proven effective for seizure disorders and various acute and chronic neurological disorders (25, 31). Its well known that restricting brain glucose by administering insulin in the absence of ketones causes rapid seizures in animal models and humans and increases vulnerability to evoked seizures. This phenomenon is observed with CNS-OT, whereby insulin-induced hypoglycemia enhances vulnerability to HBO₂-induced seizures (1). It is clear that hyperoxic stress increases neuronal excitability (19) and thus produces greater metabolic demands and substrate utilization (49). Our data suggest that BD-AcAc₂-induced delay in CNS-OT is conferred through enhancement of brain metabolism or synaptic stability by elevation of specific ketones, namely AcAc and acetone. Supplying alternative metabolic substrates to the brain may stabilize synaptic activity through mechanisms reported previously by other investigators, including increased Szent-Györgyi-Krebs cycle intermediates, antioxidant effects, increased GABA-to-glutamate ratio, and activation of ATP-sensitive potassium channel (KATP) channels (6, 36). Additional metabolomic studies are required to investigate whether there are local or regional changes in brain ATP levels, neurotransmitters, and redox couples after administration of BD-AcAc₂.

Direct effect of specific ketones. In previous studies, Chavko et al. (9) demonstrated that an elevation of the primary ketone body BHB (via 1,3-butandiol injection) did not delay CNS-OT. This observation is consistent with the finding that inducing ketosis by administration of BHB does not prevent seizures in animal models (6). It is well known that BD produces ketosis, but primarily through the generation of BHB, and thus produces only low levels of AcAc and acetone (47). However, elevation of AcAc and acetone prevents acutely provoked seizures (e.g., chemical, electrical, audiogenic) in animal models (32, 42). Acetone is relatively nontoxic (LD₅₀ 5 g/kg; rat) and has an anticonvulsant effect at subnarcotic concentrations (23). Endogenous acetone levels are typically very low unless prolonged starvation is achieved (8). Collectively, these studies demonstrate that AcAc and acetone, but not BHB, have greater anticonvulsant properties in standardized animal models of seizures. Therefore, we chose to develop and test a ketone ester (BD-AcAc₂) that elevated all three ketone bodies, but with the highest potential to elevate and sustain blood levels of AcAc (12, 21), which by spontaneous decarboxylation, would elevate acetone.

Our data show that preferential utilization of AcAc and acetone, elevated by BD-AcAc₂, delays CNS-OT. Evidence exists for a direct effect of these ketone bodies on hyperpolarizing neuronal membrane potential and reducing synaptic release of excitatory neurotransmitters (55). These data support the idea that KATP channels are activated in the presence of ketone bodies (BHB and AcAc), but the mechanism of this activation is largely unknown. Work by Juge et al. (28) demonstrates that AcAc inhibited glutamate release by competing with Cl⁻ at the site of allosteric regulation. Very little is known about the anticonvulsant mechanism of acetone. Like other solvents, acetone can alter plasma membrane fluidity, which may counteract hyperoxia-induced alterations in plasma membrane function and structure (18). Future hyperbaric atomic force microscopy and hyperbaric fluorescence microscopy studies will be needed to determine how ketones alter neuronal excitability and plasma membrane viscoelasticity during HBO₂ (17).

Perspectives and Significance

There has been much confusion about ketosis in the medical community, especially the metabolic function of ketones and the physiological state of nutritional ketosis (51). Many of these concerns result from viewing ketones as “metabolic poison” and the association of therapeutic ketosis with the metabolic derangement of diabetic ketoacidosis (DKA). The pathological state of DKA produces “runaway ketosis” and results in ketone concentrations of 20 mM or greater but is quickly reversed with insulin administration. A major concern that frequently arises with regard to ketosis is related to the mild metabolic acidosis caused by the accumulation of ketone
bodies in the bloodstream. Normal blood pH range is 7.35 to 7.45 and may transiently drop lower during the initial stages of ketosis (54). However, blood pH typically rebounds to normal range as long as ketones are maintained <10 mM (54). Our data and others (12, 21, 41) have demonstrated that the mild H+ load from acute administration of BD-AcAc2 does not induce a pathological metabolic acidosis. It needs to be determined how the chronic administration of BD-AcAc2 influences blood pH and physiological cardiopulmonary parameters. As with the KD, we would expect compensatory physiological and metabolic adjustments to buffer the H+ load associated with sustained ketosis. Furthermore, we would expect chronic BD-AcAc2 administration to upregulate ketone transporters and further augment the anticonvulsant effects of ketone esters. Future experiments are needed to confirm if greater anticonvulsant effects can be achieved with long-term administration of ketone esters and if this influences systemic physiological parameters such as systemic arterial blood pressure and cerebral blood flow.

In conclusion, the beneficial effects of fasting or KD-induced ketosis have been demonstrated in a variety of neurological disorders (22). Many seizure disorders and neurological disorders are linked pathophysio logically to energy dysregulation (46), and BD-AcAc2 represents an innovative strategy to normalize aberrant energy metabolism associated with CNS-OT. Recent evidence suggests that oral consumption of ketone esters is safe in humans (15) and may provide ergogenic effects by virtue of enhanced bioenergetics associated with ketone metabolism (44, 53). Similar to the KD, it is unlikely that the anticonvulsant effect of ketone esters can be unified into a single mechanism or a final common pathway. Evidence for BD-AcAc2 working through novel mechanisms is supported by the fact that BD-AcAc2 works when most AEDs fail or are required in high doses Thus BD-AcAc2 may activate mechanisms other than those targeted by any specific AED or even combinations of AEDs. Surprisingly, no commercially available AEDs attempt to mimic the effect of the KD by exploiting the anticonvulsant and neuroprotective effects of therapeutic ketosis with ketone esters. The development and testing of ketone esters represents a promising therapeutic mitigation strategy for CNS-OT. Ketone esters may represent the sought-after “ketogenic diet in a pill” (43), especially if future experiments can demonstrate efficacy in other models of seizures.

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DISCLOSURE

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AUTHOR CONTRIBUTIONS


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KETONE ESTER DELAYS HYPEROXIA-INDUCED SEIZURES