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Duration of Heightened Blood Ketone Levels after Consumption of a Ketone Ester Supplement Drink: Implications for Studying Performance

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STATEMENTS

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The views expressed in this technical report reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

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Human Research/Institutional Review Board (IRB) statement

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Abstract

Ketosis is a state that occurs when the level of ketone bodies increases, which occurs naturally during starvation or prolonged exercise. Ketosis can also be induced in humans through adherence to a ketogenic diet (i.e., low carbohydrate and high fat) or through the consumption of a ketone ester tablet or drink. Emerging research has begun to suggest that inducing ketosis through these methods can result in therapeutic outcomes in individuals with epilepsy, neurodegenerative diseases, traumatic brain injury, and cognitive aging. Thus, induced ketosis may have neuroprotective qualities that can be harnessed for a range of performance outcomes. The current study represents a first step in studying the potential positive effects of ketone supplements on performance. Healthy adult participants consumed a ketone ester drink and their resulting blood ketone levels were tested for up to 3.5 hours. These results were compared to consumption of a placebo drink. Participants also rated a variety of symptoms in both conditions to assess tolerability. Results showed that blood ketone levels peaked at 30 minutes and stayed elevated at that level for up to 120 minutes before beginning to decline. At 3.5 hours post consumption, ketone levels were still significantly elevated above that of the placebo drink. These results suggest that the dose used here would be appropriate for performance research that utilizes a relatively short timeframe, i.e., on the order of hours. Future research should examine the neuroprotective factors of ketone ester supplements on operationally relevant stressors that impair performance.

Duration of Heightened Blood Ketone Levels after Consumption of a Ketone Ester Supplement Drink: Implications for Studying Performance

Ketone bodies, D-β-hyrdroxybutyrate (βHB) and acetoacetate (AcAc), are the water-soluble molecules that are produced by the liver from fatty acids. While ketone bodies are produced continuously under normal physiological conditions, blood ketone concentrations increase during starvation (Cahill, 1970), when consuming a “ketogenic” (i.e., low carbohydrate, high fat) diet (Gilbert et al., 2000), or following prolonged exercise (Koeslag et al., 1980). When the production rate of ketone bodies exceeds the rate of utilization, their concentration in the blood increases, which is known as ketonemia. Ketonemia is followed by ketonuria, which is the excretion of ketone bodies in the urine. Together, these processes are referred to as ketosis. Ketone levels can be measured in the blood, urine, or breath and are generally between 0.5 and 3.0 mM in physiologic ketosis (Laffel, 1999).

Evidence suggests that ketosis may provide a more efficient source of energy with respect to the amount of oxygen required by the brain and therefore may have potential therapeutic outcomes for a variety of neurologic disorders and/or human performance (Veech et al., 2001). There are two main approaches to inducing ketosis in humans, 1) nutritional (i.e., a ketogenic diet, or 2) consumption of a ketone ester (KE) drink or tablet. Given the difficulty in adherence to a ketogenic diet, there has been much advancement in the development of KE supplements for the purpose of therapeutic exploration. Consumption of KE supplements has been shown effective in raising plasma ketone body levels to >2mM. When administered orally, KEs can produce plasma levels comparable to those achieved by the strictest ketogenic diet, thus providing a safe, convenient, and versatile new approach to the study and potential treatment of a variety of diseases, including epilepsy, Alzheimer’s disease (AD), and Parkinson’s disease (PD; Hashim & VanItallie, 2014).

Traditionally, a state of ketosis was seen in a negative light as an indication of starvation or a complete insulin deficiency in diabetes. However, in the early 20th century it was proposed

that a ketogenic diet may have therapeutic effects for individuals suffering from epilepsy (Veech et al., 2001). Ketone bodies have been shown to block both induced and recurrent seizures in various animal models, although the exact mechanism of this neuroprotection is still unclear (e.g., Viggiano et al., 2015; Viggiano et al., 2016; for a review see, Simeone et al., 2017). Moreover, therapeutic ketosis has been shown to significantly delay central nervous system oxygen toxicity seizures in rats (D'Agostino et al., 2013). This initial line of inquiry then led to further exploration of the therapeutic effects of ketosis on neurodegenerative diseases.

AD is a chronic, neurodegenerative disease characterized by progressive decline in memory, as well as other cognitive and behavioral symptoms. In general, dementias are associated with hypometabolism in several brain regions, particularly the hippocampus for AD (Constantini et al., 2008; Foster et al., 1983), and mitochondrial function (Blass et al., 2000). Decreased cerebral glucose utilization is an early part of AD pathology and can precede symptoms and other pathological markers by decades (Blass, 2001). It has been suggested that this metabolic imbalance can be bypassed by providing ketone bodies as an alternative fuel source for neurons. However, the results to date are mixed on the effectiveness and/or the mechanism of these potential effects. For example, in a rat model of AD, it has been found that a ketogenic diet improves motor performance, but it does not change the underlying neural pathology (Brownlow et al., 2013). On the other hand, in a mouse model, studies have found that a ketogenic diet decreased anxiety, improved learning/memory performance, and decreased AD pathology (Kashiwaya et al., 2012; Yin et al., 2016). Preliminary work in humans suggests that administration of a ketogenic supplement improves verbal memory and processing speed in adults with mild to moderate AD, compared to a placebo supplement (Ota et al., 2019). Thus, the use of a ketogenic diet or supplement to counteract the pathology and/or symptoms of AD is still being explored and applied to other neurodegenerative diseases as well.

Several studies have examined the mechanism(s) by which ketone bodies may exert their therapeutic effects on AD, PD (Veech et al., 2001), and other neurodegenerative diseases.

For example, ketone bodies protect neurons from defects in mitochondrial energy generation that are part of the pathophysiology of AD and PD (Kashiwaya et al., 2000). For AD specifically, ketone bodies have been found to block the entry into neurons of amyloid-β 42, a pathogenic hallmark protein of AD. More recently, research has examined individual differences in response to therapeutic ketosis in these neurodegenerative diseases. One such study found that there are individual differences in the response to induced ketosis in patients with AD, such as genotypes variations in the APOE4 and IL1B genes (Henderson & Poirier, 2011). For example, a randomized-controlled trial of a 45 day trial of a ketogenic supplement changed cerebral blood flow specifically in AD patients lacking the APOE4 allele (Torosyan et al., 2018; for a review see, Sharma et al., 2014).

Initially, ketosis was investigated as a therapeutic approach for disease states; however, additional investigations have illustrated the positive effects of ketosis in healthy individuals, such as improvements to athletic or cognitive performance. In animal models, a novel ketone diet improved both physical and cognitive performance in rats (Murray et al., 2016).
In humans, one study found that consumption of a KE tablet improved physical endurance in high-performance athletes by altering fuel competition for oxidative respiration (Cox et al., 2016). Similarly, it was found that consumption of a KE drink increased post-exercise insulin levels, glucose uptake, and muscle glycogen synthesis (Holdsworth et al., 2017; Vandoorne et al., 2017; Volek et al., 2016).

In addition to application for athletic performance, therapeutic ketosis has also been studied with respect to improved cognitive performance. One study found that a ketogenic diet improves cognition (maze running performance) in mice and that these improvements were linked to biochemical changes in prefrontal cortex (Hernandez et al., 2018). One implication of these findings is that therapeutic ketosis may aid cognition, not only in older individuals with dementia, but also in healthy older adults experiencing normal aged-related cognitive decline (Ota et al., 2016; for a review see, Balietti et al., 2010). Some work has taken this idea a step

further and suggested that ketone bodies supplementation may have the same effect of caloric restriction on aging, i.e., extending the lifespan (Veech et al., 2017).

Given the promise of ketone supplements in enhancing performance there are many potential applications for improving performance that is relevant in military operational contexts. For example, military personnel encounter a number of environmental stressors that can include: low-oxygen exposure in aircraft, extreme temperatures, fatigue, and exposure to various toxins. All of these environmental stressors are known to impair performance and therefore pose significant risks to individuals who are exposed. Given that many of these performance impairments relate to decreased perceptual, cognitive, or motor performance, it is possible that increased ketone bodies via a supplement or ketogenic diet may serve as a neuroprotective factor in the face of these types of environmental stressors.

One important first step in testing the potential neuroprotective efficacy of KE supplements against environmental stress is to determine the adequate dosing and tolerability of a given supplement and determine the dose needed to study the stressor of interest. One previous study examined the dosing and tolerability of a KE supplement and found that doses of 140, 357, and 714 mg/kg reached maximum plasma levels of ketones within 1–2 h, reaching 3.30 mM and 1.19 mM for βHB and AcAc, respectively, at the highest dose tested (Clarke et al., 2012). They did this three times a day for five days. Here we sought to replicate these findings with the specific goal of determining an appropriate dose to study an acute hypoxia exposure lasting several hours in order to simulate the duration that a pilot may be exposed to moderate hypoxic conditions during flight.

Method

Participants

A total of 17 healthy adults (age: *M*=32.0, *SD*=5.6; 10 males) participated for monetary compensation ($300). Participants gave written informed consent approved by the Institutional Review Board of the Naval Medical Research Unit - Dayton. Two participants were disqualified

from the study prior to any experimental procedures for medical reasons and three participants only completed one of the two necessary sessions leaving 12 complete datasets.

Procedures

Participants completed two separate visits (i.e., experimental and control) on different days in counterbalanced order. In preparation for the study, participants were asked to fast for at least 8 hours and to get a good night's sleep. Participants reported fasting for an average of 12.00 hours (*SD*=1.99*, range*=9-16). Participants also reported an average of 7.21 hours of sleep (*SD*=0.66*, range*=6-8.5) the night prior to their participation. During the experimental visit, participants drank a solution that contained HVMN® Pure ΔG™ Ketone Ester (herein referred to as KE) mixed with Ensure to mask the flavor and blind the experimental condition. The amount of KE was based on the participants' body weight with an average dose of 283.23 mg/kg (*SD*=2.61). During the control visit, participants drank Ensure in the same volume mixed with a control fluid that tasted similar to the KE solution, but did not contain any active ingredients. For both visits, participants had blood drawn via a finger stick to test blood levels of ketones at eight different time points: 15 minutes before consumption (i.e., baseline), and then 30, 60, 90, 120, 150, 180, and 210 minutes after consumption. During both visits, participants also completed the Environmental Symptoms Questionnaire (ESQ; Sampson et al., 1983). The ESQ is a 68-item assessment of potential symptoms (e.g., I feel lightheaded, I feel alert), where the participant rates the degree to which they are currently experiencing that symptom on a 0-5 scale (0 = not at all, 5 = extreme). The ESQ was administered at four different time points: 15 minutes before consuming the solution (i.e., baseline), and then 60, 120, and 210 minutes after consuming the solution.

Results

Ketone-level Blood Analysis

At 8 different time points, blood was drawn via a finger-stick and tested via Precision Xtra® blood β-Ketone test strips (Abbott) to determine the level of ketones in the blood in mM/L.

Two tests were completed on each blood sample, at each time point. The average of the two readings was then taken for each time point and each condition separately. Two participants were tested only at the first three time points and their data are included in the below analyses where possible. To examine differences in conditions and across time, a 2 (condition: experimental vs. control) x 8 (time: baseline, 30, 60, 90, 120, 150, 180, and 210) repeated-measures ANOVA was tested. The main effect of condition was significant, $F(1,9)=259.95$, $p<0.001$, $\eta_p^2=0.97$, where ketone levels were higher in the experimental condition compared to the control. The main effect of time was also significant, $F(7,3)=25.22$, $p<0.001$, $\eta_p^2=0.74$, with ketone levels decreasing with time. Importantly, the condition x time interaction was significant, $F(7,3)=27.10$, $p<0.001$, $\eta_p^2=0.75$. Planned follow-up *t*-tests showed that at baseline, there was no difference between the experimental and control conditions, $t(11)=0.71$, $p=0.49$, but at all subsequent time points, ketone levels were greater in the experimental compared to the control condition, all ts≥4.14, all ps<0.001. Figure 1 illustrates these results.

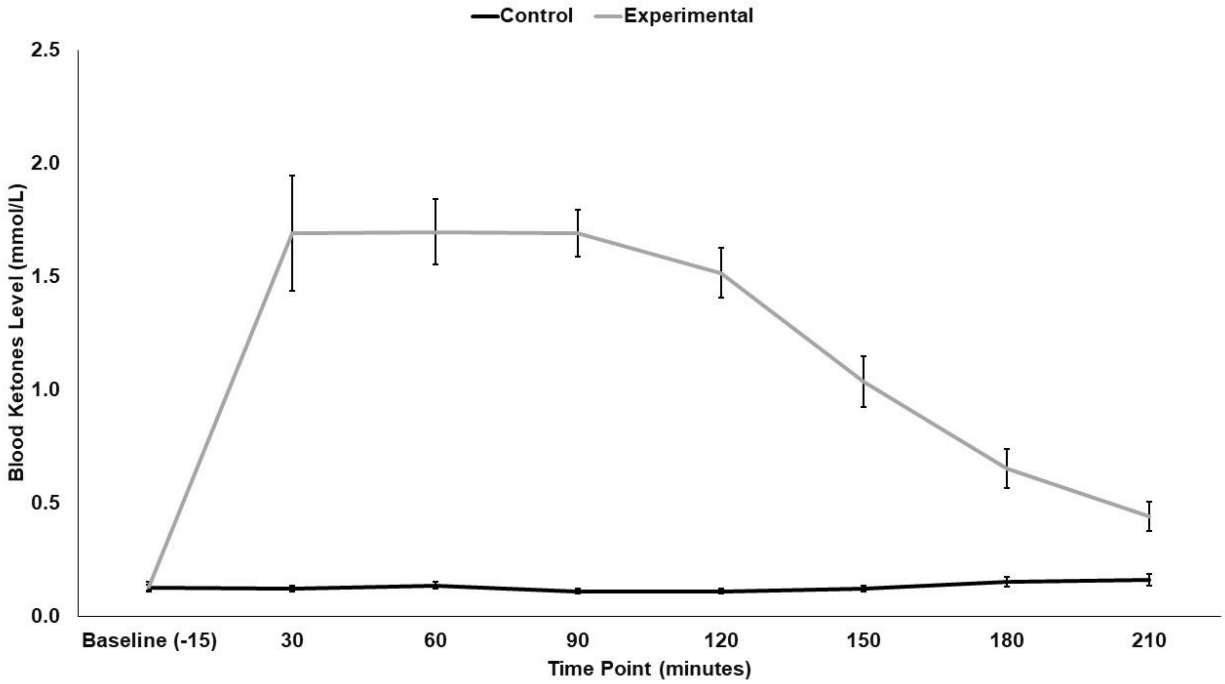

Figure 1. Blood ketone levels over time for the control and experimental conditions separately. Error bars represent standard error of the mean (SEM).

ESQ

The ESQ has nine separate sub-scales (Sampson, Kobrick, & Johnson, 1993) that include the following symptom clusters: cerebral, respiratory, ears/nose/throat (ENT), cold, distress, alert, exert, muscle, and fatigue. Table 1 contains descriptive statistics for each condition and time point. For each of these factors, a 2 (condition: experimental vs. control) x 3 (time: 60, 120 and 210 min post-drink) repeated-measures ANOVA was tested.

ESQ: Average Rating (SEM)						
Symptom Sub-scale	**Control: 60 min**	**Control: 120 min**	**Control: 210 min**	**Exp: 60 min**	**Exp: 120 min**	**Exp: 210 min**
Cerebral	0.21 (0.07)	0.05 (0.02)	0.05 (0.03)	0.06 (0.03)	0.07 (0.04)	0.06 (0.03)
Respiratory	0.12 (0.05)	0.09 (0.04)	0.08 (0.04)	0.07 (0.03)	0.10 (0.03)	0.12 (0.04)
ENT	0.03 (0.02)	0.01 (0.01)	0.00 (0.00)	0.07 (0.03)	0.08 (0.04)	0.07 (0.04)
Cold	0.05 (0.03)	0.03 (0.02)	0.04 (0.02)	0.05 (0.03)	0.08 (0.05)	0.10 (0.04)
Distress	0.15 (0.07)	0.08 (0.03)	0.13 (0.05)	0.12 (0.05)	0.16 (0.06)	0.16 (0.07)
Alert	4.01 (0.13)	4.05 (0.12)	3.99 (0.13)	3.96 (0.15)	3.93 (0.19)	3.87 (0.22)
Exert	0.01 (0.01)	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.00 (0.00)	0.02 (0.02)
Muscle	0.09 (0.05)	0.09 (0.04)	0.11 (0.04)	0.10 (0.04)	0.08 (0.04)	0.07 (0.04)
Fatigue	1.60 (0.03)	1.59 (0.03)	1.57 (0.04)	1.61 (0.07)	1.56 (0.07)	1.54 (0.07)

Table 1. ESQ sub-scale averages (SEM) for each condition and time point after consumption of the solution. Each question was scored on a 1-5 scale.

For the cerebral sub-scale, neither the main effect of condition, $F(1,11)=0.04$, $p=0.84$, nor of time, $F(2,10)=1.04$, $p=0.37$, reached significance. The time x condition interaction, $F(2,10)=1.62$, $p=0.22$, was also not significant. For the respiratory sub-scale, neither the main effect of condition, $F(1,11)<0.01$, $p=1.0$, nor of time, $F(2,10)=0.12$, $p=0.89$, reached significance. However, the time x condition interaction, $F(2,10)=3.67$, $p=0.04$, $\eta_p^2=0.25$, was significant. Follow-up paired-samples t-tests demonstrated no significant difference between the experimental and control conditions at any of the time points, all ts≤ 1.25, all ps≥ 0.24. For the

ENT sub-scale, neither the main effect of condition, $F(1,11)=3.42$, $p=0.09$, nor of time, $F(2,10)=0.76$, $p=0.48$, reached significance. The time x condition interaction, $F(2,10)=0.81$, $p=0.46$, was also not significant. For the cold sub-scale, neither the main effect of condition, $F(1,11)=3.30$, $p=0.1$, nor of time, $F(2,10)=0.85$, $p=0.44$, reached significance. The time x condition interaction, $F(2,10)=1.08$, $p=0.36$, was also not significant. For the distress sub-scale, neither the main effect of condition, $F(1,11)=0.82$, $p=0.38$, nor of time, $F(2,10)=0.45$, $p=0.65$, reached significance. The time x condition interaction, $F(2,10)=0.96$, $p=0.40$, was also not significant. For the alert sub-scale, neither the main effect of condition, $F(1,11)=0.94$, $p=0.35$, nor of time, $F(2,10)=0.59$, $p=0.56$, reached significance. The time x condition interaction, $F(2,10)=0.22$, $p=0.81$, was also not significant. For the exert sub-scale, neither the main effect of condition, $F(1,11)=0.65$, $p=0.44$, nor of time, $F(2,10)=0.65$, $p=0.53$, reached significance. The time x condition interaction, $F(2,10)=0.65$, $p=0.53$, was also not significant. For the muscle sub-scale, neither the main effect of condition, $F(1,11)=0.51$, $p=0.49$, nor of time, $F(2,10)=0.22$, $p=0.81$, reached significance. The time x condition interaction, $F(2,10)=0.90$, $p=0.42$, was also not significant. For the fatigue sub-scale, the main effect of condition, $F(1,11)=0.17$, $p=0.69$, was not significant, but the main effect of time did reach significance, $F(2,10)=3.67$, $p=0.04$, $\eta_p^2=0.25$, with fatigue scores decreasing over time for both conditions. The time x condition interaction, $F(2,10)=0.45$, $p=0.64$, was also not significant. Together, these results indicate that there was no difference in any reported symptoms following ingestion of the KE drink, as measured by the ESQ.

Discussion

The current study demonstrated that the dose of KE used here elevated blood ketone levels for up to 2 hours before seeing significant decline. Moreover, participants did not report any significant symptomatology based on the ESQ after ingesting the KE supplement compared to the control drink. These results suggest that the dose used here is tolerable and would be sufficient for a study of performance impairment under environmental stress (e.g., hypoxia) that

lasts up to 2 hours in duration. The results also showed that even after 3.5 hours, blood ketone levels were still significantly elevated above baseline.

While the current study represents a simple first step in dosage and tolerability, the results here provide a way forward to explore the potential neuroprotective ability of KE supplements in response to a variety of operational stressors. Previous work has shown that induced ketosis has therapeutic benefits for epilepsy (Simeone et al., 2017), neurodegenerative diseases (Hashim & VanItallie, 2014), traumatic brain injury (Prins, 2008), cancer (Poff et al., 2014), and cognitive aging (Balietti et al., 2010). Moreover, induced ketosis has been shown to improve cognition and athletic performance in healthy individuals, as well as protect against impairment after exposure to chronic stress (Brownlow et al., 2017).

Animal models of the therapeutic effects of KE, as well as studies on athletic performance during ketosis, suggest that induced ketosis changes the underlying metabolic functioning of the brain. Specifically, ketosis seems to provide a more efficient source of energy with respect to the amount of oxygen required by the brain. Therefore, a potential operational application would be to use induced ketosis as a neuroprotective factor against hypoxia during flight. Currently, military aircraft are not outfitted with a warning system that can alert a pilot to conditions that may cause or exacerbate hypoxia in the cockpit. Additionally, there is a whole body of research that shows the deleterious effects of hypoxia on performance. Future research should explore the effectiveness of KE supplements on performance-related impairments during acute hypoxia exposure.

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