Exogenous Ketosis as a Countermeasure for Acute Hypoxic Exposure

Tyler S. McClure, BSc MSc

Doctor of Philosophy

Dublin City University
School of Health and Human Performance

Supervisor: Dr. Brendan Egan

Submitted to Dublin City University, August 2023

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: ID No: Date:

Acknowledgements

The study of exercise physiology and ketone metabolism has grown into a great passion of mine. I would like to take a moment to acknowledge the people who have helped me grow and develop this passion as well as the people who have helped me accomplish all this work.

First of all, to my family, Erica and Steve, thank you for your continued love and support and for believing in me when I doubted I could achieve anything as close to this accomplishment. I would have never made it this far without you.

Dr. Andrew Koutnik, thank you sharing your passion, experiences, advice and always being that positive voice in my ear, this has all been vital to my success during my PhD.

Collaborators at IHMC and Grove City College, thank you for all the effort towards our work together in this PhD and the manuscripts that will arise from this work. It has proven vital to my success.

Mark Evans, Your original ketone review “Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation” (with Brendan Egan and Karl Cogan) is what really sparked my interest into this field and without it I don’t know what direction I would have wondered off into, so thank you for that foundation literature review.

To the whole lab group at DCU, David, Mark, Ketone Mark, Ian, Aidan, Katherine. You welcomed me with open arms and made me feel at home over here in Dublin and provided me with memories I won’t forget, thank you.

And last but certainly not least, Brendan Egan. I greatly appreciate all work and guidance you have given me over the years, as this PhD path has been by no means a normal path, but you continually helped me through every twist and turn along the way. You are an amazing supervisor who allowed me to work freely but was always available with advice and guidance when needed. You are an excellent role model as you are a truly humble and ethnically sound researcher who just wants to learn and move science forward. Your supervision has impacted me both as a researcher and a person, thank you for everything you have taught me.

Conference Proceedings Arising from this Thesis

Society For Brain Mapping and Therapeutics (SBMT)

(Los Angeles, CA USA March, 2022)

Oral Presentation

Cognitive & neurological resilience with exogenous ketones
Tyler S. McClure

All-Ireland Postgrad Conference in Sport Science, Phys Activity and Physical Education

(Dublin, Ireland September 2022)

Oral Presentation

Exogenous ketone monoester ingestion attenuates declines in cognition and oxygen saturation during severe hypoxia
Tyler S. McClure

All-Ireland Postgrad Conference in Sport Science, Phys Activity and Physical Education

(Cork, Ireland May 2023)

Oral Presentation

Exogenous ketone monoester ingestion attenuates declining oxygen saturation during weighted treadmill ruck exercise at simulated high altitude but does not impact measures of cognitive performance
Tyler S. McClure

Co-authored Articles Arising from this Thesis

1. Evans M, **McClure TS**, Koutnik AP, Egan B. Exogenous Ketone Supplements in Athletic Contexts: Past, Present, and Future. Sports Med. 2022 52(Suppl 1):25-67. doi: 10.1007/s40279-022-01756-2.

Other Co-authored Articles Independent from this Thesis

1. Prins PJ, Buxton JD, **McClure TS**, D'Agostino DP, Ault DL, Welton GL, Jones DW, Atwell AD, Slack MA, Slack ML, Williams CE, Blanchflower ME, Kannel KK, Faulkner MN, Szmaciasz HL, Croll SM, Stanforth LM, Harris TD, Gwaltney HC, Koutnik AP. Ketone Bodies Impact on Hypoxic CO_2 Retention Protocol During Exercise. Front Physiol. 2021 12:780755. doi: 10.3389/fphys.2021.780755.
2. Buga A, Crabtree CD, Stoner JT, Decker DD, Robinson BT, Kackley ML, Sapper TN, Buxton JD, D'Agostino DP, **McClure TS**, Berardi A, Cline S, Fleck T, Krout J, Newby D, Koutnik AP, Volek JS, Prins PJ. Metabolic and ruck performance effects of a novel, light-weight, energy-dense ketogenic bar. Exp Physiol. 2023 108(5):715-727. doi: 10.1113/EP091029.

Table of Contents

List of Abbreviations

β-hydroxybutyrate (βHB)

3-hydroxybutyrate dehydrogenase (BDH)

acetoacetate (AcAc)

acetoacetyl CoA (AcAc-CoA)

acetyl CoA (Ac-CoA)

acetyl CoA acetyltransferase (ACAT)

acute mountain sickness (AMS)

adenosine triphosphate (ATP)

blood brain barrier (BBB)

carbohydrate (CHO)

carbonic anhydrase (CA)

cerebral blood flow (CBF)

chronic obstructive pulmonary disease (COPD)

cytochrome c oxidase (COX)

electron transport chain (ETC)

exogenous ketone supplements (EKS)

hypobaric hypoxia (HH)

hypoxia-inducible factor (HIF-1)

hypoxic ventilatory response (HVR)

internal carotid artery (ICA)

ketogenic diet (KD)

ketone bodies (KB)

ketone diester (KDE)

ketone monoester (KME)

ketone salts (KS)

lactate dehydrogenase A (LDHA)

medium chain fatty acids (MCFA)

nitric oxide (NO)

normobaric hypoxia (NH)

oxygen (O_2)

oxygen saturation (SpO_2)

oxygenated hemoglobin (Hb)

partial pressure of oxygen (PaO_2)

partial pressure of carbon dioxide ($PaCO_2$)

phosphofructokinase (PFK)

pyruvate dehydrogenase (PDH)

rating of perceived exertion (RPE)

reactive oxygen species (ROS)

succinyl-CoA:3-oxoacid CoA transferase (OXCT)

tricarboxylic acid (TCA) cycle

medium chain triglycerides (MCT)

vertebral artery (VA)

von Hippel-Lindau (pVHL)

List of Figures

List of Tables

Exogenous Ketosis as a Countermeasure for Acute Hypoxic Exposure

Tyler S. McClure

Abstract

Exogenous ketones are a supplemental form of ketone bodies that rapidly alter metabolism, providing extrahepatic tissues with an alternate fuel source, and can affect blood gases and pH balance. This thesis investigated whether consumption of exogenous ketone supplements (EKS) can be applied as a countermeasure to hypoxic exposure by mitigating the decline in oxygen saturation and cognitive performance as hypoxic exposure has shown to decrease both physical and cognitive performance.

Study 1: Investigated the effects of various forms of EKS on metabolism, blood gases, respiration, heart rate variability, hemodynamics, and cognitive performance in healthy male and female populations at rest. Findings include rapid effects on metabolism, blood gases, pH, and no effect on cognitive performance.

Study 2: Investigated the application of a ketone monoester (KME) as a potential countermeasure for oxygen saturation and cognitive declines during severe hypoxic exposure (6096 m, 9.7%O_2 for 20 min) in healthy male participants at rest. Findings included enhanced resiliency to the declines in oxygen saturation and cognitive performance after KME ingestion.

Study 3: A follow-up investigation to the prior study evaluated a lower dose of KME and a different domain of cognitive performance (vigilance/attention) during severe hypoxic exposure (6096 m, 9.7%O_2 for 15 min) in healthy male participants at rest. Findings included enhanced resiliency to the declines in oxygen saturation and cognitive performance after KME ingestion.

Study 4: Investigated the use of KME as a countermeasure to declines in oxygen saturation, cognitive performance, and effects of subjective exercise intensity during severe hypoxia at rest and during moderate intensity exercise in healthy males. Findings included enhanced resiliency to the decline in oxygen saturation, but no effect on cognitive performance after KME ingestion.

Collectively, these investigations show promise for KME as a countermeasure for acute exposure to severe hypoxia both at rest and during exercise, with further work needed to establish optimal dosing and effectiveness in other populations and specific use cases.

Chapter 1
Literature review[1]

[1] **Please note:** Portions of this literature review are adapted from the following published article.
Evans, M., **McClure, T. S.**, Koutnik, A. P., & Egan, B. (2022). Exogenous Ketone Supplements in Athletic Contexts: Past, Present, and Future. *Sports Medicine* 52(Suppl 1), 25–67. https://doi.org/10.1007/s40279-022-01756-2

1.1 INTRODUCTION

Hypoxia can arise as a result of altitude exposure or in response to a pathological condition such as cardiovascular disease, asthma, chronic obstructive pulmonary disease (COPD), or respiratory virus infections. The accompanying decrease in oxygen (O_2) availability and accompanying reduction in mitochondrial respiratory rate and increases oxidative stress and inflammation (Pasiakos et al., 2021) may have a deleterious influence on various organ and tissue systems, including the brain and musculoskeletal system (Chaillou, 2018, Goodall et al., 2014, Michiels, 2004). Acute hypoxic exposure can also result in a decrease in circulating hemoglobin O_2 saturation (SpO_2), which can disrupt brain metabolism and impair cognitive function (Williams et al., 2019). Acute impairments in working memory (Legg et al., 2016, Malle et al., 2013), cognitive flexibility (Asmaro et al., 2013, Turner et al., 2015), attention (Asmaro et al., 2013, Stepanek et al., 2014, Turner et al., 2015), executive function (Turner et al., 2015), and auditory processing (Beer et al., 2017). have been associated with moderate-to-severe hypoxia exposure.

Countermeasures that aim to reduce or eliminate the effects of hypoxia could have far-reaching consequences for those who are affected by or at risk of hypoxia. Attenuating drops in circulation and tissue O_2 concentrations is a viable target for alleviating acute hypoxia symptoms and minimizing the harmful effects of long-term hypoxia. For example, during environmental hypoxia, descending to a lower altitude and increasing breathing rate by hyperventilation might ameliorate acute altitude-related hypoxia symptoms because SpO_2 values return to normal (>95%) (Shaw et al., 2021). In COPD patients with chronic pathological hypoxia, daily O_2 therapy has been shown to enhance cognition and mortality rates (Karamanli et al., 2015, Pavlov et al., 2018). Thus, whether acute or chronic, increasing oxygen availability is the most effective way to alleviate hypoxic symptoms. Furthermore, the carbonic anhydrase inhibitor acetazolamide, which generates moderate acidosis and increases respiratory rate, has

been shown to increase O_2 saturation and alleviate symptoms of acute mountain sickness (AMS) (Wang et al., 2015).

Although acetazolamide is the standard of care for treating AMS and has been shown to reduce the incidence of AMS at high altitude, studies have also shown that carbon anhydrase inhibitors have a negative effect on sub-maximal and maximal physical performance (Posch et al., 2018) as well as learning, memory, and attention (Sun et al., 2001, Wang et al., 2013a). Treatments that are helpful at low-to-moderate altitudes are frequently found to have little or no effect at higher altitudes (Robach et al., 2008).

These constraints highlight a significant gap for particular populations such as military aircraft pilots, mountain warfare operators, and rescue operational people, among others, who must undertake cognitively demanding missions at altitudes of up to and, on occasion, surpassing 6096 m (Shaw et al., 2021). Even small deterioration in these systems can significantly reduce the likelihood of success under these harsh conditions, with potential serious implications. Given these characteristics, there is an urgent need to develop more effective and practical hypoxia exposure countermeasures.

In pre-clinical models, a ketogenic diet (KD), characterized by an increase in circulating ketone bodies (KB) known as ketosis, has shown to be efficacious as a countermeasure in hypoxic environments (Brownlow et al., 2017, Gom et al., 2021). A KD necessitates a multiweek adaption period making long-term compliance problematic (Burke, 2021). Acute administration of KBs to induce ketosis via exogenous ketone supplements (EKS) may be a more realistic alternative because they have been shown to rapidly boost blood R-βhydroxybutyrate (R-βHB) levels without necessitating lifestyle or dietary changes (Evans et al., 2022, Poff et al., 2020). A recent study observed that the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (R-BD R-βHB) ketone monoester (KME) could reduce the deleterious effects of acute moderate hypoxia exposure (5029 m) on SpO_2 and cognitive performance (Coleman

et al., 2021). However, military operators, mountaineers, and occupations such as pilots are often required to maintain high levels of cognitive performance across diverse tasks to maintain safety even when severe hypoxia exposure occurs.

The current PhD studies sought to investigate whether acute ingestion of R-BD R-βHB KME could mitigate declines in SpO_2 and cognitive performance from severe hypoxia by investigating

1. The effects of various forms of EKS on pH, ventilation, blood gases, heart rate variability, and cognitive performance at rest in normoxia
2. The effect of acute ingestion of R-BD R-βHB KME during acute severe hypoxic exposure (simulated 6096 m altitude; 9.7%O_2) on performance in selected cognitive performance tests
3. The utility of R-BD R-βHB KME as a countermeasure to hypoxic exposure when an additional stressor that upregulates oxygen consumption, namely moderate exercise, is involved

Hypotheses

1. Ingestion of KME will decrease pH and PCO_2, increase ventilation and heart rate, but have no effect on cognitive performance at rest, whereas other EKS will not affect blood gases, ventilation, heart rate or cognitive performance at rest.
2. Ingestion of KME will increase blood βHB concentrations and decrease blood glucose concentrations in addition to attenuating declines in SpO_2 and cognitive performance compared to PLA condition at rest.
3. Ingestion of KME will increase blood βHB concentrations and attenuate the increase in blood glucose concentrations in addition attenuating declines in SpO_2 and cognitive performance compared to PLA condition at rest and after exercise.

CHAPTER II

LITERATURE REVIEW

The purpose of the literature review is to provide (i) an overview of ketone body metabolism, (ii) detailed descriptions of currently-researched EKS, (iii) cognitive performance and its relevance to occupational and performance contexts, (iv) the effects of acute hypoxic exposure on cognitive performance, and (v) a rationale for why EKS are worthy of investigation

1.2 KETONE BODIES, KETOSIS, AND EXOGENOUS KETONE SUPPLEMENTS

The KBs, namely acetoacetate (AcAc), βHB and acetone, are lipid-derived, water-soluble organic compounds produced almost exclusively in the liver, and whose production is amplified most obviously during physiological states characterized by low carbohydrate (CHO) availability i.e. starvation, prolonged fasting or undertaking ketogenic diets (Robinson and Williamson, 1980, Laffel, 1999, Poff et al., 2020). AcAc and βHB have pleiotropic effects in multiple organs including brain, heart, and skeletal muscle by modulating substrate utilisation, inflammation, oxidative stress, catabolic processes, and gene expression (Newman and Verdin, 2017, Poff et al., 2020, Puchalska and Crawford, 2021).

In vivo administration of ketogenic compounds was first conducted in patients with paediatric malabsorption disorders associated with chronic pancreatitis and cystic fibrosis; (Hashim et al., 1962, Kuo and Huang, 1965) prior to the discovery that they could elevate systemic [KB] in humans (Freund and Weinsier, 1966). The development of novel synthetic compounds for the *in vivo* administration of KBs from exogenous sources has been for ~40 years (Birkhahn et al., 1977, Birkhahn and Border, 1978, Birkhahn et al., 1979, Birkhahn, 1983), initially in the context of parenteral nutrition (Brunengraber, 1997), and more recently with more broad therapeutic applications (Cahill and Veech, 2003, Veech, 2004, Hashim and VanItallie, 2014). Early forms included glycerol monobutyrate (Birkhahn et al., 1977), monoacetoacetin, a monoester of glycerol and AcAc (Birkhahn and Border, 1978, Birkhahn et

al., 1979), triesters of glycerol and AcAc, and mono- and triesters of glycerol and βHB (Brunengraber, 1997). As well as glycerol, R,S-1,3-butanediol (BD) can be esterified to βHB or AcAc, with BD itself in turn elevating [βHB] given its action as a ketogenic precursor (Kies et al., 1973, McCarthy et al., 2021a).

This initial work on administration of KBs from exogenous sources and their potential role in parenteral nutrition, primarily via intravenous infusion, informed the more recent development of ingestible ketone salts and ketone esters, now collectively referred to as exogenous ketone supplements (EKS). βHB is a chiral molecule with R- and S-, enantiomers, also known as D- and L-, respectively. R-βHB is the circulating and primary form of βHB (Tsai et al., 2006, van Rijt et al., 2021), with S-βHB contributing ~3% of [total βHB] even in individuals adhering to a ketogenic diet (Kackley et al., 2020). Upon entry into peripheral tissues R-βHB is re-oxidised to AcAc by mitochondrial 3-hydroxybutyrate dehydrogenase (BDH) and then rapidly catabolized to acetyl CoA via the ketolytic pathway involving succinyl-CoA:3-oxoacid CoA transferase (OXCT) and acetyl CoA acetyltransferase (ACAT) before entering the tricarboxylic acid (TCA) cycle (Robinson and Williamson, 1980, Evans et al., 2017). The metabolic pathways of ketogenesis and ketolysis are in explained in greater detail later in subsequent sections.

S-βHB is biologically-present in small quantities. It is not a substrate for BDH and is therefore not directly metabolised to AcAc. Current evidence, indicates that the relative contribution of S-βHB enantiomer to energy production is relatively small (Scofield et al., 1982). The metabolism of S-βHB remains somewhat poorly described, but is likely to be involved in the hepatic synthesis of free fatty acids and sterols, and with a large proportion being converted to R-βHB (Webber and Edmond, 1977, Lincoln et al., 1987).

S-βHB itself does exhibit bioactivity through G-protein coupled receptors (Taggart et al., 2005) and shares similar molecular interactions and intracellular signal transduction

cascades with R-βHB (Youm et al., 2015). It is likely therefore that changes in the circulating concentration R-βHB is likely to have physiological consequences. Enantiomer-specific effects have been reported on oxidative phosphorylation in the brain (Tieu et al., 2003), lifespan extension in *Caenorhabditis elegans* (Edwards et al., 2014), glucose utilisation in cardiomyocytes (Tsai et al., 2006), and insulin-stimulated glucose uptake in oxidative skeletal muscle (Yamada et al., 2010), whereby in each instance effects of R-βHB were not similarly observed for S-βHB. For example, Edwards et al. (2014) found an increased in the lifespan of *C. elegans* nematodes at R-βHB concentrations of 2 mM, 10 mM, and 20 mM and no effect of any dose of S-βHB (Edwards et al., 2014).

Blood [KB] are typically ≤0.1 mM in the postprandial state, and ~0.1 to ~0.4 mM after an overnight fast (Robinson and Williamson, 1980, Balasse and Fery, 1989, Laffel, 1999). Circulating concentrations may reach ~1.0 and ~5.0 mM after 24 h and 1 week of fasting respectively, ~0.5 to 3.0 mM on a ketogenic diet, and >14.0 mM in a state of diabetic ketoacidosis (Robinson and Williamson, 1980, Balasse and Fery, 1989, Evans et al., 2017, Poff et al., 2020). Hyperketonaemia was accepted originally as circulating [KB] exceeding 0.2 mM (Robinson and Williamson, 1980), whereas circulating [R-βHB] ≥0.5 mM has more recently been proposed as an operational definition of "nutritional ketosis" (Volek et al., 2015, Poff et al., 2020). Depending on the method of producing acute nutritional ketosis, there can be divergent responses in [R-βHB], [S-βHB], and [AcAc] such that total [KB] may meaningfully differ from [R-βHB]. In that context, the threshold of [R-βHB] ≥0.5 mM is arguably arbitrary. For example, infusion of R-βHB in healthy young men to a concentration of as little as ~ 0.2 to ~ 0.5 mM elicits changes in whole-body metabolism including attenuation of estimated hepatic glucose output and adipose tissue lipolysis, and increases in cerebral R-βHB uptake (Mikkelsen et al., 2015).

This initial work on administration of KBs from exogenous sources and their potential role in parenteral nutrition, primarily via intravenous infusion, informed the more recent development of EKS. The broad category encompassed by EKS currently includes ketogenic precursors such as BD and medium chain fatty acids (MCFA) and triglycerides (MCT), isolated KBs in the form of R-βHB and R,S-βHB ketone salts (KS), and ketone esters (described in more detail in Table 1.1). Ketone esters have been prominent in the exercise science literature (Cox et al., 2016, Leckey et al., 2017, Vandoorne et al., 2017, Holdsworth et al., 2017, Evans and Egan, 2018, Evans et al., 2019, Dearlove et al., 2019, Stubbs et al., 2019, Faull et al., 2019, Poffé et al., 2019, Poffé et al., 2020, Martin-Arrowsmith et al., 2020, Dearlove et al., 2021a, Dearlove et al., 2021b, Poffé et al., 2021a, Poffé et al., 2021b, Poffé et al., 2021c, McCarthy et al., 2021b, Whitfield et al., 2021, Waldman et al., 2022, Peacock et al., 2022, Dearlove et al., 2022), and include the R-BD R-βHB KME (Clarke et al., 2012b, Cox et al., 2016, Stubbs et al., 2017), originally developed to improve the physical and cognitive performance in combat soldiers (Ford and Glymour, 2014), and the R,S-1,3-butanediol acetoacetate (R,S-BD AcAc) ketone diester (KDE) (Desrochers et al., 1995, Kesl et al., 2016, Leckey et al., 2017). Other ketone esters that have been reported in the peer-reviewed literature to date include a compound of βHB and the short chain fatty acid butyrate (βHB-BA) (Cavaleri and Bashar, 2018), and a diester of hexanoic acid (a ketogenic MCFA) and R-1,3 butanediol (BH-BD) (Stubbs et al., 2021a, Chen et al., 2021, Crabtree et al., 2022). Given the numerous possible combinations of AcAc and βHB with ketogenic precursors (including BD, MCFAs, glycerol, and ketogenic amino acids), it is likely that additional forms of EKS will be developed in the future.

Ingestion of EKS in athletic contexts is undertaken primarily with the aim of elevating circulating [R-βHB], an effect that can occur within minutes of ingestion and be maintained for several hours depending on form and dose of EKS. The degree of change in [R-βHB], following ingestion of EKS is dependent on a number of other factor (Figure 1.1), including

being fasted or fed, and being at rest or exercising(see Table 1.1). Ingestion of EKS, provides an alternative method to increase [R-βHB], and to a lesser extent [AcAc] (Stubbs et al., 2017, Leckey et al., 2017, Evans et al., 2018, Prins et al., 2020a, McCarthy et al., 2021b, Crabtree et al., 2022), without injections or intravenous infusions (White et al., 2021), both of which would be impractical or illegal in most occupational and performance contexts.

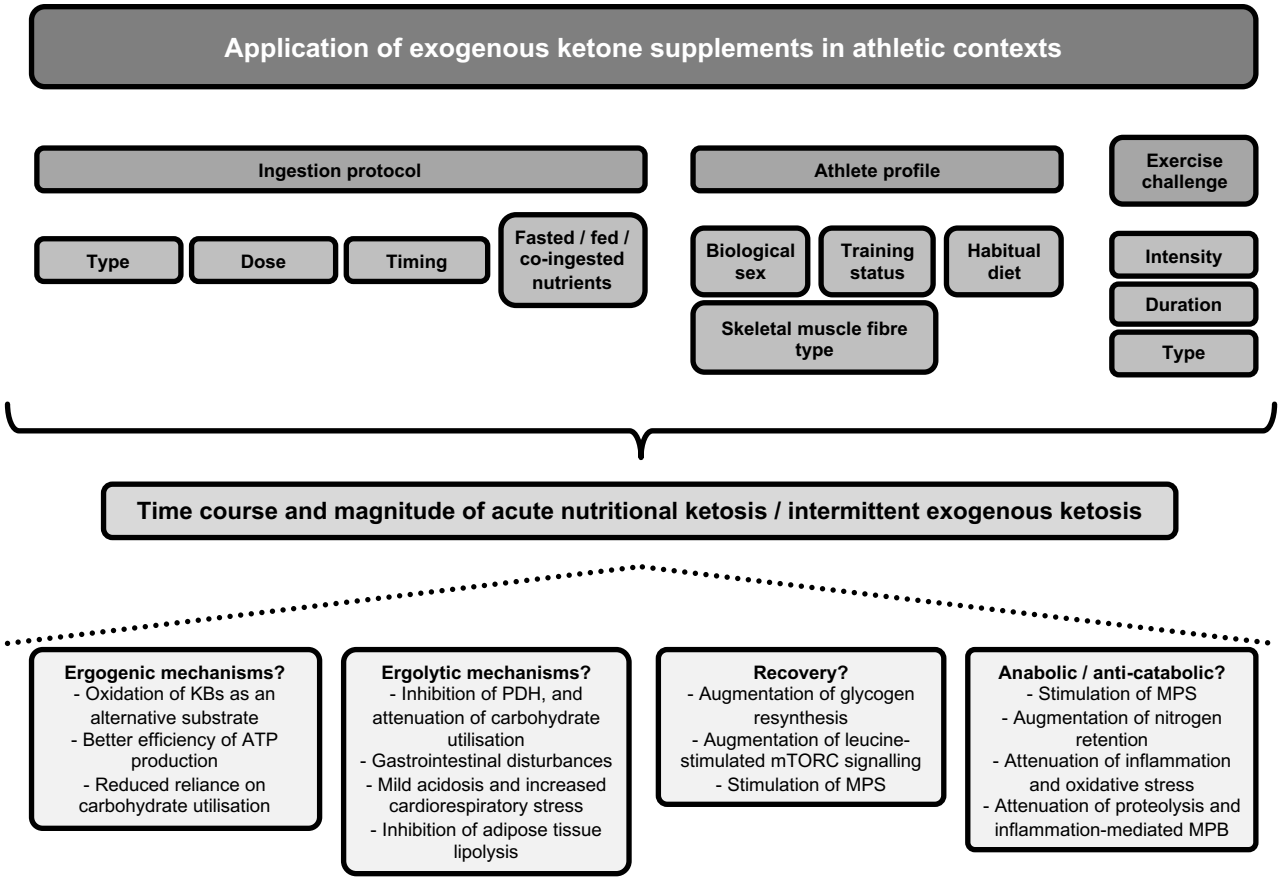

Figure 1.1 Factors influencing the time course and magnitude of transient changes in circulating concentrations of ketone bodies after acute ingestion of exogenous ketone supplements, and mechanisms of potential benefit and impairment of consequent effects in athletic contexts.
Abbreviations: ATP, adenosine triphosphate; KB, ketone bodies; MPB, muscle protein breakdown; MPS, muscle protein synthesis; mTORC, mechanistic target of rapamycin complex; PDH, pyruvate dehydrogenase.

After ingestion of EKS, the consequent acute transient increase in circulating [R-βHB] and [AcAc], which has been termed "acute nutritional ketosis" (Cox and Clarke, 2014) or "intermittent exogenous ketosis" (Poffé et al., 2020, Poffé and Hespel, 2020), has been

consistently observed to impact metabolism both at rest, and during and after exercise (Cox et al., 2016, O'Malley et al., 2017, Rodger et al., 2017, Leckey et al., 2017, Stubbs et al., 2017, Vandoorne et al., 2017, Holdsworth et al., 2017, Evans and Egan, 2018, Evans et al., 2018, Myette-Côté et al., 2018, Waldman et al., 2018, Fischer et al., 2018, Stubbs et al., 2018, Evans et al., 2019, Myette-Cote et al., 2019, Scott et al., 2019, Shaw et al., 2019, Dearlove et al., 2019, Stubbs et al., 2019, Faull et al., 2019, James and Kjerulf Greer, 2019, Waldman et al., 2020, Norwitz et al., 2020, Prins et al., 2020a, Prins et al., 2020b, Kackley et al., 2020, Rittig et al., 2020, Poffé et al., 2020, Martin-Arrowsmith et al., 2020, Dearlove et al., 2021a, Dearlove et al., 2021b, Prins et al., 2021, Poffé et al., 2021a, Poffé et al., 2021b, Poffé et al., 2021c, McCarthy et al., 2021b, Clark et al., 2021, Walsh et al., 2021a, Walsh et al., 2021b, Coleman et al., 2021, Svart et al., 2021, Whitfield et al., 2021, Vestergaard et al., 2021, Løkken et al., 2022, Waldman et al., 2022, Cameron et al., 2022, Jo et al., 2022, Qazi et al., 2022, Quinones and Lemon, 2022a, Peacock et al., 2022, Dearlove et al., 2022). These effects, combined with interest in KBs as an alternative substrate in the failing heart (Yurista et al., 2021) and aging brain (Myette-Côté et al., 2021), has therefore led to considerable interest in EKS as beneficial agents in athletic performance, recovery and beyond (Evans et al., 2017, Koutnik et al., 2019, Margolis and O'Fallon, 2020, Mansor and Woo, 2020, Shaw et al., 2020, Valenzuela et al., 2021, Puchalska and Crawford, 2021, Daines, 2021, Brooks et al., 2022, Falkenhain et al., 2022).

In the time since 2016 when the first peer-reviewed research papers detailing the effects of acute ingestion of EKS on exercise metabolism and endurance performance in humans were published (Cox et al., 2016), there has been a dramatic increase in the number of studies that have investigated the effects of acute ingestion of EKS of various types on exercise metabolism, physical and cognitive performance, and recovery from exercise (Cox et al., 2016, O'Malley et al., 2017, Rodger et al., 2017, Leckey et al., 2017, Vandoorne et al., 2017, Holdsworth et al., 2017, Evans and Egan, 2018, Evans et al., 2018, Waldman et al., 2018, Fischer et al., 2018,

Evans et al., 2019, Scott et al., 2019, Shaw et al., 2019, Dearlove et al., 2019, Stubbs et al., 2019, Faull et al., 2019, James and Kjerulf Greer, 2019, Waldman et al., 2020, Prins et al., 2020a, Prins et al., 2020b, Kackley et al., 2020, Poffé et al., 2020, Martin-Arrowsmith et al., 2020, Dearlove et al., 2021a, Dearlove et al., 2021b, Prins et al., 2021, Poffé et al., 2021a, Poffé et al., 2021b, Poffé et al., 2021c, McCarthy et al., 2021b, Clark et al., 2021, Whitfield et al., 2021, Waldman et al., 2022, Cameron et al., 2022, Quinones and Lemon, 2022a, Qazi et al., 2022, Peacock et al., 2022), and in other studies investigating short-term (~10 d to 6 weeks) daily consumption (Poffé et al., 2019, Soto-Mota et al., 2019, Walsh et al., 2020b, Walsh et al., 2021a, Soto-Mota et al., 2021, Buga et al., 2021, Jo et al., 2022, Dearlove et al., 2022). The global market for EKS has grown since becoming commercially available in the latter half of the last decade and is projected to reach ~ USD$918 million by 2030, with a compound annual growth rate of 7.7% during this period with much of the market currently based in the USA and Asia–Pacific regions (ResearchAndMarkets.com, 2021).

1.3 SYNTHESIS AND UTILIZATION OF KETONE BODIES

1.3.1 Ketogenesis

The primary physiological role of the amplification of ketogenesis during low CHO availability is to provide an additional substrate in the form of KB for cerebral metabolism and to a lesser extent cardiac and skeletal muscle (Robinson and Williamson, 1980, Laffel, 1999, Puchalska and Crawford, 2021). KBs are water soluble molecules that can pass through the blood brain barrier (BBB) via monocarboxylate transporter 1 (MCT1)-mediated transport (Pierre and Pellerin, 2005) and their uptake is increased by increasing concentrations in the circulation (Courchesne-Loyer et al., 2017).

KBs are created predominately from FFAs, although they can be derived from ketogenic amino acids such as leucine, lysine, phenylalanine, isoleucine, tryptophan, and tyrosine but these likely contribute less than 5% of circulating KBs (Thomas et al., 1982). During low CHO

availably, plasma glucose and insulin are reduced, increasing lipolysis, and thus releasing more FFAs into circulation. Ketogenesis is stimulated by an elevated glucagon-to-insulin ratio and declines in hepatic glycogen concentration, whereas reduced blood flow to the liver or elevations in KBs suppress ketogenesis (Laffel, 1999, Robinson and Williamson, 1980). Ketogenesis (see Fig 1.2) involves a series of sequential enzymatic reactions in the liver, beginning with acetyl CoA (Ac-CoA) and acetoacetyl CoA (AcAc-CoA), ultimately producing AcAc. FFAs are converted to fatty acyl CoA (FA-CoA), enter hepatic mitochondria via CPT1-mediated transport and undergo β-oxidation to acetyl CoA. Sequential reactions of condensation of Ac-CoA molecules to acetoacetyl CoA (AcAc-CoA) by mitochondrial thiolase activity of Ac-CoA acetyltransferase (ACAT), generation of hydroxymethylglutaryl-CoA (HMG-CoA) by hydroxymethylglutaryl CoA synthase (HMGCS), and decomposition of HMG-CoA, liberating AcAc and Ac-CoA, in a reaction catalysed by HMG-CoA lyase (HMGCL). AcAc is the central KB, and some will be exported to the circulation but the majority is reduced to βHB in an NAD^+-NADH-coupled near equilibrium reaction catalysed by BDH, in which the equilibrium constant favours βHB formation. Additionally, some acetone formation occurs, which is readily generated by the spontaneous decarboxylation of AcAc, although contribution to energy provision is negligible (Evans et al., 2017).

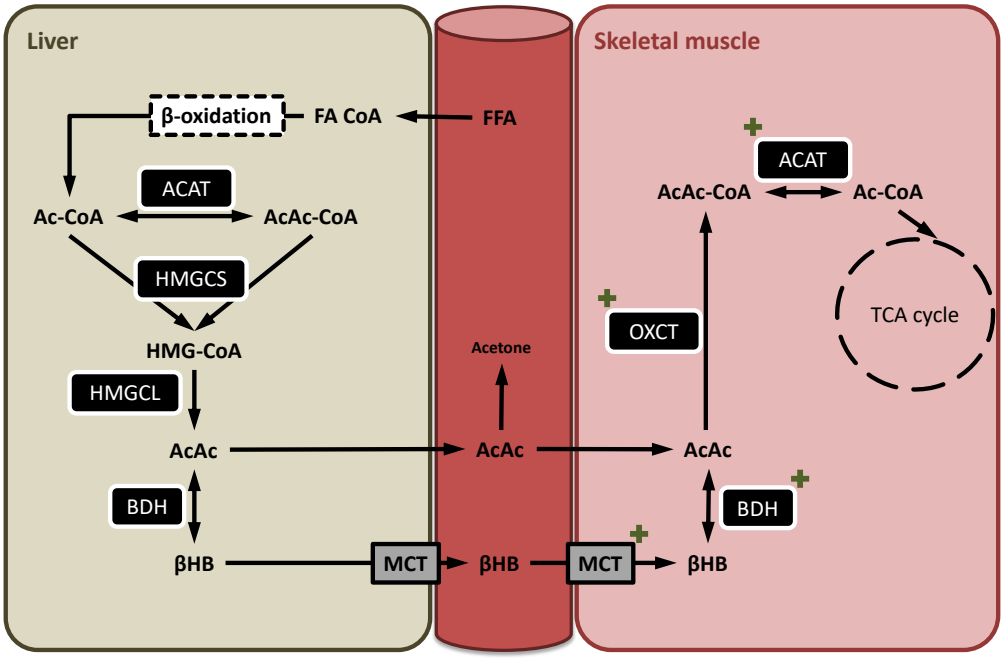

Figure 1.2. Metabolic pathways of ketone body metabolism in liver and skeletal muscle
Ketogenesis: FFAs are converted to fatty acyl CoA (FA-CoA), enter hepatic mitochondria via CPT1-mediated transport and undergo β-oxidation to acetyl CoA. Sequential reactions of condensation of Ac-CoA molecules to acetoacetyl CoA (AcAc-CoA) by mitochondrial thiolase activity of Ac-CoA acetyltransferase (ACAT), generation of hydroxymethylglutaryl-CoA (HMG-CoA) by hydroxymethylglutaryl CoA synthase (HMGCS), and decomposition of HMG-CoA, liberating AcAc and Ac-CoA, in a reaction catalysed by HMG-CoA lyase (HMGCL). AcAc is the central KB, and some will be exported to the circulation but the majority is reduced to βHB in an NAD+–NADH-coupled near equilibrium reaction catalysed by BDH, in which the equilibrium constant favours βHB formation. Ketolysis: The only metabolic fate of βHB is inter-conversion with AcAc, and upon entry into peripheral tissues it is re-oxidised to AcAc. Covalent activation of AcAc by CoA is catalysed by succinyl-CoA:3-oxoacid CoA transferase (OXCT) resulting in generation of AcAc-CoA. This near equilibrium reaction exchanges CoA between succinate and AcAc, with succinyl-CoA acting as a CoA donor. Because the free energy released by hydrolysis of AcAc-CoA is greater than that of succinyl-CoA, the equilibrium of this reaction thermodynamically favours the formation of AcAc. Two molecules of Ac-CoA are liberated by thiolytic cleavage of AcAc-CoA by ACAT, after which Ac-CoA is incorporated into the TCA cycle. Protein content and enzyme activity that are higher in exercise-trained skeletal muscle are indicated by the green cross (+).

The above description refers largely to effects consequent to fasting, starvation, and ketogenic diets, whereby elevated FFAs provide an *endogenous* substrate for ketogenesis. However, it is also possible to provide *exogenous* substrates for ketogenesis in the form of molecules such as medium chain fatty acids, and 1,3-butanediol as described later in the Chapter, and this mechanism of stimulating ketogenesis is an important consideration in the formulation of EKS.

1.3.2 Ketolysis

KB metabolism predominately occurs in peripheral tissues in the form of βHB, as it is transported into the mitochondrial matrix via MCT1 mediated transport. The only metabolic fate of βHB is inter-conversion with AcAc, and upon entry into peripheral tissues it is re-oxidised to AcAc. Covalent activation of AcAc by CoA is catalysed by succinyl-CoA:3-oxoacid CoA transferase (OXCT) resulting in generation of AcAc-CoA. This near equilibrium reaction exchanges CoA between succinate and AcAc, with succinyl-CoA acting as a CoA donor. Because the free energy released by hydrolysis of AcAc-CoA is greater than that of succinyl-CoA, the equilibrium of this reaction thermodynamically favours the formation of AcAc. Two molecules of Ac-CoA are liberated by thiolytic cleavage of AcAc-CoA by ACAT, after which Ac-CoA is incorporated into the TCA cycle.

Ketolysis does not occur to a significant extent in the liver as hepatic tissues contain a very low quantity of succinyl-CoA:3-oxoacid CoA transferase (OXCT), which is an essential enzyme for ketolysis that is more abundantly found in extra-hepatic tissues (Robinson and Williamson, 1980). The heart and kidney have the highest OXCT activity in the body, followed by skeletal muscle and the brain, but because of the proportion of body mass comprised of skeletal muscle (~40%), total KB oxidation is highest in overall quantity in skeletal muscle (Balasse and Fery, 1989, Laffel, 1999).

1.4 TYPES OF EXOGENOUS KETONE SUPPLEMENTS

The broad category encompassed by EKS currently includes ketogenic precursors such as BD, MCFA and MCT, isolated KBs in the form of R-βHB and R,S-βHB KS, and ketone esters, which I will now describe in more detail below. Effects that have been observed for each form of EKS in human participants are summarized in Table 1.1. Direct comparison of different

types of EKS in repeated measures designs are lacking with only one such investigation of time course and metabolic effects having been completed to date (Stubbs et al., 2017).

1.4.1 Medium chain fatty acids (MCFA) / Medium chain triglycerides (MCT)

MCTS contain fatty acids that vary in length, ranging from 6 to 12 carbons (i.e. MCFAs), and examples include caproic acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12) (Schönfeld and Wojtczak, 2016). MCTs are natural compounds that contribute to cell metabolism and are found in animal and plant tissues, such as plant oils and milk (Marten et al., 2006). In contrast to long-chain fatty acids (LCFA) that are enter the lymphatic system esterified to glycerol in nascent chylomicrons, MCFA are absorbed , MCFAs can be absorbed via hepatic portal circulation and enter the hepatic mitochondria without requiring carnitine transport, where they are rapidly metabolized to acetyl CoA and subsequently to KBs (Clegg, 2010). Both MCTs and MCFAs are considered are considered ketogenic fats as they result in ketogenesis without requiring dietary CHO restriction. However high levels of MCT consumption are impalpable and can lead to gastrointestinal distress (Jeukendrup et al., 1998).

1.4.2 1,3-butanediol (BD)

BD was initially developed as an alternative source of energy intake for manned space travel. Following ingestoin, BD is converted by β-hydroxybutyraldehyde in the liver, and oxidised to R,S-βHB via the action of alcohol dehydrogenase and aldehyde dehydrogenase, respectively (McCarthy et al., 2021a). Suprapharmacological concentrations of BD need to be ingested to elicit a significant increase in R-βHB (>1.0 mM) and are associated with adverse effects such as body mass loss, dehydration, and hepatic sinusoidal dilation (McCarthy et al., 2021a). Ingestion of BD can increase circulating R-βHB in isolation or can augment increases in circulating R-βHB in response to ketone ester ingestion when present as an esterified component of R-BD R-βHB KME or R,S-BD AcAc KDE with βHB or AcAc, respectively.

1.4.3 Ketone salts (KS)

A KS is a KB in the form of R/S-βHB bound to sodium, potassium, magnesium, and/or calcium to help minimize the acid load associated with the KB. Most available KS formulations are currently racemic mixtures, consisting of equal quantities of the R- and S- enantiomers of βHB (Stubbs et al., 2017). Racemic KS ingestion induces ketosis in the 0.5-1.0 mM range in ~60 min, which is often considered below the threshold to elicit therapeutic and performance benefits (Evans et al., 2017). Despite this, KS are more commercialized than other EKS products as they are cheaper, easier to produce, and more palpable for consumption. The limitations in achieving high levels of ketosis without a high salt load has shifted the focus to using other EKS such as KME (Evans et al., 2022, Saris and Timmers, 2022). Future work is required to evaluate the ingestion and uptake capacity of non-racemic KS containing only the R-βHB enantiomer.

1.4.4 Medium chain fatty acids co-ingested with ketone salts (MCFA+KS)

The co-administration of MCT and KS is a strategy used to elicit a higher level of ketosis than either supplement can provide alone without a high salt load or gastrointestinal distress (Prins et al., 2020a, Prins et al., 2020b). This approach allows for lower dosing of individual components, with lesser potential for side effects from high intake of individual EKS or minerals. The co-administration MCFA+KS is typically a combination of the respective compounds in 1:1 or 2:1 ratio (Kesl et al., 2016, Prins et al., 2020a, Prins et al., 2020b). Early work in rodent models found that co-administration results in a more sustained induction of nutritional ketosis due to the fact that KBs are delivered directly in the form of KS, while ketogenesis is stimulated by MCFAs (Kesl et al., 2016). The co-administration of MCFA and KS has failed to consistently elevate ketosis beyond 1.0 mM in human volunteers (Table 1.1) (Prins et al., 2020a, Prins et al., 2020b).

1.4.5 (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (R-BD R-βHB) ketone monoester (KME)

R-BD R-βHB KME is a KB (R-βHB) bound to R-BD via an ester bond. This ketone ester is salt-free, has 99% chiral purity (Clarke et al., 2012a, Stubbs et al., 2017, Shivva et al., 2016). Once ingested, KME is hydrolyzed by gut enzymes into R-βHB, which is readily absorbed, and R-BD, which is also absorbed and converted to R-βHB in the liver. Absorption of both R-βHB and R-BD results in a rapid rise in BHB blood concentration, which becomes available to the various extra-hepatic tissues (Clarke et al., 2012a, Stubbs et al., 2017, Shivva et al., 2016) and has consistently demonstrated the ability to increase circulating R-βHB concentrations >2 mM within 30 min (Table 1.1) (Evans et al., 2022).

1.4.6 R,S-1,3-butanediol acetoacetate (R,S-BD AcAc) ketone diester (KDE)

R,S-BD AcAc is a ketone diester containing two KB (AcAc) bound to R-BD via an ester bond. It is produced by the transesterification of t-butylacetoacetate with R,S-1,3-butanediol (D'Agostino et al., 2013, Desrochers et al., 1995) and is a non-ionized sodium-free and pH-neutral precursor of AcAc.

1.4.7 Bis hexanoyl (R)-1,3-butanediol (BH-BD) ketone diester (KDE)

BH-BD is a ketone diester consisting of hexanoic acid (a 6-carbon ketogenic MCFA also known as caproic acid) and R-1,3-butanediol (Chen et al., 2021, Crabtree et al., 2023, Stubbs et al., 2021c). The two ester bonds are expected to undergo enzymatic hydrolysis by intestinal esterase enzymes to release the ketone precursors: hexanoic acid and (R)-1,3-butanediol. Both of these ketogenic precursors are expected to undergo metabolism primarily in the liver to generate BHB (see Table 1.1) (Stubbs et al., 2021a, Stubbs et al., 2021b, Stubbs et al., 2021c).

Table 1.1 Form of exogenous ketone supplements and transient changes in circulating concentrations of ketone bodies after acute ingestion

Supplement type	Dose and changes in circulating [KB]
Medium chain fatty acids (MCFA) / Medium chain triglycerides (MCT)	-Ingestion of MCFA increases circulating [R-βHB] in a dose-dependent manner, with ~25 to ~30 g and ~85 g elevating concentrations to ~0.5 mM and ~0.9 to ~1.5 mM during submaximal

	exercise, respectively (Jeukendrup et al., 1996, Van Zyl et al., 1996, Jeukendrup et al., 1998).
1,3-butanediol (BD)	-In the fasted state, ingestion of 2 x 0.35 $g.kg^{-1}$ (~48 g) BD elevates whole blood [R-βHB] to ~0.5 mM following 85 min steady state at 85% of participants' VT_2 and reaching peak concentrations of 1.38±0.35 mM 60 min after a subsequent TT (Shaw et al., 2019). -Similarly, in the fasted state, ingestion of 0.5 $g.kg^{-1}$ BD (~34 g) ingested alongside 60 g CHO elevates whole blood [R-βHB] to ~0.8 mM following a 1 h pre-load at 75%VO_{2max} and reached peak concentrations of ~0.8 mM following a subsequent running TT (Scott et al., 2019).
Ketone salts (KS)	-Several studies have reported circulating [R-βHB] of ~0.4 to ~1.0 mM in response to ingestion of racemic and non-racemic KS at doses ranging from ~10 to ~40 g of βHB (Stubbs et al., 2017, O'Malley et al., 2017, Rodger et al., 2017, Fischer et al., 2018, Evans et al., 2018, Waldman et al., 2018, Waldman et al., 2020, Kackley et al., 2020, Quinones and Lemon, 2022a). -The majority of commercially-available KS being racemic makes them less effective at elevating the R-βHB enantiomer, yet produce larger (~two-fold greater than R-βHB) and sustained (~2.0 mM at 90 to 120 min) increases in [S-βHB] (Stubbs et al., 2017).
Medium chain fatty acids co-ingested with ketone salts (MCFA+KS)	-Such formulations are available in popular commercialized EKS, but have not been extensively evaluated in human trials. -Two studies have reported whole blood [R-βHB] of ~0.6 mM 60 min after ingestion of a ~7 to ~9 g R,S-βHB salt with ~7 g MCFA (Prins et al., 2020b, Prins et al., 2020a). -Whole blood [R-βHB] was ~0.1 mM higher after ingestion of double the above dose (Prins et al., 2020a).
(R)-3-hydroxybutyl (R)-3-hydroxybutyrate (R-BD R-βHB) ketone monoester (KME)	-Ingestion in the fasted state produces a rapid and dose-responsive increase in whole blood [R-βHB] e.g. ~1.5 mM 20 min after ingestion of 141 $mg.kg^{-1}$ ~2.8 mM 60 min after ingestion of 282 $mg.kg^{-1}$ ~3.0 mM 30 min after ingestion of 482 $mg.kg^{-1}$ ~3.5 mM 10 min after of 573 $mg.kg^{-1}$ and reaching ~6.0 and ~6.5 mM after 40 and 70 min, respectively (Cox et al., 2016, Stubbs et al., 2017, Myette-Côté et al., 2018). -Feeding status alters the [R-βHB] response to ingestion, with a prior meal attenuating the increase in circulating [R-βHB] by ~30% (Stubbs et al., 2017). -AcAc kinetics follow a similar time course to R-βHB, but with [R-βHB]:[AcAc] being ~6:1 when ingested fasted, and ~4:1 when ingested fed at rest (Stubbs et al., 2017), and ~2:1 during exercise when ingested fed (McCarthy et al., 2021b).
R,S-1,3-butanediol acetoacetate (R,S-BD AcAc) ketone diester (KDE) [a]	-Only one human study has reported circulating [KB] after acute ingestion of R,S-BD AcAc KDE (Leckey et al., 2017). -Ingestion of 0.5 $g.kg^{-1}$ (2x 0.25 $g.kg^{-1}$ 20 min apart) had only modest effects on serum [R-βHB] by increasing to ~0.3 to ~0.6 mM, although POC measurement of whole blood [R-βHB] was two- to three-fold higher. -Serum [AcAc] was increased to ~0.4 mM.
Bis hexanoyl (R)-1,3-butanediol (BH-BD) ketone diester (KDE)	-Ingestion in the fed state produces a rapid and dose-responsive increase in plasma [R-βHB] (Chen et al., 2021, Crabtree et al., 2022) e.g.

	~0.4 to ~0.8 mM 30 to 60 min after ingestion of 12.5 g ~1.0 to ~1.7 mM 60 min after ingestion of 25.0 g.

Notes:*Abbreviations: AcAc, acetoacetate; βHB, β-hydroxybutyrate; CHO, carbohydrate; POC, point-of-care; TT, time-trial; VT_2, second ventilatory threshold; VO_{2max}, maximum rate of oxygen uptake.*
[a] Not currently (Q3 2023) commercially-available

1.5 EFFECTS OF EXOGENOUS KETOSIS ON METABOLISM

1.5.1 EKS effects on BHB

Although all EKS increase circulating levels R-βHB, the level of ketosis achieved, and uptake period varies between ketone esters, KS and precursor supplements such as MCT and BD (Table 1.1). Ingestion of a racemic KS induces ketosis in the ~0.5-1.0 mM range, typically reaching peak concentration at around 60 min (Stubbs et al., 2017). This level of ketosis can elicit potential beneficial effects although it is outside the theorized optimal range (~2-4 mM) for therapeutic and performance benefits (Evans et al., 2017). Investigations have sought to increase the level of ketosis achieved during KS ingestion by co-administering with MCFAs and MCTs to further enhance the availability of FFAs and ideally increase endogenous ketogenesis, although average concentrations achieved were still below 1.0 mM even when administering a double dose of KS (Prins et al., 2020a, Prins et al., 2020b). Increasing the quantity of ingested racemic KS any further could potentially lead to gastric distress due to the high salt load associated with the KS itself (Veech, 2004). KS could potentially reach optimal ranges if non-racemic mixtures containing 100% of the R-βHB enantiomer are administered, as this doubles the availability in a single dose without increasing the cation load. However, this remains to be tested and needs further investigation to evaluate the tolerability and uptake of a non-racemic KS.

Ingestion of the R-BD R-βHB KME rapidly induces ketosis (~2-4 mM) within 30 min without the associated cation load that accompanies the intake of KS (Stubbs et al., 2017). The level of ketosis achieved falls into the theorized optimal range which has led to most of the focus in EKS research to revolve around the R-BD R-βHB KME. The rapid influx of R-βHB

into the circulation creates challenges in maintaining normal pH ranges during rest and exercise (Stubbs et al., 2017, Dearlove et al., 2019, Dearlove et al., 2021a, Poffé et al., 2020, Poffé et al., 2021a, Poffé et al., 2021b, Poffé et al., 2021c). The adoption of co-administration strategies with sodium bicarbonate have been used to enhance acid buffering capacity when ingesting R-BD R-βHB KME, resulting in maintaining normal pH ranges and a greater level of ketosis. (~0.5 mM) (Poffé et al., 2021a, Poffé et al., 2021b, Poffé et al., 2021c). The only investigation into human consumption of the KDE (2 doses 250 mg/kg) reported increases of ~1.0 to 1.5 mM in R-βHB although the KDE formulation at that time was highly in tolerated, leading to decreases in physical performance (Leckey et al., 2017). Similar to KME, BD ingestion alone has demonstrated the ability to increase R-βHB (~1.0-1.5 mM) within 30 min. The peak levels are however lower due to the fact that KBs are derived from the single ketogenic pathway as opposed to the independent effects of R-βHB and ketogenesis from BD(Crabtree et al., 2023, Lowder et al., 2023).

1.5.2 EKS effects on glucose

EKS, mainly in the form of R-BD R-βHB KME and KS has been found to rapidly decrease circulating glucose concentrations in both the fed and fasted state (Stubbs et al., 2017). Acute administration of R-BD R-βHB KME (482 mg/kg body mass) has been shown to attenuate the rise in blood glucose measured by area under the curve (AUC) during an oral glucose tolerance test in healthy populations (AUC -16%) (Myette-Côté et al., 2018), obese populations (AUC -11%) (Myette-Cote et al., 2019) and in populations with impaired glucose tolerance (AUC -16%) (Nakagata et al., 2021). Moreover, in healthy active populations R-BD R-βHB KME attenuates the rise in glucose when co-administered with CHO at rest and during exercise (Evans and Egan, 2018, Evans et al., 2019). The glucose-lowering effect lasts up to at least 4 h and is approximately 3 times greater during R-BD R-βHB KME consumption compared to KS (Falkenhain et al., 2022).

Acute and chronic EKS's have been found to reduce and attenuate circulating glucose in multiple populations with varying degrees of glucose tolerance and insulin sensitivity. Pre-meal consumption of R-BD R-βHB KME (12 g) 3 times a day for 14 days lowered average daily glucose and postprandial glucose (Walsh et al., 2021b). Soto-Mota et al. (2021) completed a 28 d interventional study in T2D participants, consisting of R-BD R-βHB KME consumption (25 g) 3 times a day, eliciting an average R-βHB response of ~3.5 mM (Soto-Mota et al., 2021). Consumption of R-BD R-βHB KME improved glycemic control markers, including fructosamine (335±60 to 290±49 μM), HbA1c (7.7±0.9% to 7.2±0.9%), mean daily glucose (7.8±1.4 to 7.4±1.3 mM) and time in range (67±11% to 69±10%) (Soto-Mota et al., 2021).

While the exact mechanisms underlying the glucose-lowering effect are not fully understood, it has been theorized that ketones directly increase insulin secretion (Madison et al., 1964). A recent meta-analysis found that acute increases in plasma insulin post-consumption of EKS (Falkenhain et al., 2022), supporting the concept that EKS have a direct stimulatory effect on the pancreatic β -cells, leading to insulin secretion and glucose-lowering effects (Madison et al., 1964). Other mechanisms likely exist, as infusion-based studies have observed glucose lowering without elevations in insulin (Rittig et al., 2020, Sherwin et al., 1976). Sherwin et al. (1976) utilized a 3 h infusion of Na DL-β-hydroxybutyrate in healthy volunteers and insulin-dependent diabetics during the postabsorptive state (Sherwin et al., 1976). Blood glucose levels were significantly reduced in both groups highlighting the role of other potential mechanisms in the regulation of glucose homeostasis (Sherwin et al., 1976). Rittig et al. (2020) found that dietary but not intravenous KS increased insulin secretion in healthy populations despite both conditions eliciting similar levels of R-βHB (~1.2 mM) and reductions in glucose (Rittig et al., 2020). These findings strongly suggest that other insulin-independent mechanisms may play a role in controlling the glucoregulatory effects of ketones. Other putative mechanisms affecting glucose concentrations include EKS ability to decrease gluconeogenesis

and hepatic glucose output via inhibiting lipolysis (Senior and Loridan, 1968, Taggart et al., 2005, Reaven, 2005), and to reduce the availability of gluconeogenic precursors (L-alanine) (Soto-Mota et al., 2022), in turn increasing peripheral glucose uptake, and modulating the sympathetic nervous system (Kimura et al., 2011). Together these findings highlight the efficacy of EKS to lower glucose in various populations (healthy or diseased) or contexts (fed, fasted, rest, exercise, etc.). Further work is required to address mechanisms outside of direct effects on insulin secretion.

1.5.3 EKS effects on lactate

EKS, mainly in the form of KME, has been shown to attenuate the rise in blood lactate during exercise. This effect has been observed in several studies employing ketone esters (Cox et al., 2016, Leckey et al., 2017, Evans and Egan, 2018, Dearlove et al., 2019, Dearlove et al., 2021a, Poffé et al., 2021c, Peacock et al., 2022), although not all studies (Evans et al., 2019, Poffé et al., 2020, Poffé et al., 2021a, McCarthy et al., 2021b), and is generally interpreted as indicative of altered CHO utilization due to increased KB availability to extra hepatic tissues. The mechanistic basis for the attenuation of CHO utilisation during exercise, whether by high dose caffeine, pre-exercise high fat feeding, adherence to high fat and ketogenic diet, or acute nutritional ketosis is largely attributed to the inhibition of the enzymatic activity of key metabolic enzymes including phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) (Graham et al., 2008, Evans et al., 2017, Shaw et al., 2020, Burke, 2021). KBs are proposed to inhibit glycolysis and increase the conversion of glucose to glycogen as demonstrated in rat skeletal muscle *in vitro* (Maizels et al., 1977), and a perfused heart model in dogs (Laughlin et al., 1994). These effects are mediated by inhibition of PFK and PDH by increases in NADH:NAD^+, acetyl-CoA:CoA and/or citrate as a consequence of the metabolism of AcAc in mitochondria (Randle et al., 1964, Maizels et al., 1977, Laughlin et al., 1994, Kashiwaya et al., 1997).

In contrast to a proposed benefit of attenuated CHO utilisation during exercise, it has been proposed that nutrition strategies that attenuate CHO utilisation could paradoxically also negatively impact performance i.e., an ergolytic effect (Evans et al., 2017, Shaw et al., 2020, Burke, 2021). Colloquially, this has been framed as a question of whether such nutrition strategies 'spare' CHO, or 'impair' CHO utilisation. Data from studies of low CHO-high fat and ketogenic diets often supports the ergolytic effect of attenuated CHO utilisation, especially during high intensity exercise. This evidence is extensively reviewed elsewhere (Burke, 2021). It is worth highlighting that impairment of performance may result from attenuated activity of PDH, which was found to be reduced during 20 min of cycling at 70%VO_{2max} and during 1 min supramaximal cycling at 150%W_{max} following a 6 d adaptation period to a high fat diet (Stellingwerff et al., 2006). Measurement of enzymatic activities of PFK and PDH during exercise after ingestion of EKS will be required to elucidate the mechanism, but it is notable that PDH activity is reduced in cardiac muscle of rats whose diet is supplemented with R-BD R-βHB KME (Murray et al., 2016).

To date, two studies have reported impaired performance during high intensity, short duration (~10 to 30 min) cycling time trials after ingestion of R,S-βHB salts (O'Malley et al., 2017) or R-BD R-βHB KME (Poffé et al., 2021c), although the mechanism of impaired performance was not established in either study. A third study demonstrating impaired cycling TT performance after R,S-BD AcAc KDE attributed the decrement to severe gastrointestinal (GI) disturbances (Leckey et al., 2017). Two more recent studies also reported that trends towards impaired high intensity, short duration performance coincided with increased GI disturbances (Evans and Egan, 2018, McCarthy et al., 2021b). As a result, GI disturbances are another important consideration for potential ergolytic effects of EKS. This effect on metabolism is important in terms of fuel utilization during exercise although a not is a non-factor at rest when metabolism is predominately met by fat metabolism.

1.5.4 EKS effects on pH and blood gases

βHB and AcAc are weak organic acids. Unsurprisingly ingestion of EKS in the form of ketone esters can produce a mild metabolic acidosis evidenced by a decrease in blood pH (~0.05 to 0.10) at rest (Stubbs et al., 2017) and during exercise (Dearlove et al., 2019, Poffé et al., 2021a, Poffé et al., 2021b). The rapid influx of KBs into the circulation creates challenges in maintaining a normal acid-base balance leading to decreases in circulating [bicarbonate] (Stubbs et al., 2017, Dearlove et al., 2019, Prins et al., 2021). In most performance contexts, an increased H^+ load prior to and during exercise is suboptimal for performance as it would limit overall buffering capacity needed to combat the increased H^+ load during high intensity exercise (Caciano et al., 2015). Mild acidosis is therefore associated with ergolytic effects on exercise performance (Carr et al., 2011), whereas increasing buffering capacity through acute ingestion (e.g., sodium bicarbonate; BIC) or chronic supplementation (e.g. β-alanine) is often ergogenic (Lancha Junior et al., 2015, de Oliveira et al., 2022).

An increase in minute ventilation has been observed during 30 min of steady-state cycling exercise at VT (McCarthy et al., 2021b), 3 h of intermittent intensity cycling exercise in normoxia (Poffé et al., 2021a) and hypoxia (Poffé et al., 2021b), and short-duration incremental cycling exercise (Dearlove et al., 2019), after ingestion of R-BD R-βHB KME ([R-βHB] ~2 to ~4 mM). In a related analysis of the latter study (Dearlove et al., 2019), subjective reporting of anxiety of breathing and intensity of leg discomfort were higher during acute nutritional ketosis (Faull et al., 2019). Mixed effects modelling revealed that pH and [R-βHB] were predictors of these responses, but whole-body ratings of perceived exertion (RPE) were not higher during acute nutritional ketosis despite the localised perceptual effects (Faull et al., 2019). This lack of effect on RPE is consistent with most studies of acute ingestion of EKS observing no differences in RPE compared to control conditions. When there has been higher RPE observed (Poffé et al., 2020, McCarthy et al., 2021b, Waldman et al., 2022), outcomes of

the performance tests were not negatively impacted. Another avenue of research is whether declines in pH elicited by KME could have performance benefits in extreme environments such as hypoxia and voluntary hypoventilation (Poffé et al., 2021b, Coleman et al., 2021, Prins et al., 2021). Pre-exercise R-BD R-βHB KME ingestion elicits a range of related effects including increasing the ventilation rate during exercise, lowering PCO_2 (~5 to 10 mmHg) and shifting the oxygen saturation curve to the left leading to an ~3% to ~6% advantage in O_2 saturation, both in circulation and skeletal muscle (Dearlove et al., 2019, Poffé et al., 2021b, Coleman et al., 2021, Prins et al., 2021, McCarthy et al., 2021b). Thus, rather than being ergolytic, in hypoxia and altitude the mild acidosis induced by R-BD R-βHB KME ingestion may be a putative ergogenic benefit of EKS. This is explored further in section 1.8.5.1.

1.6 OXIDATION OF KBS AS AN ALTERNATIVE SUBSTRATE

1.6.1 Oxidation of KBs in skeletal and cardiac muscle

The majority of information regarding the oxidation of KBs in skeletal muscle comes human studies from the late 1960s - 1980s using infusions of KBs and/or fasting of various durations (Hagenfeldt and Wahren, 1968, Hagenfeldt and Wahren, 1971, Fery et al., 1974, Balasse et al., 1978, Fery and Balasse, 1983, Wahren et al., 1984, Fery and Balasse, 1986, Fery and Balasse, 1988). These early studies have made a significant contribution to the understanding of KB metabolism and substrate utilisation during exercise (Balasse and Fery, 1989, Evans et al., 2017).

Briefly, disposal of KBs into skeletal muscle is increased as much as fivefold during exercise in the fasted state, and is reflected by a decrease in circulating [KB] at the onset of exercise (Balasse and Fery, 1989). This decrease largely reflects R-βHB being the primary KB extracted from circulation, whereas a net production of AcAc in skeletal muscle has also been observed (Balasse and Fery, 1989). These studies were almost exclusively performed in the fasted state, including with prolonged elevations of [KB], which is in contrast to the acute

transient increase achieved by EKS and the fact that most competitive athletic performance takes place in the fed state (Burke and Hawley, 2018). Interestingly, the metabolic clearance of KBs is enhanced with insulin infusion, at least in the resting state (Keller et al., 1989), which suggests that co-ingestion of EKS with CHO could potentially augment the oxidation of KBs. Alternatively, because KBs are unlikely to be preferentially used over glucose in skeletal muscle (Miller et al., 2020, Petrick et al., 2020), co-ingestion could potentially attenuate the oxidation of KBs.

Traditional stoichiometric equations used to calculate substrate utilisation from the respiratory exchange ratio (RER), oxygen consumption, and carbon dioxide production provide data on absolute oxidation rates ($g.min^{-1}$) and percentage contribution to energy provision for CHO and fat, but assume negligible contributions from other substrates including amino acids, KBs and lactate (Frayn, 1983, Jeukendrup and Wallis, 2005, Kuo et al., 2005). During acute nutritional ketosis, careful interpretation of RER during exercise is needed because the stoichiometry of AcAc, the final step in KB oxidation, is 1.00 i.e. similar to that of CHO, whereas the equivalent value for βHB is 0.89 (Frayn, 1983). However, estimation of oxidation rates of KBs is possible using indirect methods if values for the volume distribution of KBs (i.e., total amount of KBs in the body divided by plasma [KB]), and uptake of KBs into skeletal muscle are known (Frayn, 1983).

The first study to estimate the oxidation of KBs after acute ingestion of EKS using these methods analyzed the contribution of βHB to total oxygen consumption in trained athletes during 45 min of cycling at 40% and 75% maximal power output (W_{max}) (Cox et al., 2016). Ingestion of R-BD R-βHB KME (573 $mg.kg^{-1}$) increased [R-βHB] to ~3.0 mM at the start of exercise, where it remained throughout the 40%W_{max} trial, but declined by ~1.1 mM during the 75%W_{max} trial. Rates of R-βHB oxidation were estimated to account for ~18% and ~16% of total oxygen consumption (i.e. energy provision) during the steady-state exercise at 40% and

75%W_{max}, respectively, with oxidation rates increasing from ~0.35 g·min^{-1} at the lower intensity to ~0.5 g·min^{-1} at the higher intensity (Cox et al., 2016). These percentage contributions and oxidation rates were several-fold higher than had been reported in earlier studies using labelled tracers and the measurement of metabolic clearance rate (Balasse and Fery, 1989). This discordance may have been an artefact of the necessity for several assumptions to be made in order to calculate substrate oxidation from gas exchange data using traditional stoichiometric equations that are otherwise unsuitable for use in ketogenic or ketotic states (Frayn, 1983, Jeukendrup and Wallis, 2005). For example, uptake of KBs into skeletal muscle following EKS ingestion was estimated using the difference between incremental area-under-the-curve of blood [R-βHB] between resting and exercising conditions (Cox et al., 2016), but such a method does not account for how much of this difference between conditions would be explained by KBs being stored in the form of D-3-hydroxybutyrylcarnitine (also known as ketocarnitine) (Soeters et al., 2012), or lost in the breath and urine (Robinson and Williamson, 1980).

Another potential site of disposal of KBs that is unaccounted for by this method is the utilisation of KBs by the heart. R-βHB can become a major contributor to energy metabolism in cardiac muscle (Karwi and Lopaschuk, 2021), especially when circulating concentrations are increased (Little et al., 1970, Kim et al., 1991, Gormsen et al., 2017, Ho et al., 2021), as would be achieved by acute nutritional ketosis. To my knowledge, myocardial utilisation of KBs during exercise has not been described, but is an area of relevant interest in the context of EKS (Kolwicz, 2018, Abdul Kadir et al., 2020).

Shifts in myocardial substrate utilisation analogous to skeletal muscle do occur with the onset of increased contractile activity, and increasing exercise intensity and duration (Carlsten et al., 1961, Wahlqvist et al., 1973, Gertz et al., 1988, Goodwin et al., 1998a, Goodwin et al., 1998b, Kemppainen et al., 2002). Yet in principle, KBs can alter substrate utilisation in the heart, at least at rest, by reducing reliance on glucose metabolism via product inhibition of key

glycolytic and mitochondrial enzymes (Williamson and Krebs, 1961, Newsholme et al., 1962, Garland et al., 1963, Randle et al., 1964, Russell et al., 1997). Whether cardiac muscle is an important site of KB utilisation during exercise, and whether this utilisation is altered by acute ingestion of EKS are intriguing unanswered questions.

Subsequent investigations by the same research group have used ^{13}C-labelled R-βHB for a more accurate determination of rates of R-βHB oxidation via breath analysis (Dearlove et al., 2021a, Dearlove et al., 2021b). These studies in trained endurance athletes with various manipulations of [KB], exercise intensity, and circulating and intramuscular substrate availability, have reported rates of R-βHB oxidation increasing by ~five- to ~ten-fold above rest during aerobic exercise, and absolute values of ~0.06 to ~0.10 g·min^{-1} (Dearlove et al., 2021a), and ~0.2 to ~0.3 g·min^{-1} (Dearlove et al., 2021b). The approximately three-fold difference between the two studies remains unexplained at present. The respective studies estimated the contribution of R-βHB oxidation to energy provision to average ~2.5 to ~4.5% (Dearlove et al., 2021a), and ~7.4 to ~8.4% (Dearlove et al., 2021b). These observations are more consistent with values of ~2 to ~10% reported in the early infusion and prolonged fasting studies (Balasse and Fery, 1989, Evans et al., 2017). The data indicate that KBs make only a minor contribution as a direct source of ATP provision in skeletal muscle during exercise (Balasse and Fery, 1989, Evans et al., 2017), even when [R-βHB] is acutely elevated in the range of ~1.7 to ~4.5 mM by ingestion of EKS (Dearlove et al., 2021a, Dearlove et al., 2021b). The minor contribution of KB's as a direct source of ATP provision is not surprising given recent *in vitro* data from permeabilised muscle fibres and isolated mitochondria from skeletal muscle demonstrating that KBs make a minimal contribution to mitochondrial respiration, particularly when other substrates (e.g. pyruvate) are readily available (Miller et al., 2020, Petrick et al., 2020)

1.6.2 Oxidation of KBs in the brain

The brain consumes a high amount of the body's energy (~20-23%) proportional to its small mass (~2%) compared to other organs and is predominately fuelled by glucose metabolism (Rolfe and Brown, 1997). KBs can pass through the blood brain barrier and enter the brain via monocarboxylic acid transporter, whereas glucose is transported in via GLUT1. Monocarboxylic acid transporter expression responds rapidly to increases in ketosis, allowing for increased uptake capacity (Pan et al., 2001). When no KBs are present, the brains energy supply will come from glucose oxidation. The uptake of KBs by the brain is directly proportional to the circulating concentrations over at least the range of 0.02-12.0 mM (Courchesne-Loyer et al., 2013, Cunnane et al., 2016b).

KBs ability to meet the energy demand of the brain has been known since the 1960s (Cahill et al., 1968). At this time the only methods of achieving hyperketonemia were through long-duration fasting/starvation, ketogenic diets or infusion of KBs themselves as this was prior to the creation of EKS. These early investigations were the foundational for the current understanding of ketone metabolism in the brain and how availability of KBs dictate what percent of the energy demand is being met by glucose or KBs (Cahill, 2006). Subsequent investigations have examined short term starvation (3.5 d) (Hasselbalch et al., 1995), short term ketogenic diets (Hasselbalch et al., 1996, Courchesne-Loyer et al., 2017) or infusion of KBs (Pan et al., 2001, Mikkelsen et al., 2015, Svart et al., 2018) to further understand how KB concentration effects cerebral metabolism, cerebral blood flow (CBF), oxygen utilization/availability and fuel preference. Although decreases in glucose availability and/or increases in KB availability alter the fuel preference in the brain, this preference varies depending on nutritional status (fed, fasted, starvation) and/or method of inducing ketosis (fasting, ketogenic diet, infusion of KBs, ingestion of EKS) (Hasselbalch et al., 1996, Courchesne-Loyer et al., 2017, Pan et al., 2001, Cahill et al., 1968).

The brain's ability to change fuel utilization from glucose to KBs appears to be rapid. Short-term starvation and diets have been found to result in a 6-10 fold increase in KB metabolism in 3-4 days. Short term starvation (3.5 d) in healthy subjects alters βHB influx into the brain greater than 10-fold (Hasselbalch et al., 1995). Moreover, a short 4-day ketogenic diet increased cerebral AcAc oxidation 6-fold while decreasing glucose oxidation by 20%, resulting in 33% of total cerebral energy coming from KB oxidation after just 4 days (Courchesne-Loyer et al., 2017). Infusion based studies have noted similar alterations in fuel selection. Hasselbalch et al. (1996) reported a 5-fold increase in KB oxidation, ~33% decrease in glucose oxidation an increase 39% increase in CBF post-infusion (Hasselbalch et al., 1996). Subsequent work by other labs reported similar trends in reducing glucose oxidation (Mikkelsen et al., 2015, Svart et al., 2018), increasing KB oxidation (Pan et al., 2001) and CBF (Svart et al., 2018) in infusion-based models. Infusion of healthy volunteers with sodium R/S-βHB (0.22 g/kg/hour) for 4 hours elicits an increase in circulating βHB (5.5 ±0.4 mM), increased CBF (30%), and decreased cerebral glucose consumption (14%) without altering O_2 consumption (Svart et al., 2018). Furthermore, infusion of sodium R- βHB for 3 hours in a step wise manor (4.7 μmol/kg/min, 9.4 μmol/kg/min, 18.8 μmol/kg/min) results in a linear uptake in cerebral βHB uptake and oxidation and reductions in glucose appearance (14±2%) and lipolytic rate (37±4%) (Mikkelsen et al., 2015).

Research into the physiology underpinning KB metabolism in the brain is growing in parallel to the prevalence of cognitive impairment and Alzheimer's disease being on the rise (Cunnane et al., 2016b). In theory, altering ketone availability would increase CBF and uptake into the brain, and thereby bridge the gap in energy demand caused by impaired glucose metabolism, which in turn could thereby alleviate associated declines in cognitive function (Cunnane et al., 2016b, Cunnane et al., 2011). Despite this theoretical basis, further work is needed to understand the mechanisms affecting CBF, differences in fed vs. fasted conditions,

and applications of various EKS on cerebral metabolism. Moreover, the concept of using KBs as a countermeasure to cognitive declines is expanding beyond dementia and Alzheimer's, such as other physiological states that elicit cognitive declines such as prolonged endurance exercise (Evans and Egan, 2018, Poffé et al., 2023, Quinones and Lemon, 2022b) or hypoxia (Coleman et al., 2021). Such conditions could potentially benefit from EKS through the same mechanisms, and the results of these studies are described in more detail in later sections.

1.7 COGNITIVE PERFORMANCE, ITS MEASUREMENT, AND RELEVANCE TO OCCUPATIONAL AND PERFORMANCE CONTEXTS

A certain level of cognitive performance is required to accomplish tasks of daily living to support a high quality of life and independence across the lifespan (Murman, 2015). Physical and environmental conditions that stress overall physiology such as exhaustive exercise (Lim and Kwak, 2019), sleep deprivation (Killgore, 2010), hypoxia (McMorris et al., 2017), heat (Schmit et al., 2017), and cold exposure (Falla et al., 2021a) can negatively affect cognitive performance. Elite athletes also benefit from higher level executive function capabilities which can translate to better decision-making during game play and ultimately better performance (Vestberg et al., 2017, Voss et al., 2010). Together, these examples highlight a wide application for cognitive performance testing that traverses multiple populations across their life span.

A few valid classically used cognitive tests such as the clock drawing test (CDT) and the Montreal Cognitive Assessment (MoCA) are still administered directly via paper (Aprahamian et al., 2009, Dautzenberg et al., 2020), whereas more modern approaches use tablet or computer based cognitive tests to allow for easier administration, higher retake reliability, more accurate reaction times and the ability to track eye movement, blink rate and duration (Committee on Psychological Testing et al., 2015, Liu et al., 2021, Pradhan et al., 2021, Rosa et al., 2014). The automated neuropsychological assessment metrics (ANAM) is a library

of valid cognitive tests that are either computer or tablet based and are designed to test a wide range of cognitive domains (Jones et al., 2008, Woodhouse et al., 2013)

Cognitive tests are specifically designed to test certain domains of cognitive performance or the combination of multiple domains and are classified into 3 main categories: language, executive function, and memory/attention (Harvey, 2019). These domains are often categorized by regional brain structures involved or by a hierarchy based on complexity of the operations in the following order: language/verbal skills, processing speed, executive function, memory, attention/concentration, motor skills, perception, sensation.

Language consists of receptive and productive abilities along with the ability to understand language, access semantic memory, to identify objects with a name, and to respond to verbal instructions with behavioural acts. Language skills are assessed with measures of fluency (e.g., name as many animals as possible), object naming, and responding to instructions (Harvey, 2019). Executive function, which is commonly referred to as reasoning and problem solving; includes tasks such as: problem solving, planning, manipulating mazes, and other complex tasks where management of multiple cognitive abilities are required (Diamond, 2013). Working memory/attention is the most multifaceted cognitive domain consisting of multiple sub domains (working memory, episodic/declarative/explicit memory, procedural memory, semantic memory, prospective memory). Briefly, working memory is the ability to hold information in consciousness for adaptive use. Episodic/declarative/explicit memory interacts with working memory storage processes to encode, maintain, and retrieve information into and out of longer-term storage. Procedural memory is for motor actions or skills (e.g., riding a bike). Semantic memory is often referred to as long-term memory, consisting of storage of verbal information, whereas perspective memory is the ability to remember performing tasks in the future (e.g., taking medication) (Harvey, 2019).

Maximizing cognitive performance and mitigating declines in cognition during extreme environments or in response to ageing are of great interest and have sparked interest in the development of potent countermeasures that are applicable in a wide range of settings and populations. EKS have emerged as having potential utility to mitigate declines in cognitive performance observed in aging populations (Cunnane et al., 2016a), prolonged exercise (Evans and Egan, 2018, Poffé et al., 2023, Quinones and Lemon, 2022b) or in extreme environments such as hypoxia (Coleman et al., 2021).

1.7.1 Mitigation of cognitive decline by EKS

Multiple studies have utilized acute EKS to mitigate declines in cognitive performance, both with KS (Prins et al., 2020a, Waldman et al., 2018, Waldman et al., 2020) and R-BD R-βHB KME (Coleman et al., 2021, Evans and Egan, 2018, Evans et al., 2019, Poffé et al., 2023, Prins et al., 2021, Quinones and Lemon, 2022b, Walsh et al., 2021a). To date, 6 out of 10 investigations reported a significant effect of acute EKS in mitigating declines in cognitive performance. One investigation using KS (co-administered with MCT) reported improved reaction time performance during a Stroop Word and Colour test prior to exercise (Prins et al., 2020a), whereas two studies utilizing KS without MCT observed no differences in reaction time performance during a Stroop Word and Colour test prior to exercise in healthy populations (Waldman et al., 2018, Waldman et al., 2020) (Table 1.2).

The majority of studies investigation the have shown that R-BD R-βHB KME improves, or mitigates declines in, cognitive performance (Coleman et al., 2021, Evans and Egan, 2018, Evans et al., 2019, Poffé et al., 2023, Prins et al., 2021, Quinones and Lemon, 2022b, Walsh et al., 2021a) (Table 1.2). Administration of R-BD R-βHB KME 3 times a day over a 14 day period in obese individuals improved performance during a digit-symbol substitution task (+2.7 correct responses) (Walsh et al., 2021a). Specifically for acute ingestion, R-BD R-βHB KME has shown promise in mitigating cognitive declines from prolonged endurance exercise in

healthy populations (Evans and Egan, 2018, Poffé et al., 2023, Quinones and Lemon, 2022b). Poffé et al. (2023) evaluated cognitive performance with R-BD R-βHB KME immediately before and after ultra-endurance exercise (100 km) (Poffé et al., 2023). R-BD R-βHB KME ingestion attenuated the rise in reaction time (KE: 362±41 vs. CON: 419±54 ms), movement time (KE: 192±47 vs. CON: 245±64 ms), and false alarms during a reaction time task and rapid visual information processing task (Poffé et al., 2023). Evans & Egan (2018) observed a similar mitigation of decline in cognitive performance during a multi-tasking test after exhaustive intermittent running exercise (Evans and Egan, 2018). Quinones & Lemon (2022) observed similar results during a choice reaction time test, again during a simulated soccer match protocol that induced mental fatigue (Quinones and Lemon, 2022b). Acute administration of R-BD R-βHB KME was also found to mitigate the decline in cognitive performance during severe hypoxia (20 min, 5029 m), (Coleman et al., 2021). Hypoxia and its effects on physiology, metabolism, and cognitive performance will be covered in further detail in the next section (Section 1.8)

Table 1.2 Effects of exogenous ketone supplements on markers of cognitive performance after acute ingestion

Supplement used, Study	Participant profile	Supplement dose and timing	Cognitive test(s) and timing	Methodological features	Performance outcomes
Ketone salt (KS)					
KS+MCT (KETO//OS 2.1 Pruvit, TX, USA) (Prins et al., 2020)	13 recreational male distance runners (age 24.8±9.6 y, 72.5±8.3 kg, VO2max 60.1±5.4 ml/kg/min)	Low dose: 22.1g of KS + MCT (KS1) High dose: 44.2g of KS + MCT (KS2) Single dose ingested -60 min pre-exercise	ANAM (Vista Life Sciences, United States): Stroop Word-Color Test (congruent and incongruent), Switching Task (manikin and mathematical processing) -30 min prior to exercise and 5 min post 5km	Double blinded: Yes Placebo: taste/volume matched Blinding success: Unclear Randomised: Yes Crossover: Yes Dietary control: yes Fasted or Fed: fed Validated cognitive test: yes Familiarisation trial: yes βHB measurement: POC	KS2 significantly improved stroop incongruent RT pre-exercise ($P<0.05$) KS1 and PLA significantly improved from pre- to post exercise ($P<0.05$)
KS (Perfect Keto.; New York, NY) (Waldman et al., 2018)	15 healthy college aged males (age: 23.1±2.4 y, height: 165.4±2.0 cm, mass: 81.4±9.2 kg)	KS: 11.38g Single dose: -30 min pre-exercise	FitLights™ (FL) (FITLIGHT Sports Corporation, Aurora, Ontario, Canada) Administered during exercise	Double blinded: Yes Placebo: taste/volume Blinding success: Unclear Randomised: Yes Crossover: Yes Dietary control: Fasted or Fed: fasted Validated cognitive test: yes Familiarisation trial: yes βHB measurement: POC	No difference in cognitive performance between KS and PLA
KS (Perfect Keto.; New York, NY) (Waldman et al., 2019)	16 healthy college aged males (age: 21.9±1.9 y; body mass: 80.6±7.4 kg; height: 1.76±0.09 m; VO_2peak: 40.5±8.4 ml/kg/min)	KS: 0.38 g/kg body mass Split dose: -60 and -15 min pre dual stress challenge	Mental arithmetic challenge (MAC) Modified Stroop Color Word task During exercise (+10 min)	Double blinded: Yes Placebo: noncaloric Blinding success: Unclear Randomised: Yes Crossover: Yes Dietary control: Fasted or Fed: fasted Validated cognitive test: yes Familiarisation trial: yes βHB measurement: POC	No difference in cognitive performance between KS and PLA

Ketone monoester (KME): (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (R-BD R-βHB)					
KME (DeltaG; HVMN) (Coleman et al., 2021)	11 healthy male subjects (age: 24.3±1.2 y; weight: 85.2±11.1 kg)	KME: 550 mg/kg of body mass Split dose: 1st 400 mg/kg 2nd 150 mg/kg 1st -20 pre normoxia 2nd -20 pre hypoxia	DANA (Anthrotronix, Silver Spring, MD, USA): simple RT, procedural RT, code substitution-simultaneous test RightEye (Bethesda, MD, USA): Simple RT test, Choice RT test, Discriminate RT test	Double blinded: no Placebo: noncaloric Blinding success: yes Randomised: Yes Crossover: Yes Dietary control: yes Fasted or Fed: fed Validated cognitive test: yes Familiarisation trial: yes βHB measurement: POC	KME significantly improved performance during code substitution task compared to PLA during hypoxia ($P<0.05$)
KME (KE4; KetoneAid) (Evans & Egan, 2018)	11 male team sport athletes (age: 25.4±4.6 y; height:1.80±0.05 cm; body mass: 78.6±5.3 kg; VO_2max: 53.9±2.2 ml/kg/min)	KME + CHO: 750 mg/kg KME + 1.2 g/min CHO PLA: 1.2 g/min CHO Multiple (50:25:25; − 30 min pre-exercise, 15 and 30 min into exercise)	Battery of cognitive tests (CANTAB Cognition, UK): RT task, multi-tasking test, rapid visual information processing task -45 min pre exercise, post exercise	Double blinded: Yes Placebo: Not iso-caloric Blinding success: 27% Randomised: Yes Crossover: Yes Dietary control: yes Fasted or fed: fed Validated cognitive test: Yes Familiarisation trial: Yes βHB measurement: Direct/ laboratory assay	KME significantly attenuated the decline in cognitive performance (multi-tasking test) after exhaustive exercise compared to CHO ($P<0.05$)
KME (DeltaG; HVMN) (Evans et al., 2019)	8 trained, middle- and long-distance runners (male/female, 7/1; age: 33.5±7.3 y; height: 1.79±0.07 m; body mass: 68.8±9.7 kg; body fat: 8.0±4.1%; VO_2max: 62.0±5.6 ml/kg/min)	KME + CHO: 573 mg/kg KME + 1.0 g/min CHO PLA: 1.0 g/min CHO Multiple (50:25:25; − 30 min pre-exercise, 20 and 60 min into exercise)	Battery of cognitive tests (CANTAB Cognition, UK): RT task and multi-tasking test -75 min pre exercise, post 10km time trial	Double blinded: Yes Placebo: Not iso-caloric Blinding success: 75% Randomised: Yes Crossover: Yes Dietary control: yes Fasted or fed: fed Validated cognitive test: Yes Familiarisation trial: Yes βHB measurement: Direct/ laboratory assay	No difference in cognitive performance between conditions
KME (KE4; KetoneAid)	18 male recreational runners [control group (n=9); age: 34.1±8.7 y; height:	KME: 25g per dose (non-exercise), 12.5g per dose (start of run and every 30 min after)	Battery of cognitive tests (CANTAB Cognition, UK): RT task and rapid visual information processing task	Double blinded: Yes Placebo: non-caloric Blinding success: < 50% Randomised: Yes Crossover: no Dietary control: yes	KME Significantly attenuated the rise in reaction (KE: 362±41 vs. CON: 419±54ms, $p=0.04$) and movement time (KE:

(Poffé et al., 2023)	1.83±0.08 m; body mass: 73.8±8.2 kg] [KME group (n=9); age: 37.1±7.2 y; height: 1.80±0.05 m; body mass: 73.1±6.0 kg]	Multi-dose: -30 min before exercise, at start of exercise, every 30 min of exercise, immediately after exercise, 30 min before sleep, 30 min before breakfast, lunch and sleep the following day	Immediately prior to exercise and post exercise	Fasted or fed: fed Validated cognitive test: Yes Familiarisation trial: Yes βHB measurement: POC	192±47 vs. CON: 245±64ms, p=0.049) compared to CON KME attenuated increase in number of false alarms compared to CON (pre to post test)
KME (DeltaG; HVMN) (Prins et al., 2021)	15 recreational male distance runners (age: 20.6±2.1 y; height: 180.1±7.8 cm; body mass: 76.7±9.6 kg; body fat: 12.8±5.7 %)	KME: 573 mg/kg Single dose: -45 min pre exercise	ANAM (Vista Life Sciences, United States): Stroop Word-Color Test (congruent and incongruent), Switching Task (manikin and mathematical processing) Pre, 30 min and end of exericse	Double blinded: Yes Placebo: Isocaloric Blinding success: Unclear Randomised: Yes Crossover: Yes Dietary control: yes Fasted or Fed: fed Validated cognitive test: yes Familiarisation trial: yes βHB measurement: POC	No difference in cognitive performance between conditions
KME (DeltaG; TdeltaS) (Quinones & Lemon, 2022)	9 recreationally active men (age: 30±3 y; height: 174.3±4.2 cm; weight: 76.6±7.4 kg; body fat: 14.2±5.5 % VO_2max: 55±5 ml/kg/min)	KS + CAF + AA: 7g R,S-βHB, 120 mg caffeine, 2.7g taurine, 2.1g leucine KS + AA: 7g R,S-βHB, 2.7g taurine, 2.1g leucine PLA: Isocaloric CHO (~ 11g) Single (−30 min pre-exercise)	Stroop color-word test Choice reaction time (CRT) test Pre, during and post exercise	Double blinded: Yes Placebo: Isocaloric CHO Blinding success: Unclear Randomised: Yes Crossover: Yes Dietary control: None Fasted or fed:Fasted Validated cognitive test: Familiarisation trial: Yes βHB measurement: POC	KME significantly attenuated declines in cognitive performance during CRT compared to PLA (P<0.05)
KME (DeltaG; HVMN) (Walsh et al., 2021)	14 obese adults (10 females/4 males: age: 56±12 y; body mass index: 33.8±6.9 kg/m)	KME: 12g PLA: taste matched Multi dose: 3 times a day for 14 days	Stroop color-word test Digit-symbol substitution (DSST) Task-switching test	Double blinded: Yes Placebo: taste matched Blinding success: Unclear Randomised: Yes Crossover: Yes Dietary control: yes Fasted or fed: Fed Validated cognitive test: Yes	Improved DSST task (+2.7 correct responses; P<0.001)

			Pre and post supplemental period	Familiarisation trial: Yes βHB measurement: POC	

A number of mechanistic explanations have been speculated to explain the observed effects of EKS on cognitive performance; more efficient fuel source for cerebral metabolism per O_2 molecule oxidized (Veech, 2004), increases CBF (Walsh et al., 2021a), enhances brain network stability (Mujica-Parodi et al., 2020) and increases circulating dopamine (Poffé et al., 2023). Together, these mechanisms could allow for a 'better' physiological environment for cognitive performance, especially in situations where cerebral metabolic rate appears to be altered via environment (hypoxia), disease (Alzheimer's), or injury (traumatic brain injury, TBI)) (Bernini et al., 2020, Cunnane et al., 2016a, Vestergaard et al., 2016).

1.8 ACUTE HYPOXIC EXPOSURE AS A CHALLENGE TO HOMEOSTASIS AND WHY COUNTERMEASURES ARE NEEDED

Oxygen is essential for aerobic organisms for homeostasis and cell survival as it plays a critical role in adenosine triphosphate (ATP) synthesis via oxidative phosphorylation (Gnaiger, 2001). Hypoxia is defined as reduced oxygen availability or inability to use oxygen, leading to homeostasis dysregulation (Zander and Vaupel, 1985). Currently no agreeable definition of hypoxic expose exists in the literature (Saxena and Jolly, 2019). For the purpose of this review and thesis, acute hypoxia will be considered for exposures lasting <24 h. Acute hypoxia usually occurs during sudden-onset illnesses like acute respiratory distress syndrome (ARDS), stroke, cardiac arrest, or environmental alterations that influence oxygen uptake capacity (Wang et al., 2022).

Hypoxia is measured through pulse oximetry devices. It is a rapid and non-invasive method to quantify arterial oxygen saturation (SpO_2), with normal sea level ranges including 96% to 100% (Torp et al., 2023). Briefly, this measurement is based on the principle that deoxygenated and oxygenated haemoglobin absorb light at different wavelengths, thus allowing for the measurement of oxygen saturation (Torp et al., 2023).

1.8.1 Types of environmental acute hypoxia

Environmental hypoxia can occur simply by physically traveling or ascending to higher altitudes, or can be simulated in a variety of ways, including hypoxia tents, re-breathing devices, metabolic carts altering oxygen availability, as well as hypobaric and normobaric chambers (Garver et al., 2018). Although all methods can induce hypoxia by lowering the partial pressure of oxygen (PaO_2), differences in physiological response can occur due to the method of inducing hypoxia. When utilizing 'real-world' settings, it is challenging to simulate acute hypoxia as some locations can only be reached by foot, which allows time to acclimate to the environment during ascent (Taylor, 2011). Additional concerns in field-based studies include logistics of equipment use and transport at high altitudes, and the influence of cold environments and weather, thus limiting the control and amount of data that can be collected at high altitudes. In response to these challenges a greater number of acute hypoxia studies are conducted in a laboratory setting utilizing normobaric or hypobaric chambers. Hypobaric chambers simulate the decline in atmospheric pressure at higher altitudes, whereas normobaric chambers do not (Bliemsrieder et al., 2022). Differences between physiological responses to normobaric hypoxia compared to hypobaric hypoxia are still a topic of debate and are discussed in further detail in Section 1.9.

1.8.2 Environmental hypoxia effects on whole body physiology

Barometric pressure begins to drop as altitude increases, with PaO_2 dropping to~70% of sea level value at 3000 m and ~50% at 5000 m (West, 2004). The change in the partial pressure creates a challenging environment for oxygen uptake and utilization that involves multiple adaptations to maintain oxygen homeostasis. Rapid ascent without prior adaptation to a hypoxic environment is a physiological stressor with the severity of stress depending on the degree of altitude exposure, rate of ascent, and length of time exposed (Taylor, 2011). Acute physiological changes include an increase in ventilatory response (Taylor, 2011), alterations in

cerebral blood flow that appear to vary by region of the brain (Hoiland et al., 2016, Lumb and Slinger, 2015), and down-regulation of oxidative phosphorylation (Semenza, 2007).

During acute hypoxic exposure, the body rapidly alters multiple systems to adapt to environmental conditions. Once exposed, PaO_2 begins to decline, and $PaCO_2$ begins to rise. Interestingly, hypercapnia ($PaCO_2$ >45 mmHg) drives the physiological response to hypoxia as increases in CO_2 and hydrogen ions (i.e., lowering of pH) in the blood are sensed via central and peripheral chemoreceptors of carotid bodies (Taylor, 2011). This leads to activation of sympathetic nervous system activity that in turn increases cardiac output, blood pressure, and ventilation, all of which enhance O_2 uptake and utilization (Bärtsch and Gibbs, 2007).

The increased ventilatory drive is referred to as Hypoxic Ventilatory Response (HVR). Hyperventilation accelerates CO_2 removal and produces respiratory alkalosis by lowering $PaCO_2$ and raising the pH in the blood, thus decreasing pulmonary vasoconstriction (Taylor, 2011). Following restoration of pH, HVR drive is reduced as alkalosis decreases the respiratory rate. HVR is time-dependent and often begins to decline within an hour of exposure, limiting its ability to counter acute hypoxic exposure (Steinback and Poulin, 2007).

Alterations in blood flow via vasoconstriction or vasodilation occur during acute hypoxic exposure, primarily in the skeletal muscle (Dinenno, 2016), pulmonary (Lumb and Slinger, 2015), and cerebral (Hoiland et al., 2016) vasculature. The pulmonary vasculature constricts during acute hypoxic exposure in response to changes in $PaCO_2$ and PaO_2, which diverts blood to better-oxygenated lung segments allowing for more optimal ventilation-perfusion matching and systemic oxygen delivery (Dunham-Snary et al., 2017). In contrast, the skeletal muscle and cerebral vasculature dilates to increase tissue perfusion and oxygen delivery (Allen et al., 2009).

The vasodilation in skeletal muscle is theorized to be controlled by nitric oxide (NO). Briefly, oxygenated hemoglobin (Hb) becomes desaturated, causing the allosteric release of

NO bioactivity in the form of S-nitrosothiols (SNOs). Red blood cells (RBCs) containing deoxygenated Hb enter the venous system where SNO-Hb and other sources in the plasma recharge the HbFe-NO stores, forming metHb-containing hybrids (FeIII/NO). Reoxygenation in the lungs replaces NO from the hemes and restores dilator capacity of the RBCs (Allen et al., 2009).

In contrast, cerebral vasculature increases blood flow when PaO_2 is below ~50 mmHg (Hoiland et al., 2016), vasodilates during hypercapnia (>45 mmHg), and vasoconstricts during hypocapnia (Mardimae et al., 2012). Mardimae et al. (2012) investigated how hypoxia alters CBF by inducing progressive hypoxia (ranging PaO_2 100-40 mmHg) repeated at three different randomly-ordered CO_2 tensions (30, 40 and 50 mmHg) (Mardimae et al., 2012). Their investigation found that CBF increases progressively with hypoxia, and that CO_2 and hypoxia act synergistically to regulate CBF. Interestingly, the increases in CBF are not uniform. Lastly, Ogoh et al. (2013) investigated the effects of hypoxia and isocapnic hypoxia (gas-controlled mixture) on CBF using ultrasonography to measure the internal carotid artery (ICA) and vertebral artery (VA) blood flow (Ogoh et al., 2013). During hypoxia, ICA blood flow was unchanged, and VA blood flow increased (+10.3±3.1%), whereas, during isocapnic hypoxia, both ICA (+14.5±1.4%) and VA (+10.9±2.4%) blood flow increased. During hypoxia, the lack of blood flow change in ICA was attributed to the vasodilation drive of low PaO_2 ablating the vasoconstriction drive of low PaCO2, whereas in isocapnic hypoxia $PaCO_2$ was maintained, allowing for ICA vasodilation (Ogoh et al., 2013). These findings highlights that the relative contribution of $PaCO_2$ to CBF regulation during hypoxia could potentially be greater than PaO_2. The VA perfuses brain regions primarily responsible for autonomic function (e.g. cerebellum, brainstem, putamen, pallidum, caudate nucleus, and thalamus), which are influenced by pH during hypercapnia (Kuschinsky et al., 1981) and hypoxia (Lockwood et al., 1989) in rodent models. Although the exact mechanisms remain unclear, preserving VA blood flow could be a

survival adaptation to hypoxia that protects brain regions involved with homeostatic processes such as respiration and cardiac function (Ogoh et al., 2013).

1.8.3 Alterations in metabolism

Oxygen is critical combustion fuel for oxidative metabolism. Any state that limits the uptake or utilization of oxygen, such as hypoxia, can affect the metabolic rate (Wheaton and Chandel, 2011). Organisms relying upon aerobic metabolism have developed survival mechanisms to adapt to hypoxia, referred to as oxygen conformance. They downregulate their metabolic rate when oxygen supply begins to decline in order to persevere oxygen and limit reactive oxygen species (ROS) formation in the electron transport chain (ETC) (Hochachka et al., 1996). One mechanism involves decreasing the rate of mitochondrial oxygen consumption in the ETC by altering complex IV isoforms (Fukuda et al., 2007). This mechanism is observable at oxygen levels well above (1-3%O_2) the threshold where oxygen becomes limiting (<0.3%O_2) to cytochrome c oxidase (COX) (complex IV), which is the primary O_2 utilizing enzyme in the ETC for ATP generation (Hochachka et al., 1996).

Additionally, hypoxia activates the transcriptional activity of hypoxia-inducible factor (HIF-1), which regulates ATP-generating pathways such as glycolysis and the tricarboxylic acid cycle (Semenza, 2007). HIF-1 is a transcription factor that aids in regulating oxygen homeostasis and regulates hundreds of human genes involved in a wide array of cellular processes, but especially those related to energy metabolism (Elvidge et al., 2006). It comprises an oxygen-regulated HIF-1α subunit and an expressed HIF-1β subunit. During normoxia (aerobic conditions), HIF-1α is rapidly hydroxylated, ubiquitinated, and degraded. Briefly, HIF-1α is involved with the hydroxylation of two proline residues (Pro402 and Pro564 in human HIF-1α) by dioxygenases that utilize O_2 and α-ketoglutarate as reaction substrates, where one oxygen atom is inserted into a proline, and the other is used to convert α-ketoglutarate to succinate. The hydroxylation of prolines allows for the binding of von Hippel-

Lindau (pVHL) protein, which promotes the ubiquitination and proteasomal degradation of HIF-1α (Semenza, 2007). During hypoxia, HIF-1α hydroxylation is inhibited by O_2 limitation (Epstein et al., 2001) and/or oxidation of the prolyl hydroxylases, which contain Fe(II) in their active site, by ROS generated in the mitochondria by electron-transport Complex III (Chandel et al., 2000, Guzy et al., 2005). HIF-1α regulates glycolysis during hypoxia through two main mechanisms: upregulation of the glycolytic enzymes and pyruvate dehydrogenase kinase 1 (PDK1).

HIF-1α upregulates gene expression of glucose transporters as well as virtually all glycolytic enzymes, including hexokinase, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3, aldolase, phosphoglycerate kinase, enolase, and lactate dehydrogenase A (LDHA). These changes promote and enhance the capability of the glycolytic pathway by upregulating the transport and use of glucose (Semenza, 2007, Semenza et al., 1994). Additionally, HIF-1α upregulates PDK1, inhibiting pyruvate dehydrogenase (PDH), which controls the conversion of pyruvate to acetyl-CoA; thus limiting the ability of pyruvate to enter the TCA and generate reducing equivalents such as NADH and $FADH_2$ (Wheaton and Chandel, 2011). These changes ultimately reduce the reliance on oxidative phosphorylation in the ETC. Moreover, increasing LDHA catalyzes the conversion of pyruvate generated from glycolytic metabolism to lactate and concurrently generates NAD+, a cofactor essential in permitting continued glycolytic activity (Semenza et al., 1996).

Both PDK1 and LDHA alter the path of pyruvate away from the TCA cycle by decreasing the production of acetyl-CoA from pyruvate and increasing lactate production (Wheaton and Chandel, 2011). The produced lactate can be removed from the cell via plasma membrane transporter, monocarboxylate transport 4 (MCT4), to avoid competitive inhibition of LDHA. This modulation in metabolism allows for reduced utilization of O_2 and the formation of ROS from the ETC (Ullah et al., 2006). Animal models have supported these metabolic

alterations as an adaptive survival response to hypoxia. Kim et al. (2006) placed HIF-1α-deficient mouse embryo fibroblasts in hypoxic conditions for 72 h and observed dramatic increases in ROS levels (~5-fold), leading to cell death (Kim et al., 2006). However, subclones transfected with an expression vector encoding PDK1 observed significantly reduced levels of ROS and apoptosis, which highlights the importance of this shift in cellular metabolism as a survival technique that limits toxic ROS formation and programmed cell death (apoptosis) (Wheaton and Chandel, 2011).

Moreover, ETC complexes such as complex IV alter isoform expression between COX4-1 and COX4-2 proteins in response to changes in cellular O_2 concentrations (Fukuda et al., 2007). Current research suggests that COX4-1 isoform is expressed under normoxic conditions, whereas COX4-2 is expressed during hypoxia. HIF-1 regulates this process through activating transcriptions of the genes encoding COX4 and LON, a mitochondrial protease required for COX4-1 degradation (Fukuda et al., 2007). Fukuda et al. (2007) were the first to demonstrate that COX4-2 and LON mRNA expression were induced by hypoxia in the liver and lungs of mice exposed to 10%O_2 (Fukuda et al., 2007). During hypoxia, COX4-2 was replaced with COX4-1, leading to significant decreases in O_2 consumption, COX activity, and ATP concentrations (Fukuda et al., 2007).

Additional research is needed to establish the effects of acute hypoxia on metabolism, through HIF-1α regulation and altered COX-4 expression as most research has investigated chronic effects with little focus on acute hypoxic exposure. Van Thienen et al. (2017) exposed monozygotic twins to acute hypoxic exposure (20 min, ~5000 m) at rest and during submaximal exercise (~30% of sea level VO_2max) and analyzed the HIF-1 pathway, glycolytic and oxidative enzymatic response (Van Thienen et al., 2017). Demonstrating that HIF-1α protein expression increases by ~120% at rest and ~150% during exercise. Moreover, hypoxia increased muscle mRNA content of HIF-1α (+50%), PHD2 (+45%), pVHL (+45%), PDK4 (+1200%). However,

PHD1, LDH-A, PDH-A1, and COX-4 expressions were not altered by hypoxia or exercise (Van Thienen et al., 2017). These findings highlight the rapid upregulation of HIF-1α. Longer exposure duration or higher intensity is likely required to stimulate downstream processes such as COX-4 expression.

Finally, hypoxia reduces cellular ATP demand via reducing Na-K-ATPase activity and inhibiting mammalian target of rapamycin (mTORC1)-dependent translation which are two major ATP-consuming processes in the cell. Briefly, Na-K-ATPase is a plasma membrane that transports sodium (Na^+) and potassium (K^+), consumes ATP and accounts for 20-70% of the cellular oxygen expenditure (Milligan and McBride, 1985). Hypoxic exposures as short as 15 min decreases Na-K-ATPase activity (Zhou et al., 2008). The increase in mitochondrial ROS production in response to hypoxia, activates AMP-activated protein kinase (AMPK) resulting in a decrease in Na-K-ATPase activity and inhibition of mTORC1 (Wheaton and Chandel, 2011).

In conclusion, diminished O_2 availability via lowered PaO_2 from hypoxic exposure reduces metabolism during hypoxia in order to maintain homeostasis. This is achieved through the downregulation of multiple ATP consuming processes such as protein synthesis and Na-K-ATPase activity while reducing carbon flux into the TCA and electron flux through the ETC by activating HIF-1, upregulation of PDK1 and switching of COX subunit4 (Wheaton and Chandel, 2011). The changes, ultimately limit ATP production and slow the rate of oxygen depletion by reducing TCA and ETC activity during hypoxic exposure.

1.9 ADVERSE EFFECTS OF ACUTE HYPOXIA

1.9.1 Normobaric versus hypobaric hypoxia

Acute hypoxic exposure can take the form of either normobaric or hypobaric hypoxia depending on the experimental design and methods employed. Indeed, the question of whether

humans experience physiological differences between normobaric and hypobaric hypoxia are still being debated (Coppel et al., 2015). As a decline in barometric pressure is the primary driving force of enviromental hypoxia, there is likely a physiological difference between hypoxia-induced with and without pressure changes. Short-term studies have found lower SpO_2 during hypobaric hypoxia (HH) than normobaric hypoxia (NH) (Boos et al., 2016, Savourey et al., 2003). Savourey et al. (2003) simulated 4500m in an altitude chamber for 40 min and found that hypoxemia (HH: 6.38; NH: 6.90 kPa), hypocapnia (HH: 4.65; NH: 5.06 kPa), and blood alkalosis (HH:7.46 pH; NH 7.44 pH) were greater in HH compared to NH and that SpO_2 is lower (HH: 85%, NH: 88%) (Savourey et al., 2003). Additionally, Boos et al. (2016) compared genuine high altitude (GA) with simulated HH and NH at 3,375m and observed that resting SpO_2 was lower in GA (89.3±3.4%) and HH (89.0±3.1%) than NH (92.9±1.7%) (Boos et al., 2016).

In a comprehensive review Coppel et al. (2015) reported differences in ventilation, NO, fluid retention, and AMS-related in response to HH compared with NH. During short-term exposure, oxygen saturations were lower in HH (Savourey et al., 2003, Self et al., 2011), whereas during long-term exposure, there were no differences between HH and NH (Savourey et al., 2007, Naughton et al., 1995). Interestingly, PaO_2 did not differ between HH and NH at any altitude (Savourey et al., 2003, Savourey et al., 2007, Self et al., 2011, Naughton et al., 1995). HH exposure was associated with a decrease in minute ventilation and alveolar ventilation, due to lower tidal volumes, however breathing frequencies varied among studies in HH (Savourey et al., 2003, Savourey et al., 2007) (Faiss et al., 2013, Loeppky et al., 1997, Tucker et al., 1983). Despite decreased ventilation and oxygen saturation in HH, $PETCO_2$ levels did not change significantly between NH and HH (Savourey et al., 2003, Savourey et al., 2007, Faiss et al., 2013, Loeppky et al., 1997, Tucker et al., 1983). AMS symptoms were more severe during HH than NH (Loeppky et al., 1997, Roach et al., 1996). Potentially caused by the

increased plasma pH, thus blunting the HVR effect during HH observed in other studies (Savourey et al., 2003, Savourey et al., 2007, Tucker et al., 1983). Additionally, HH had lower exhaled and systemic NO levels (Faiss et al., 2013, Hemmingsson and Linnarsson, 2009), which was likely the cause of increased oxidative stress observed in HH (Faiss et al., 2013).

1.9.2 Effects on cognitive performance

Acute hypoxia adversely affects cognitive performance, with this adverse effect likely caused by increased glycolysis, oxidative stress, calcium overload, and inflammation (Wang et al., 2022). Carotid bodies rapidly release adenosine and dopamine, in response to hypoxic exposure. The neurotransmitters activate sensory vagal afferents to initiate systemic cardiorespiratory reflex resulting in an increase in ventilation rate, elevation in heart rate, dilation of blood vessels, and modulation of CBF regionally (Sharp and Bernaudin, 2004), ultimately leading to hyperventilation (Ogoh et al., 2014), and dynamic cerebral autoregulation impairment induced by acute hypoxia (Subudhi et al., 2010). Cerebral autoregulation that normally compensates for fluctuations in perfusion pressure in order to maintain consistent blood flow (Paulson et al., 1990), may be impaired by acute hypoxia (Subudhi et al., 2009, Subudhi et al., 2010), potentially disrupting the blood brain barrier leading to vasogenic edema (Lassen and Harper, 1975) and worsening of acute mountain sickness symptoms (Ainslie et al., 2008).

Hypoxia activates HIF-1α, promotes anaerobic glycolysis, and drastically decreases the production of cellular ATP (Semenza, 2007). These hypoxic induced alterations potentially leads to an accumulation of lactate which promotes the production of ROS (Millar et al., 2017). Moreover, decreased oxygen availability hinders oxidation phosphorylation, which in turn may contribute to the production of superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite, NO, and carbon monoxide (CO) (Kalyanaraman, 2013). The overproduction of free radicals can disrupt the basement membrane of the blood-brain barrier, causing vasogenic

edema (Wilson et al., 2009). The accumulation of NO, ROS, and free radicals can potentially inhibit the activity of Na^+/K^+ ATPase, thus inducing ion gradients and cell membrane depolarization (Bogdanova et al., 2016). Additionally, the intracellular influx of calcium via the calcium pump, Na^+/Ca_2^+ exchanger, voltage-dependent and receptor-operated Ca_2^+ channels elicit calcium overload (Pregnolato et al., 2019), leading to water being exchanged into cells, creating cytotoxic edema (Oechmichen and Meissner, 2006). Finally, ROS production can lead to neuroinflammation by activating microglia and astrocytes. Once activated, microglia cells produce excessive proinflammatory and inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6, IL-1α, and interferon γ (IFN-γ), along with glutamate (Jellema et al., 2013, Tuttolomondo et al., 2008). Production of TNF-α may accelerate leukocyte migration and glial cell activation by disrupting the blood-brain barrier and its transporters, thus damaging the extracellular matrix and neurovascular unit, and thereby disrupting the brain microenvironment (Rosenberg, 2017). These changes may eventually alter synaptic plasticity (Lim and Pack, 2014) and lead to cognitive deficits (Cheon et al., 2017) during chronic prolonged exposures.

Acute hypoxic exposure has been shown to negatively affect executive and non-executive, perception/attention, and short-term memory tasks in both normobaric and hypobaric conditions (McMorris et al., 2017). The level of impairment appears to be closely correlated with PaO_2 and most impairment occurs in the 35-60 mmHg range (McMorris et al., 2017). Differences in the physiological responses to HH and NH, have not translated to differences in cerebral oxygenation or cognitive performances (Hohenauer et al., 2022). Hohenauer et al. (2022) compared cognitive performance between NH and two HH conditions (HH1: first day of ascent; HH2: after overnight stay) at 2980 m and observed no difference in cognitive performance (throughput, accuracy, and reaction time) or cerebral oxygenation between

conditions even though there were significant differences in SpO_2 between NH and HH1 (1.7±0.5%) (Hohenauer et al., 2022).

In a recent systematic literature review (Bliemsrieder et al., 2022) indicated that a high level of cognitive impairment exists in both in-field and simulated environments, including a 64.7% impairment in lab-based hypoxic studies and a 33.6% impairment during in-field studies (Bliemsrieder et al., 2022). In hypoxic cognitive research, higher-level cognitive domains such as attentional capacity, concentration, and executive functions are the most frequently studied. This is due to the fact that the brain regions controlling these cognitive domains maybe the most susceptible to oxygen depletion and include the hippocampus, basal ganglia, and the cerebral cortex (Caine and Watson, 2000, Di Paola et al., 2008). The hippocampus and cerebral cortex are involved in learning and memory, whereas the basal ganglia and cerebral cortex aid in higher-level processes such as executive function (Chauhan et al., 2021, Lezak et al., 2012).

Executive function has been the most extensively studied domain of cognitive performance during hypoxic exposure. It is a high-level cognitive domain including willpower, planning and decision-making, goal-directed action, and effective performance (Lezak et al., 2012), which is an essential domain of cognitive performance to maintain for survival when exposed to a real-world hypoxic environment. Approximately 50% of the trials evaluating the effect of hypoxia on executive function were linked to elevated risk behaviour or executive function impairment (Bliemsrieder et al., 2022).

The Stroop task is most commonly used executive function test in studies examining the effects of hypoxia on cognitive performance. All five laboratory studies to date, found impaired cognitive function in response to altitude exposures (Asmaro et al., 2013, Chroboczek et al., 2021, de Aquino Lemos et al., 2012, Ochi et al., 2018, Turner et al., 2015) ranging from 2000 m to 7620 m. Conversely, the results from in-field studies were more equivocal. with the two lowest altitudes (3109 m; 3500 m) showing impairment (Griva et al., 2017, Weigle et al.,

2007), one showing improvement (Lefferts et al., 2020), and the two highest altitudes (4554 m; 5500 m) demonstrating no observable effects (Bjursten et al., 2010, Issa et al., 2016). The mixed findings between laboratory and in-field studies are potentially due to the length of ascent to achieve the desired hypoxic exposure. All the in-field studies used an active ascent, the slowest method to reach altitude, and allows for better acclimatization prior to cognitive testing (Schneider et al., 2002).

Outside of executive function, attentional capacity, processing speed, and working memory are the most well-studied cognitive domains during hypoxic exposure (Bliemsrieder et al., 2022). The abilities of attention, tracking, and concentration are required for goal-directed activity. Depending on the focus, they can only be measured within the framework of a cognitive activity sequence. Information must be temporarily stored, and a common feature of all storage devices is their limited capacity (Lezak et al., 2012). Three lab-based studies have used the Corsi Block Forwards and Digit Span Test-Forward at 4500 m (de Aquino Lemos et al., 2012) and 7620 m (Asmaro et al., 2013) to measure attention, and all three observed a decreased performance. In comparison, in-field studies have found mixed results for different cognitive tests designed to measure attention. Two studies using lower altitude tests (4280 m; 5100 m) observed impaired cognitive function (Harris et al., 2009, Shi et al., 2016), whereas in-field studies involving higher altitudes (6348 m; 7200 m) found no impairment (Malle et al., 2016, Petiet et al., 1988) in cognitive function.

Processing speed can be assessed using response speed tests based on reaction time and accuracy data, although slowdown is frequently associated with attentional deficiencies. The Psychomotor Vigilance Test is a commonly used to evaluate processing speed. To date, only in-field studies have been undertaken to assess the impact of hypoxic exposure on processing speed. The findings were mixed with two studies involving altitudes of 2590 m and 3269 m showing no effect of hypoxic exposure on processing speed (Falla et al., 2021b, Latshang et al.,

2013) whereas two other at 3800 m; 5050 m showing impairments (Frost et al., 2021, Pun et al., 2018). Overall, these findings highlight the potential for a threshold effect of altitude on processing, although further research involving both in-field and in-lab is needed.

Working memory, commonly referred to as mental tracking, is a cognitive function that handles more complicated cognitive tasks and permits the manipulation and maintenance of information in temporary storage (Lezak et al., 2012). In working memory literature, approximately half of the investigations demonstrated changes due to hypoxic exposure. De Aquino Lemos et al. (2012) found impairment performance in the Corsi Block Backwards, Digit Span Test-Backward, Random Number Generation, and Sequence of Numbers and Letters at 4500 m (de Aquino Lemos et al., 2012). Impairments have also been observed during the Digit Span Test-Backward at over 5334 m (Asmaro et al., 2013) and the Running Memory Continuous Performance at 4300 m (Seo et al., 2015). In contrast, in-field studies have found no effect of hypoxic exposure on working memory (Subudhi et al., 2014, Harris et al., 2009, Malle et al., 2016, Zhang et al., 2013).of

Taken together, the research findings to date highlights the potential detrimental effects hypoxic exposure on cognitive performance that vary in length and severity and these effects are observed across multiple domains. The highest-level cognitive abilities appear most susceptible to the hypoxic exposure. Altitude, duration of exposure, and degree of oxygen desaturation (Ochi et al., 2018) appear to have the most significant impact on cognitive performance. In-field studies have shown that sufficient acclimatization benefits cognitive performance, although impairments have still been observed. Countermeasures other than acclimatization are required for many occupations (Bliemsrieder et al., 2022).

1.9.3 Acute Mountain Sickness (AMS)

Decreased PaO_2 during acute hypoxic exposure could lead to adverse effects such as acute mountain sickness (AMS) and progress to life-threatening illnesses such as high altitude

pulmonary or cerebral edema if untreated (Jensen and Vincent, 2023). Symptoms of AMS include; headache variably accompanied by loss of appetite, disturbed sleep, nausea, fatigue, and dizziness beginning within 12 hours of ascent in two-thirds of susceptible subjects and within 36 hours in the remaining third (Honigman et al., 1993). The mechanisms responsible for hypoxic induced AMS are not fully understood. The major determining factors of AMS development and severity are altitude attained, individual susceptibility, rate of ascent, and degree of pre-acclimatisation (Luks et al., 2017). The incidence of AMS increases with increasing altitude. At 2850 m the occurrence rate of AMS is only 9% (Maggiorini et al., 1990) but climbs to 58% by 4730 m (Karinen et al., 2008). It appears that susceptibility to AMS is determined by the interaction of physiological responses to hypoxia and anatomical factors such as the compensatory capacity for cerebrospinal fluid and the capacity of venous outflow (Bärtsch et al., 2002). Studies have found a relation between lower PaO_2 and higher AMS incidence rates in populations exposed to hypoxia (Bärtsch et al., 1987). This is likely due to lower ventilatory drive response between individuals (Bärtsch et al., 2002). The rate and/or method of ascent alters the adaptation period while traveling to altitude as studies have shown increases in AMS symptoms during rapid ascents without prior pre-exposure adaption periods. Additionally adding pre-exposure periods has shown to reduce AMS symptoms, even in more susceptible populations (Schneider et al., 2002).

1.9.4 Countermeasures to negative consequences of acute hypoxic exposure

Carbonic anhydrase (CA) inhibitors often in the form of acetazolamide is currently the only pharmaceutical countermeasure to hypoxia:, (Imray et al., 2010). CA inhibitors induce metabolic acidosis by inhibiting enzymes that catalyze the hydration of carbon dioxide to bicarbonate and protons, thus reducing acid buffering capacity through CO_2 removal (Imray et al., 2010). Inducing metabolic acidosis increases the HVR response to hypoxia, maintaining a higher arterial PaO_2 (Leaf and Goldfarb, 2007). Larson et al. (1982) demonstrated increased

minute ventilation after acetazolamide administration compared to PLA (24.9±2.0 L/min; 16.9±3.8 L/min; respectively) during a genuine high-altitude exposure of 4394 m (Larson et al., 1982). The benefit of using acetazolamide to counter AMS symptoms during hypoxic exposure when PaO_2 increases is well documented. For example, Grissom et al. (1992) studied 12 climbers ascenting to a summit of 6150 m and divided them into an acetazolamide (n=6) and placebo (n=6) groups. Acetazolamide or PLA was consumed twice (250 mg/kg), once at baseline and once again 8 hours later. Pre and post summit (24 h) AMS and blood gases measurements were collected in both groups. All except one participant in the acetazolamide group was free of AMS whereas all climbers in the PLA group hadAMS symptoms. Additionally, acetazolamide significantly improved PaO_2 (+2.9±0.8 mmHg) compared to PLA (-1.3±2.8 mmHg) (Grissom et al., 1992).

A major drawback to CA inhibitors is that they are not well tolerated. The proportion of patients reporting side effects ranges from 7% to 76%, with the most frequently reported adverse events including paraesthesia followed by drowsiness, nausea, and dizziness (Shukralla et al., 2022). More severe side effects include acute hypercrystalluria (Shah et al., 2018), rapidly deteriorating acute kidney injury requiring hemodialysis, urological intervention, or both (Davies, 1959, Higenbottam et al., 1978, Neyra et al., 2014).

The use of CA inhibitors may hinder physical (Bradwell et al., 2018, Bradwell et al., 2014) and cognitive performance (Wang et al., 2013a). Wang et al. (2013) employed real altitude exposure (3561 m) to induce hypoxia in an acetazolamide (n=11) and placebo group (n=10) and found that 4 d of treatment with acetazolamide reduced AMS symptoms, but impaired measures of concentration, cognitive processing speed, reaction time, short-term memory, and working memory (Wang et al., 2013a). Supplementation (125 mg/kg twice daily) commenced 3 days prior to ascent and ended 1 day after. Measurements included a daily AMS symptom questionnaire and neuropsychological performance testing, assessed using the digit

symbol substitution test (DSST), paced auditory serial addition test (PASAT), operation span task, and free recall test at 6, 30, and 54 h after ascent. The acetazolamide intervention resulted in reduced AMS symptoms (acetazolamide: 1.3; PLA: 3.1), but impaired cognitive performance during DSST (acetazolamide: 136.5±13.4; PLA: 150.5±14.3), during PASAT (acetazolamide: 48.0±3.5; PLA: 53.6±3.4), and during an operation span task (acetazolamide: 0.77±0.06; PLA: 0.84±0.05) compared to PLA (Wang et al., 2013a). The authors attributed the impairments to the inhibitory effects of carbonic anhydrase, which may have adverse effects on learning and memory. For example, animal studies have shown the inhibitory effects of acetazolamide on synaptic efficacy, spatial learning, and memory (Sun et al., 2001). Mechanistically this is likely because carbonic anhydrase plays a crucial role in signal processing, long-term synaptic transformation, and attention gating of memory storage (Staley et al., 1995, Sun and Alkon, 2002). Together these observations highlight the demand for alternative countermeasure to acute hypoxia that increase oxygen availability, reduce AMS symptoms, while maintaining cognitive and physical performance without any potential adverse effects.

1.9.5 Potential for acute ingestion of EKS as a countermeasure to acute hypoxic exposure

This thesis is primary focused on the potential for the acute ingestion of EKS as a countermeasure to acute hypoxic exposure. It is proposed that acute nutritional ketosis induced by acute ingestion of the R-BD R-βHB KME potentially provides utility as a countermeasure to acute hypoxic exposure based on several potential mechanisms.

Firstly, it increases CBF in the common carotid artery (+12%) and the vertebral artery (+11%) in obese individuals (Walsh et al., 2021a). Infusion-based studies in healthy volunteers that elicit an increase in circulating βHB (5.5±0.4 mM) have been found to increased CBF by ~30% without altering O_2 consumption (Svart et al., 2018). This mechanism may potentially

ensure an adequate O_2 supply in order to maintain the required cerebral metabolic rate (Brugniaux et al., 2007).

Secondly, it induces metabolic acidosis at rest (Dearlove et al., 2020; McCarthy et al., 2021; Stubbs et al., 2017). Stubbs et al. (2017) demonstrated that a single dose eliciting a ~2-3 mM response in R-βHB at rest was enough to induce metabolic acidosis for >1 h. Currently, no investigation has been able to demonstrate increased ventilation at rest after R-BD R-βHB KME consumption. It has however been shown to increase ventilation during prolonged exercise (>3 h) in NH conditions (~1,000 m to 3,000 m) at multiple time points compared to CON (Poffe et al., 2021). Exercise is a co-founding variable that could influence this response, highlighting the need for further research. Interestingly, co-administering R-BD R-βHB KME with BIC alleviated the acid load of KME leading to an almost identical ventilatory response as the control group even at the highest level of hypoxic exposure (CON: 103±12 L/min; KME: 115±15 L/min; KME+BIC: 103±11 L/min) (Poffe et al., 2021). Together, R-BD R-βHB KME induces prolonged metabolic acidosis and potentially increases ventilatory drive leading to increases in PaO_2 during hypoxic exposure, similar to acetazolamide (Ritchie et al., 2012).

Thirdly, KBs are in theory a more efficient fuel source than glucose for cerebral metabolism per O_2 molecule oxidized (Veech, 2004) and can help supplement the increased energy demand that occurs in cerebral metabolic rate during acute hypoxic exposure. A recent investigation exposed healthy individuals to 40 min of hypoxia (10%O_2) and found a 15.5% increase in CBF accompanied by an 8.5% increase in cerebral metabolic rate and 180% in cerebral lactate (Vestergaard et al., 2016).

Fourthly, KBs can function as a direct antioxidant to hydroxyl radical (Haces et al., 2008) and as an inhibitor of mitochondrial ROS production in stressed neurons by facilitating NADH oxidation (Maalouf et al., 2007), therefore potentially reducing inflammation during hypoxia exposure, although this may not occur acutely.

Finally, R-BD R-βHB KME was recently shown to increase circulating dopamine levels 2-fold during ultra-endurance exercise and significantly attenuate the rise in reaction time (KE: 362±41 vs. CON: 419±54ms), movement time (KE: 192±47 vs. CON: 245±64ms), and false alarms during a reaction time task and rapid visual information processing task (Poffé et al., 2023). It is worth noting that circulating dopamine does not cross the blood-brain barrier. Studies in healthy populations using the dopamine precursor Levodopa, found improved reaction and movement times in healthy populations suggesting the beneficial effects of increasing dopamine in the central nervous system (Hasbroucq et al., 2003, Rihet et al., 2002). Additionally, R-BD R-βHB KME increased striatal dopamine release in a mouse model of Parkinson's (Mahajan et al., 2022). Acute hypoxia has been shown to reduce the turnover of key neurotransmitters such as dopamine as a result of the oxygen requirement during the synthesis, release, and metabolism of these neurotransmitters (Gibson and Peterson, 1982, Gibson et al., 1981). Increasing dopamine concentrations in the central nervous system during hypoxic exposure may therefore improve cognitive performance. Addition human research is is needed to support this potential mechanism.

R-BD R-βHB KME is potentially the ideal countermeasure to acute hypoxic exposure as it increases CBF, extends the HVR, aids in cerebral metabolic demand with a more efficient fuel source per O_2 molecule, and increases PaO_2. It may also reduce oxidative stress and inflammation as well as increasing dopamine levels.

At the time of commencing my PhD studies, collaborators of my supervisor Dr. Egan at the Institute for Human and Machine Cognition (Pensacola, Florida) had published preliminary work that supported the utility of the R-BD R-βHB KME in severe hypoxic exposure (20 min; 5029 m) (Coleman et al., 2021). Healthy male volunteers (n=11) completed a cognitive performance test battery under conditions of normoxia and hypoxia (20 min each) following consumption of R-BD R-βHB KME (550 mg/kg) or non-caloric PLA. Hypoxia was

simulated via a reduced oxygen breathing device (ROBD) at rest and was worn for the normoxia session. The cognitive test battery consisted of simple reaction time, procedural reaction time, code substitution test, choice reaction time, discriminant reaction time and measured blink rate and duration. Results include elevation in R-βHB ~4 mM during hypoxic exposure leading to a significant SpO_2 advantage while mitigating declines in cognitive efficiency during the code substitution task (KME: 53.0 SE 2.2; PLA: 49.7 SE 2.9) and decreased blink duration (KME 6.4, SE: 0.45 ms; PLA: 8.4, SE: 1.2 ms) although no other effects were observed in any other cognitive testing.

1.10 SUMMARY AND DIRECTION OF THESIS

EKS are an ingestible form of KBs that rapidly alter metabolism, providing extrahepatic tissues with an alternative substrate, and can affect blood gases and pH balance among a myriad of physiological effects. Recent research suggests the potential utility for acute ingestion of EKS as a countermeasure to declines induced by acute exposure to hypoxia. This thesis investigated whether acute ingestion of R-BD R-βHB KME could mitigate declines in SpO_2 and cognitive performance from severe hypoxia by investigating (i) effects of various forms of EKS on pH, ventilation, blood gases, heart rate variability, and cognitive performance at rest in normoxia (ii) whether acute ingestion of R-BD R-βHB KME during acute severe hypoxic exposure (simulated 6096 m altitude; 9.7%O_2) could impact performance in a variety of cognitive performance tests; and (iii) whether R-BD R-βHB KME has utility as a countermeasure to hypoxic exposure when an additional stressor that upregulates oxygen consumption, namely moderate exercise, is involved.

Chapter 2
Effects of acute ingestion of various forms of exogenous ketone supplements on R-βHB concentration, blood gases and pH, and cognitive performance

2.1. ABSTRACT

Acute ingestion of EKS in the form of R-BD R-βHB KME has demonstrated the ability to alter metabolism and acid/base balance at rest. A variety of new, yet unstudied, forms of EKS are commercially-available requiring further pharmacokinetic investigations at rest to better understand how these compounds modulate metabolism and acid/base balance to best suit their therapeutic or performance applications. Central to this thesis is the effect of EKS on cognitive performance, so in a single-blind randomized crossover design, twenty healthy participants (M: 10, F:10; age: 20.6±2.0 y, height: 171.7±7.5 cm, weight: 67.9±10.2 kg) performed cognitive testing at rest before, 60 and 120 min after consumption (395 mg/kg) of various EKS, namely KME, KME+sodium bicarbonate (KME+BIC), and a non-racemic ketone salt (KS), and were compared to a non-caloric taste-matched placebo (PLA). Blood metabolites, indirect calorimetry, blood gases, hemodynamics and autonomic function were measured at various time points. All EKS significantly increased blood R-βHB concentration (~2.0-3.5 mM; $P<0.01$) and reduced blood glucose concentration compared to PLA. KME ingestion decreased pH (-0.07 units), whereas KS and KME+BIC increased pH (+0.05 units) compared to PLA. Cognitive performance during the Stroop colour word test and switching task did not differ at any time point within or between conditions. These data confirm that KME induces acidosis at rest, whereas KME+BIC and KS increase pH. A novel finding is that a non-racemic KS increases blood R-βHB concentration >2.0 mM. None of the EKS alter cognitive performance under resting conditions.

2.2 INTRODUCTION

Exogenous and nutritional ketosis is a rapidly expanding area of research with potential applications for both performance and disease in a variety of environments and populations (Evans et al., 2022; Saris & Timmers, 2022). Limitations and challenges can exist during diet-induced nutritional ketosis due to multiweek adaptation timelines and overall compliance with a very low carbohydrate (CHO) diet (<50g of CHO a day) (Burke, 2021). Alternatively, exogenous ketosis is a rapidly induced acute metabolic state consequent to the ingestion of EKS that has been hypothesized to have performance (Evans et al., 2022) and therapeutic applications (Omori et al., 2022). The category of EKS is expanding with novel formulations and co-administration dosing strategies emerging to fit the potential applications in hypoxia (Coleman et al., 2021; Poffe et al., 2021; Prins et al., 2021), chronic obstructive pulmonary disease (COPD) (Norwitz et al., 2021), Parkinson's disease (Norwitz et al., 2020), cognitive impairment (Cunnane et al., 2016), traumatic brain injury (TBI) (Omori et al., 2022), metabolic efficiency (Brady et al., 2023), inflammation, and reduction of circulating glucose and lactate (Evans et al., 2022).

Currently the most researched EKS are the R-BD R-βHB KME and ketone salts (KS) (Evans et al., 2022). A ketone salt is a ketone body (KB) in the form of R/S-βHB bound to a sodium, potassium, magnesium, and/or calcium to help minimize the acid load associated with the KB (Evans et al., 2017). Most available KS formulations are currently racemic mixtures, consisting of equal quantities of both the R- and S- enantiomers of βHB (Stubbs et al., 2017). The R-enantiomer is the circulating form βHB, and is more readily oxidized fuel that the S-enantiomer (Soto-Mota et al., 2020). Pre-clinical studies have shown that the S- enantiomer plays a role in signalling that modulates metabolism of R-βHB and glucose (Tsai et al., 2006) and can act as a cellular antioxidant (Haces et al., 2008).

Consumption of KS typically leads to a peak increase in circulating R-βHB (~0.5-1.0 mM) in ~60 min which is often considered below the threshold to elicit therapeutic and performance benefits (Evans et al., 2017). This has led to co-administration dosing strategies with other ketogenic precursors such as medium chain triglycerides (MCTs) although R-βHB concentrations remain below the theorized optimal range (Evans et al., 2017; Prins et al., 2020). Alternatively, KME is an R-βHB bound to a ketone precursor (1,3-butanediol) via an ester bond and has been shown to rapidly increase R-βHB concentrations >2 mM within 30 min (Evans et al., 2022) and to decrease circulating levels of glucose concentrations in circulation, creating an altered and unique metabolic environment where both availability of glucose and high ketone concentrations are present at the tissue level (Evans et al., 2022).

A critical but somewhat understudied aspect of the impact exogenous ketosis on human physiology is its direct impact on acid-base balance. Disturbances to homeostasis that increase H+ concentrations and reduce pH result in “acidosis”, which impairs mitochondrial function and enzymatic activity, leading to altered energetic efficiency and fatigue (Lancha Junior et al., 2015). Indeed, the rapid influx of KBs into the circulation causes acute metabolic acidosis at rest (Dearlove et al., 2020; Prins et al., 2021; Stubbs et al., 2017) and during exercise (Dearlove et al., 2020; Poffé et al., 2020; Prins et al., 2021). KS slightly increases pH during rest (Stubbs et al., 2017). Decreasing pH prior to high-intensity exercise is not-optimal for performance as it reduces muscle contractability and overall buffering capacity (Lancha Junior et al., 2015). This has led to combination dosing strategies of KME with sodium bicarbonate (BIC) to increase the acid buffering capacity, potentially leading to an increase in physical performance (Poffé et al., 2020).

Relatedly, increasing R-βHB and reducing pH has been demonstrated to increase SpO_2 in hypoxic environments leading to improved cognitive performance and mitigate increases in the partial pressure of CO_2 ($PaCO_2$) during a CO_2 retention protocol, although it is unclear if

these effects are due to R-βHB and/or reducing pH (Prins et al., 2021). Thus, altered pH through administration of EKS may have antagonistic performance effects depending on pH regulation and end-use application, however the mechanism to attenuate declines in SpO_2 remains unknown. There are no data presently on how this attenuation in declines of SpO_2 may differentiate between males and females, and how "pure" R-BHB salts i.e., non-racemic KS, affect R-βHB concentration, blood gases and pH, and cognitive performance by removing the confounding effect of S-BHB metabolism.

Therefore, we sought to focus the isolated impacts of R-BHB versus pH and examine these knowledge gaps by administering multiple forms of EKS including a non-racemic R-KS and KME co-administered with BIC at rest to partially control for R-βHB, pH, racemic biochemistry and environmental confounders. This experimental approach then focused impact of R-βHB and/or pH on indirect calorimetry, ventilation, blood gases, heart rate (HR), HR variability (HRV), hemodynamics and cognitive performance in both males and females under resting conditions.

2.3 METHODS

2.3.1 Participants

Twenty male and female participants (M:10; F:10) aged 18 to 35 y were recruited to participate in the study (20.6±2.0 y, 171.7±7.5 cm, 67.9±10.2 kg). Each participant provided written informed consent to participate after being provided with written and verbal explanations of the procedures in accordance with the protocol approved by Dublin City University Research Ethics Committee (permit: DCUREC2023/070). The procedures aligned with the Declaration of Helsinki with the exception of not being registered in the trial database. Inclusion criteria were (1) healthy males and females 18 to 35 years of age, (2) currently consuming a moderate or high carbohydrate diet. Exclusion criteria were (1) cardiovascular,

musculoskeletal or metabolic disease or psychiatric disorder, (2) current smoker, (3) currently taking any performance enhancing substances (anabolic steroids, creatine, pure caffeine) or any substances that influence brain activity, (4) currently consuming EKS or following a low carbohydrate or ketogenic diet.

2.3.2 Experimental design

Utilizing a single-blind, placebo-controlled, randomized crossover design, participants visited the laboratory on five separate occasions, comprising one familiarization and four main experimental sessions with ≥7 day wash-out period between experimental sessions. During the first visit, participants were familiarized with the protocol, equipment, cognitive tests, and underwent anthropometric measurements. Height (cm) was measured using a physician's scale (Detecto, Webb City, MO). Weight (kg) and body composition (fat and lean mass) was measured using a Tanita bioelectrical impedance analyzer (BIA) (TBF-310GS Tanita Corporation of America, Arlington Heights, Illinois).

The four main experimental sessions (visits 2 to 5) consisted of metabolic, cardiac (autonomic and hemodynamic), blood gases (acid-base and gas exchange) and cognitive evaluation at rest **(**Figure 2.1). Visits 2 to 5 were identical in terms of pre-test preparation (standardized physical activity (48 h) and diet (24 h) before each visit) and cognitive testing. The visits differed only in the randomly assigned drink consumed - flavour-matched non-caloric placebo (PLA), R-BD R-βHB KME (KME), KME and sodium bicarbonate (KME+BIC), or ketone salts (KS). The primary outcome was venous blood gases and pH with secondary outcomes including indirect calorimetry, heart rate (HR), heart rate variability (HRV), hemodynamics, cognitive performance, and circulating βHB, glucose and lactate concentrations.

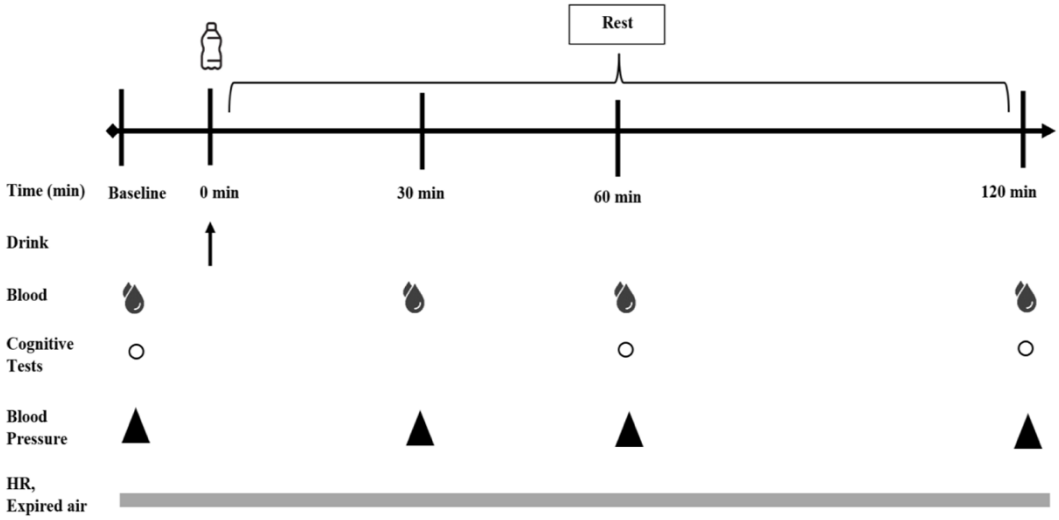

Figure 2.1. Experimental design schematic

2.3.3 Cognitive Assessments

At baseline, and 60 and 120 min after ingestion, participants performed a series of cognitive tests to assess cognitive performance. The cognitive tests were administered via ANAM-4, (Vista Life Sciences, USA), which is a brief, self-directed, computerized neuropsychological assessment battery that assesses neuropsychological functioning. A familiarization test was performed during the first laboratory visit to reduce the possibility of a learning effect. All tests were performed in a sound-insulated room under controlled conditions (i.e., appropriate lighting, as quiet as possible, and isolation from unnecessary stimuli and timing feedback). Participants were instructed to complete the battery as quickly and accurately as possible. Each trial was administered identically.

2.3.3.1 Stroop Colour and Word Task

The Stroop Colour and Word Task measures cognitive flexibility, processing speed, and executive function (Periáñez et al., 2021) and comprises of three “blocks”. In the first block (“Neutral”), the words RED, GREEN, and BLUE are presented individually in black type on the display. The user is instructed to read each word aloud and to press a corresponding key for each word (1 for RED, 2 for GREEN, 3 for BLUE). In the second block (“Congruent”), a series of XXXX's is presented on the display in one of three colors (red, green, or blue). The user is

instructed to say the color of the XXXX's aloud and press the corresponding key based on color. In the third block (“Incongruent”), a series of individual words (RED, GREEN, BLUE) are presented in a color that does not match the name of the color depicted by the word. The user is instructed to press the response key assigned to that color. Outcome measures are correct answers (*n* and %), reaction time in milliseconds (ms), reaction time for correct responses (CRT), and the interference score which measures the Stroop effect, i.e. the tendency to experience difficulty naming a physical colour when it is used to spell the name of a different colour.

2.3.3.2 Switching Task

The Switching Task requires users to alternate between two tasks: The Manikin and Mathematical Processing. Only one type of problem (Manikin or Mathematical Processing) appears on the computer screen. For mathematical processing, participants are presented with a three-digit math equation (e.g., “5+4−2”) and if the sum is greater than “5” they are instructed to click the right mouse, and if the sum is less than “5” then they are instructed to press the left mouse. For the Manikin, participants are presented with an animated character (Manikin) holding a sphere in the left or right hand. If the event that the manikin was holding the sphere in the right hand, participants are instructed to click the right mouse, and if the manikin was holding the sphere in the left hand, participants are instructed to click the left mouse. For each trial, the manikin shifts positions, so that it may be facing towards the viewer, away from the viewer, or to the side. Outcome measures included mean response time (MRT) and mean correct response time (MCRT) in milliseconds (ms).

2.3.4 Main experimental trials

Participants arrived at the laboratory at the same time for each experimental trial in a 3 h postprandial state. A baseline blood sample was taken, baseline measurements of cognitive performance, HR and blood pressure (Omron M7, OMRON, Kyoto, Japan) were recorded and

expired air was continuously collected using a metabolic cart (TrueOne 2400, ParvoMedics; USA). Participants were briefly removed from the metabolic cart and given 5 min to ingest either 395 mg/kg body mass of the KME supplement (deltaG; UK), 395 mg/kg body mass of the KS supplement (Na-BHB; Real Ketones, Saint Peterburgh, FL, USA), KME+BIC solution (KME: 395 mg/kg; BIC: 300 mg/kg NaHCO3) or a ketone-free, non-caloric flavor-matched placebo solution consisting of 3 g Truvia, 40 mL of Bitrex stock, water (500 mL), and the flavour-enhancer MiO to flavor. All drinks formulations were added to water (diluted to 500 mL) and MiO added for flavor. Following ingestion of the solution, participants sat for 120 m during which time respiratory gases, pulmonary exchange expiratory, HR and HRV (Polar; Finland) were continuously monitored.

HRV variables measured included: high frequency (HF) power, low frequency (LF) power, very low frequency (VLF) power, LF/HF ratio, root mean square of successive RR interval differences (RMSSD), and mean R-R. Venous blood samples were taken and blood pressure was measured at 30 min, 60 min and 120 min. The cognitive tests were repeated at 60 min and 120 min following ingestion.

2.3.5 Blood sample analysis

Finger capillary blood was analyzed for R-βHB, glucose (Precision Xtra, Abbott Diabetes Care Inc., Almeda, CA, United States), lactate (Lactate Plus, Nova Biomedical) concentration and blood pH, HCO_3-, PO_2, TCO_2, PCO_2, and SaO_2 (iSTAT; Abbott, Chicago, IL). Fingertip blood samples were collected using a lancet following alcohol cleaning. The first droplet was wiped away with a cotton swab, and the subsequent droplets were used for analysis.

2.3.6 Statistical analysis

All statistical analyses and graphical representation of data were performed using Prism v9 (GraphPad Software Inc, San Diego, CA USA). Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. Data are presented as mean±SD, or

mean difference (lower, higher 95% confidence limits of mean) where indicated. A two-way (Time*Condition) repeated measures ANOVA was used to identify differences between the conditions across time. When a Time*Condition interaction effect, or a main effect of Condition was observed, post-hoc pairwise comparisons were performed with Bonferroni correction applied and multiplicity-adjusted P values are reported. Sphericity was not assumed, and the Greenhouse-Geiser correction was applied to all ANOVA analyses. The threshold for statistical significance was set at $P < 0.05$ for all tests.

2.4 RESULTS

2.4.1 Blood concentrations for R-βHB, glucose and lactate

***R*-βHB**

For baseline values prior to consuming study drinks, blood *R*-βHB, glucose and lactate concentrations did not differ between the four experimental conditions (Figure 2.2). A main effect of time and condition, as well as time*condition interaction effect ($P<0.001$) were observed for blood R-βHB concentrations. KME, KME+BIC and KS significantly increased blood *R*-βHB 30 min after ingestion (KME: 2.6±0.7 mM; KME+BIC: 3.2±0.9 mM; KS: 1.5±0.4 mM; all $P<0.001$) compared to baseline and concentration remained elevated for all timepoints compared to PLA ($P<0.001$) (Figure 2.2).

Glucose

A main effect of time and condition and time*condition interaction effect ($P<0.001$) were observed for blood glucose concentrations. KME and KS ingestion significantly reduced blood glucose concentration 30 min after ingestion (KME: -15.8±14.7 mg/dL, $P<0.01$; KS: -11.6±10.3 mg/dL, $p<0.05$) compared to PLA. KME and KME+BIC reduced blood glucose concentration at 60 min (both $P<0.001$) and 120 min after ingestion ($P<0.001$, $P<0.05$; respectively) compared to PLA. At 60 min, blood glucose concentrations were significantly

lower in KS than KME and KME+BIC (-12.65 ±14.1 mg/dL, P<0.01; -11.1±12.2 mg/dL, P<0.05; respectively). Blood glucose concentrations were significantly lower in KS than KME at 120 min (-17.5±16.2 mg/dL, P<0.01) (Figure 2.2).

Lactate

A main effect of time (P<0.001) and condition (P<0.001) were observed for blood lactate concentrations. KME+BIC reduced circulating lactate concentrations at 30 (-0.5±0.5 mM, P<0.05), 60 (-0.8±0.7 mM, P<0.01), and 120 min after ingestion (-0.7±0.5 mM, P<0.001), whereas KME reduced lactate concentrations at 60 and 120 min after ingestion (-0.5±0.6 mM, -0.5±0.5 mM; both P<0.05) compared to PLA (Figure 2.2).

2.4.2 Blood gases, pH and SpO_2

Baseline pH, HCO_3^-, TCO_2, PCO_2, PO_2, SpO_2 values did not differ between the four experimental conditions (Figure 2.3). A main effect of condition (P<0.001) and condition*time interaction (P<0.001) were observed for pH concentrations. KME reduced blood pH at 30 min (-0.04±0.03, P<0.001) and the pH remained significantly decreased through 60 (-0.07±0.03, P<0.001) and 120 min (-0.03±0.02, P<0.01) after ingestion. KS increased blood pH at 60 min (0.03±0.03, P<0.05) and the pH remained elevated through 120 min (0.05±0.04, P<0.001) after ingestion compared to PLA. KME+BIC increased pH at 120 min (0.05±0.05, P<0.001) after ingestion compared to PLA. KME significantly reduced blood pH by 30 min compared to KME+BIC and KS and the pH remained decreased throughout 120 min after ingestion (P<0.001) (Figure 2.3).

A main effect of time, condition, and time*condition interaction effect (P<0.001) were observed for HCO_3^- concentrations. KME reduced HCO_3^- at 30 min (-3.00±1.77 mM, P<0.001) 60 min (-4.05±1.83 mM, P<0.001) and 120 min (-2.06±2.02 mM, P<0.01) after ingestion compared to PLA. KME+BIC and KS increased HCO_3^- concentrations at 60 (KME+BIC: 2.92±2.18 mM, P<0.01; KS: 2.66±1.84, P<0.01) and 120 min (KME+BIC: 5.24±2.58 mM,

P<0.001; KS: 4.52±2.32 mM, P<0.001) after ingestion compared to PLA. KME significantly decreased HCO_3^- concentrations by 30 min and throughout the entire 120 min (P<0.001) compared to KME+BIC and KS (Figure 2.3).

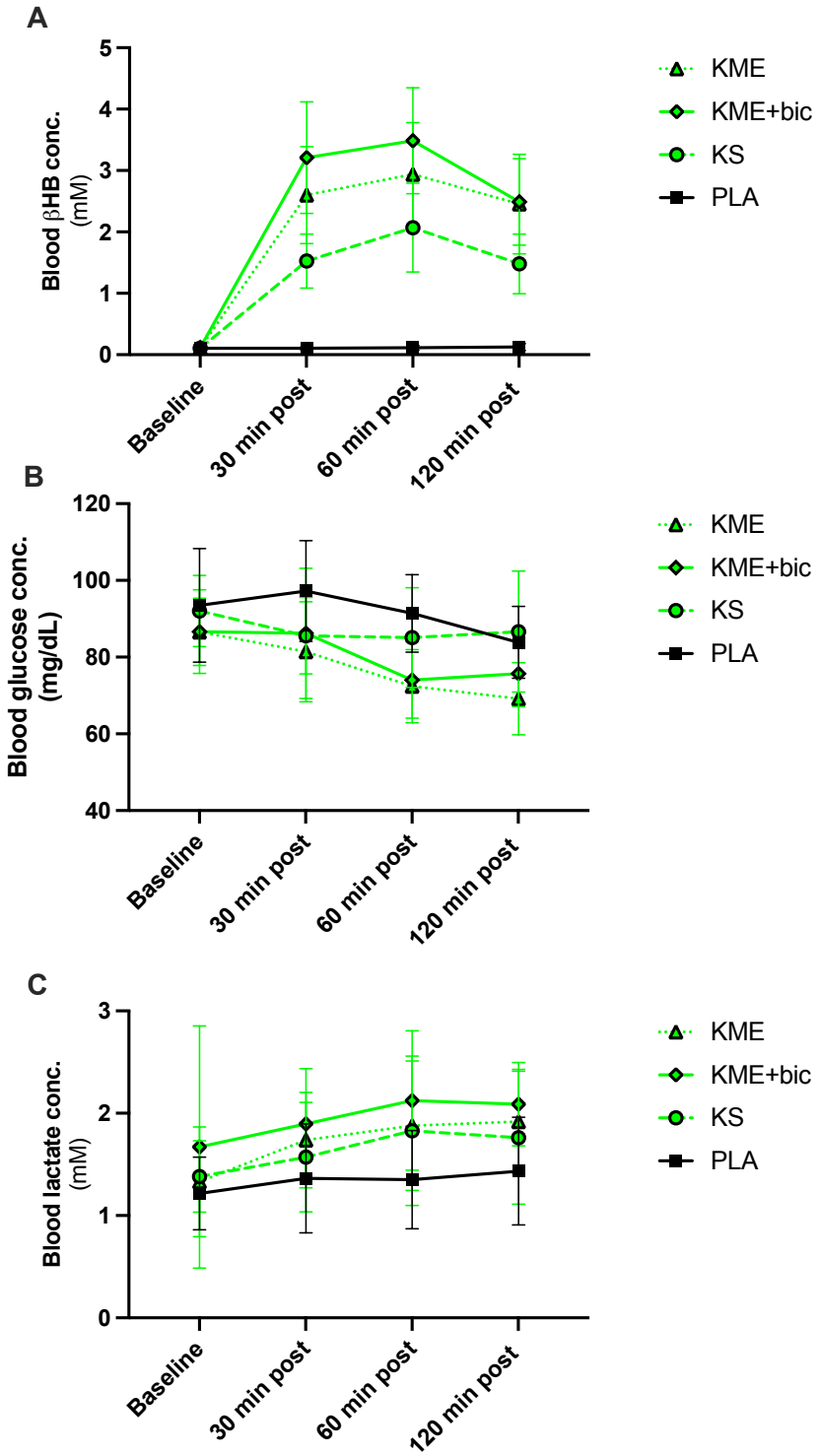

Figure 2.2. Blood metabolites. Blood R-βHB (A), glucose (B), and lactate (C) concentrations during ketone monoester (KME), ketone monoester and sodium bicarbonate (KME+BIC), ketone salt (KS)

and placebo (PLA) throughout baseline, 30, 60, and 120 min after ingestion. Data are presented as mean±SD.

A main effect of time and condition and time*condition interaction effect ($P<0.001$) were observed for TCO_2 concentrations. KME decreased TCO_2 concentrations at 30 (-3.3±2.11 mM, $P<0.001$), 60 (-3.95±2.11 mM, $P<0.001$) and 120 min (-2.05±1.66 mM, $P<0.01$) after ingestion compared to PLA. KME+BIC and KS increased TCO_2 at 60 (KME+BIC: 3.92±2.33 mM; KS: 2.65±1.92 mM, both $P<0.01$) and 120 min (KME+BIC: 6.21±2.52 mM; KS: 4.05±2.58 mM, both $P<0.001$) after ingestion compared to PLA. KME significantly lowered TCO_2 at 30, 60 and 120 min after ingestion compared to KME+BIC and KS ($P<0.001$). A main effect of time ($P<0.05$) and time*condition interaction effect ($P<0.001$) were observed for PO_2 concentrations. At 30 min after ingestion, KS significantly decreased PO_2 compared to PLA (-5.70±4.79 mmHg, $P<0.05$), whereas KME increased PO_2 compared to KS (7.45±9.46 mmHg, $P<0.05$) (Figure 2.3). A main effect of time ($P<0.001$) was observed for PCO_2 concentrations and SO_2 percentage, however no differences occurred between ketone conditions or PLA at any timepoint.

2.4.3 Indirect Calorimetry

Baseline respiratory exchange ratio (RER), VO_2, VCO_2, ventilation (VE), respiratory rate (RR), and tidal volume (Tv) values did not differ between the four experimental conditions (Figure 2.4). A main effect of time ($P<0.001$) and time*condition interaction effect ($P<0.01$) were observed for RER. At 30 min after ingestion, KME was significantly higher than KS (0.06±0.05, $P<0.01$) (Figure 2.4).

A main effect of time ($P<0.001$) and time*condition interaction effect ($P<0.01$) were observed for VE. Although there was a main effect of time for both RR ($P<0.001$) and Tv ($P<0.05$), no significant differences was found between ketone conditions or PLA at any timepoint (Figure 2.4).

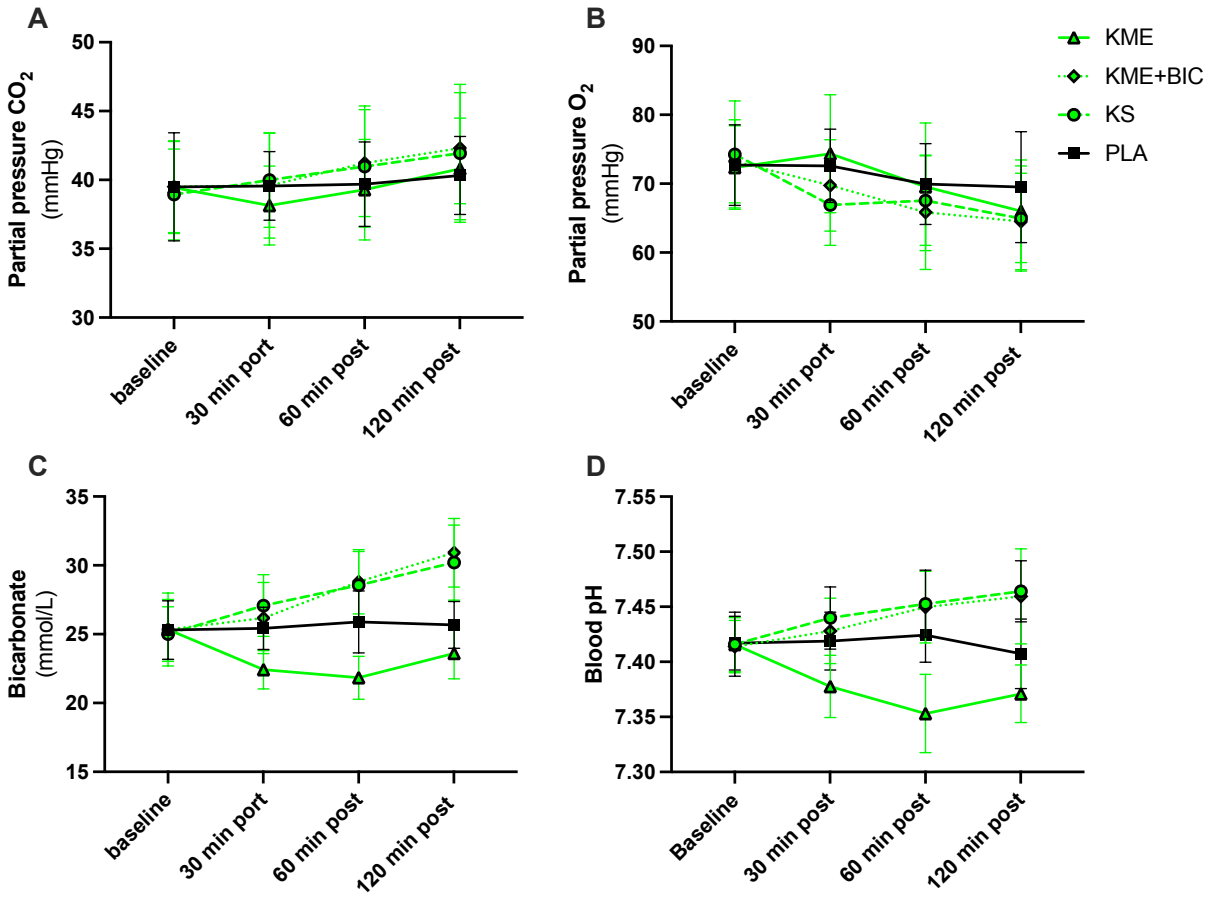

Figure 2.3. Blood Gases. Blood gases; partial pressure of carbon dioxide (PCO_2) (A), partial pressure of oxygen (PO_2) (B), bicarbonate (HCO_3-) (C), pH (D) concentrations during ketone monoester (KME), ketone monoester and sodium bicarbonate (KME+BIC), ketone salt (KS) and placebo (PLA) throughout baseline, 30 ,60 and 120 min after ingestion. Data are presented as mean±SD.

A main effect of time*condition interaction was observed for VCO_2 ($p<0.05$) whereas no effect of time, condition or time*condition interaction was observed for VO_2. No significant differences occurred between ketone conditions or PLA at any timepoint for both VCO_2 and VO_2 (Figure 2.4).

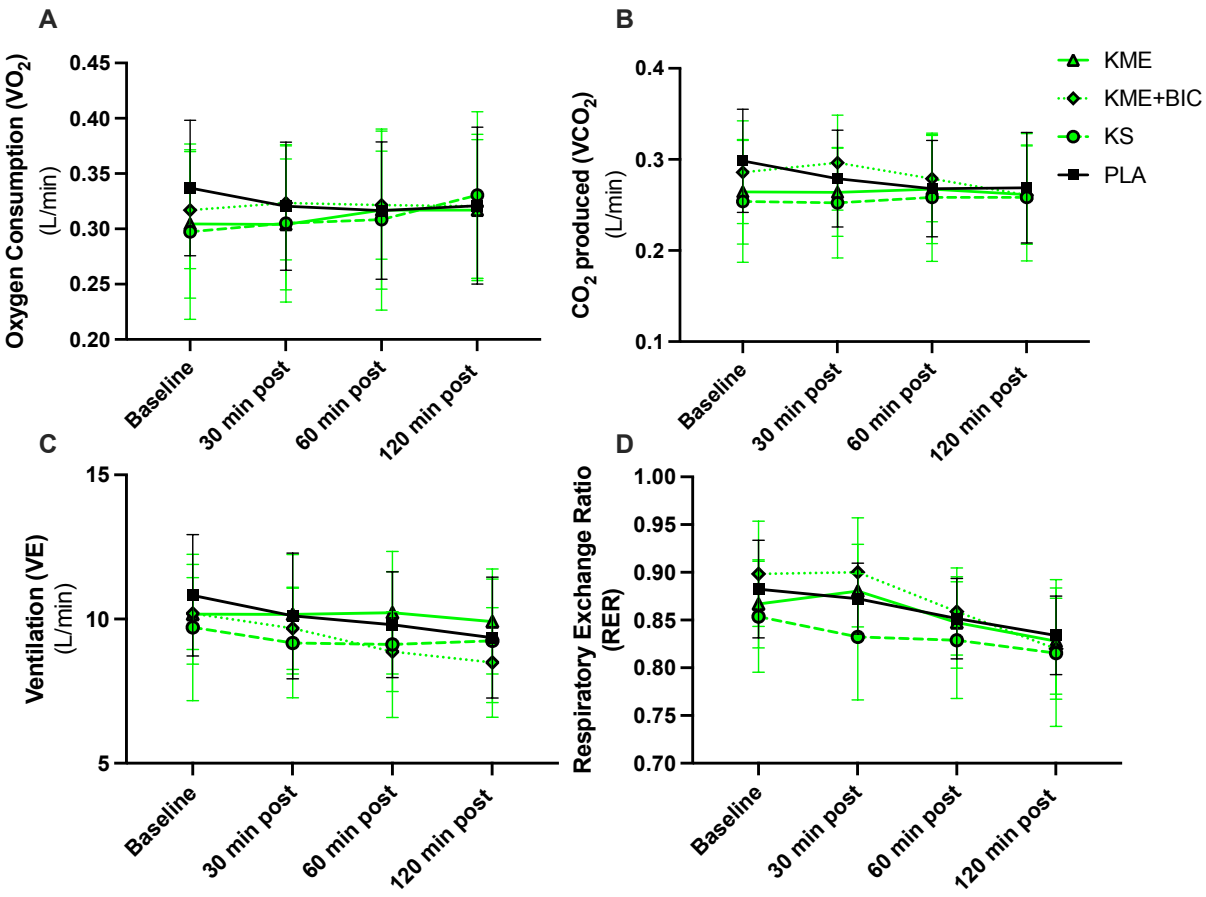

Figure 2.4. Indirect Calorimetry.; oxygen consumption (VO_2) (A), carbon dioxide produced (VCO_2) (B), ventilation (VE) (C), respiratory exchange ratio (RER) (D) concentrations during ketone monoester (KME), ketone monoester and sodium bicarbonate (KME+BIC), ketone salt (KS) and placebo (PLA) throughout baseline, 30, 60 and 120 min after ingestion. Data are presented as mean±SD.

2.4.4 Heart rate, HRV and hemodynamics

Baseline HR, mean RR, parasympathetic nervous system (PNS) index, sympathetic nervous system (SNS) index, root mean square of successive differences (RMSSD) or blood pressure (BP) values did not differ between the four experimental conditions. A main effect of time ($P<0.001$), condition ($P<0.05$) and time*condition interaction effect ($P<0.001$) were observed for HR. HR was significantly elevated at 60 (+12.1±8.4 BPM, $P<0.01$), 90 (+14.5±16.8 BPM, $P<0.001$), and 120 min (16.0±5.9 BPM, $P<0.001$) after ingestion KME+BIC compared to PLA. KME+BIC elevated HR at 90 min, compared to KME alone (10.2±11.6 BPM, $P<0.05$).

There was no time (P=0.31), condition (p=0.054) or time*condition interaction effect (P=0.39) for mean RR. KME+BIC significantly reduced mean RR compared to PLA at 60 (P<0.05), 90 (P<0.001) and 120 min (P=0.001) after ingestion.

A main effect of time, condition and time*condition interaction effect (P<0.05) were observed for PNS index. At 60 (P<0.05), 90 (P<0.001), and 120 min (P=0.001). KME+BIC reduced the PNS index compared to PLA. At 60 and 90 min, KME PNS index was significantly elevated compared to KME+BIC (P<0.05).

A main effect of time (P<0.001), condition (P<0.05) and time*condition interaction effect (P<0.001) were observed for SNS index. At 60 (P<0.01), 90 (P<0.001), and 120 min (P<0.001) KME+BIC reduced the SNS index compared to PLA. At 90 (P<0.01) and 120 min (P<0.05), KME+BIC SNS index was significantly elevated compared to KME.

A main effect of time (P<0.01), condition (P<0.05) and time*condition interaction effect (P<0.01) were observed for RMSSD. At 60 (P<0.05), 90 (P<0.01), and 120 min (P<0.01) KME+BIC reduced RMSSD compared to PLA. At 90 min, KME+BIC was reduced compared to KME and KS (P<0.05). At 120 min, KME+BIC RMSSD was reduced compared to KME (P<0.05).

There was no time (P=0.09), condition (p=0.37) or time*condition interaction effect (P=0.78) for systolic BP. A time effect (P<0.05) was observed for diastolic BP, although no significant findings at any time point compared to PLA or between acute exogenous ketosis conditions.

2.4.5 Cognitive performance

Baseline scores from Stroop Colour and Word Test [congruent and incongruent: mean reaction time (MRT) and mean correct reaction time (MRT-C)] and switching task [mean reaction time (MRT) and mean correct reaction time (MRT-C)] did not differ between the four experimental conditions (Table 2.1). No condition or time*condition interaction effects were

observed for either cognitive test at any time point compared to PLA or in between acute exogenous ketosis conditions.

Table 2.1. Cognitive outcomes

	Time			
	Baseline	**60 min post**	**120 min post**	***P* value**
Stroop incongruent RT(ms)				
PLA	703.1±138.7	695.1±145.2	648.7±136.5	T: P<0.001
KME	683.4±118.6	655.0±90.4	647.7±111.7	C: P=0.938
KME+BIC	683.8±165.9	669.9±182.3	648.5±151.4	T*C: P=0.034
KS	766.9±288.5	642.0±151.4	653.5±174.4	
Stroop incongruent correct RT (ms)				
PLA	703.9±142.8	697.0±140.0	649.0±138.8	T: P<0.001
KME	681.6±115.5	656.2±88.1	645.9±107.4	C: P=0.946
KME+BIC	679.7±160.9	671.0±183.5	646.4±148.5	T*C: P=0.078
KS	772.8±282.9	640.4±151.3	655.8±171.3	
Stroop Interference score				
PLA	20.7±8.2	22.1±7.4	24.7±8.7	T: P=0.001
KME	21.8±9.1	22.4±8.0	23.8±8.0	C: P=0.550
KME+BIC	22.9±6.3	24.0±9.5	24.2±7.4	T*C: P=0.015
KS	19.5±10.5	24.4±10.1	23.8±8.9	
Switching Task RT (ms)				
PLA	1365.6±335.5	1376.4±422.4	1300.6±351.5	T: P<0.001
KME	1507.6±477.4	1443.7±525.9	1376.6±460.7	C: P=0.844
KME+BIC	1473.8±437.7	1321.1±380.7	1264.8±345.5	T*C: P=0.013
KS	1471.3±396.4	1272.2±301.9	1324.1±350.8	
Switching Task CRT (ms)				
PLA	1374.8±392.4	1415.8±560.9	1339.6±467.4	T: P=0.001
KME	1544.5±593.2	1490.1±655.9	1424.2±620.1	C: P=0.855
KME+BIC	1503.1±545.4	1360.2±463.3	1285.0±389.6	T*C: P=0.055
KS	1464.0±400.3	1279.0±315.3	1382.8±493.4	

Data are presented as mean±SD, n=20. CRT: correct reaction time; RT: reaction time; KME: R-BD R-βHB ketone monoester; KME+BIC: R-BD R-βHB ketone monoester with sodium bicarbonate; KS: non-racemic ketone salt

2.5 DISCUSSION

EKS research has mainly focused on the application of KME and KS as an ergogenic supplement in the context of sports performance, predominately as an alternative metabolic fuel during aerobic exercise, although few studies show such benefits (Evans et al., 2022). A greater understanding of KME and KS pharmacokinetics (Stubbs et al., 2017), and their effect on pulmonary function (Dearlove et al., 2019) and blood gases (Dearlove et al., 2019; Prins et al., 2021) need further foundational work as applications and EK products are expanding.

This investigation was the first to evaluate the pharmacokinetics of a non-racemic KS, and the co-administration of KME with BIC during a prolonged resting state in both male and female populations. This work extends the only other pharmacokinetic study of EKS at rest, which include multiple doses of both R-BD R-βHB KME and a racemic KS (Stubbs et al., 2017), and provides important insights on differing responses to various EKS. Each EKS studied modulated metabolism as evidenced by increasing R-βHB to varying extents, and reducing blood glucose concentrations. Acute ingestion of these EKS altered blood gases (TCO_2 and PO_2) and pH, and when KME was co-administered with BIC, both HR and HRV responses were significantly altered. Although EK ingestion resulted in acute metabolic changes they had no impact on cognitive performance.

The absence of an effect of EKS on cognitive performance is consistent with previous work demonstrating that EKS do not alter cognitive performance at rest in healthy populations (Prins et al., 2020, 2021) without cognitive impairments (Fortier et al., 2021; Walsh et al., 2021). There is some evidence that KME ingestion can mitigate declines in cognitive performance in response to physiological stressors such as intense exercise performance (Evans & Egan, 2018; Poffé et al., 2023; Quinones & Lemon, 2022). It is likely that as reduction in cognitive performance in response to an external stressor is required before acute ingestion of EKS can

provide utility to mitigate these declines, and therefore maintain or improve cognitive performance.

The present study adds to the growing body of literature indicating that acute KME ingestion decreases pH at rest (Dearlove et al., 2020; McCarthy et al. 2023; Prins et al., 2021; Stubbs et al., 2017) whereas KS (Stubbs et al., 2017) and KME+BIC increases pH at rest (McCarthy et al., 2023). Consistent with previous findings under resting conditions, the decrease in pH in response to KME was accompanied by a decrease in TCO_2 in HCO_3^- (Prins et al., 2021). The TCO_2 concentrations increased at 60 and 120 min after KS and KME+BIC ingestion due to increases in HCO_3^-. PCO_2 remained unaltered.

In contrast to previous studies, (McCarthy et al. 2023; Prins et al., 2021) there were no observed effects on gas exchange parameters or RER in the present study. McCarthy et al., (2023) observed (in Supplemental Table 1) an increase in resting RER in response to both acute KME and KME+BIC ingestion compared to control group. These contrasting findings relating to pH, blood gases and respiration are potentially due to varying doses of both KME and BIC between studies. McCarthy et al., (2023) administered 600 mg/kg of KME and added 200 mg/kg of BIC for KME+BIC whereas the present used a dose of 395 mg/kg for KME and added 300 mg/kg of BIC for KME+BIC. Compared to McCarthy et al., the dosing strategy used in the present study provided less of an acid load for both KME and KME+BIC. There was also an enhanced acid buffering capacity in the present study during KME+BIC compared to the other investigation (McCarthy et al. 2023). This is supported by a lower pH, PCO_2 and a higher ketosis and V_E during rest in both KME and KME+BIC in the McCarthy et al., study compared to the present study. Interestingly, in both studies the average $PaCO_2$ for KME+BIC was the same as during CON/PLA, but KME conditions resulted in lower $PaCO_2$ in both studies compared to CON/PLA. $PaCO_2$ was significantly lower in McCarthy et al. (2023), highlighting that pH may be the primary mechanism for enhanced CO_2 offloading during exogenous ketosis.

Increasing ketosis in a dose-dependent manner has been shown to increase KB utilization in certain organs such as the brain (Courchesne-Loyer et al., 2017). It is therefore possible that more KBs are oxidized at higher compared to lower levels of ketosis under resting conditions, even though this does not appear to be the case during exercise (Dearlove et al., 2021). Increased oxidation of KBs would elevate RER at rest as the stoichiometry of AcAc, the final step in KB oxidation, is 1.00, i.e. similar to that of CHO, whereas the equivalent value for βHB is 0.89 (Frayn, 1983). The elucidation of the mechanisms altering gas exchange and blood gases at rest in response to varying doses of acute EK supplements, will allow for a more in-depth comparison to understand underlying mechanisms driving the enhanced CO_2 offloading of KME (McCarthy et al. 2023; Prins et al., 2021).

Two previous studies have shown an increase in resting HR following acute KME ingestion (McCarthy et al. 2023; Prins et al., 2021). In contrast, the present study is in agreement with (Dearlove et al., 2020) who found no difference in resting HR following acute KME ingestion. Conversely, KME+BIC resulted in an elevated HR 60 min after ingestion and HR remained elevated compared to PLA. KME+BIC was the only condition that significantly altered HRV, as it increased SNS index, and decreased PNS index, mean R-R, and RMSSD. These findings highlight the need for further resting studies of longer duration to allow for the analysis of multiple EKS at varying doses in order to better understand their effect on autonomic function.

A novel aspect of this investigation was the analysis the pharmacokinetics of a non-racemic KS, which was up taken more rapidly and to a higher peak concentration than a 50/50 racemic mixture (Stubbs et al., 2017). This novel finding requires further investigation into the efficacy of a non-racemic KS as an ergogenic aid due to the fact that it provides R-βHB close to the theorized range for sports performance (~2-3 mM) without reducing the acid buffering capacity prior to exercise. Multiple investigations have utilized racemic KS in attempt to

improve aerobic performance, found no effect or in some instances an impairment (O'Malley et al., 2017) in exercise performance (Clark et al., 2021; Rodger et al., 2017; Waldman et al., 2018). The lack of an ergogenic effect may be related to the low level of ketosis achieved with racemic KS consumption (<1.0 mM) with additional concerns of a high cation load with higher doses (Evans et al., 2022). Non-racemic mixtures that can reliably elicit levels of exogenous ketosis >2.0 mM and increase pH, therefore enhancing the acid buffering capacity prior to exercise could potentially enhance aerobic performance.

While each condition induced a level of ketosis, their effects on metabolite concentrations, pH, blood gases, and HRV varied between conditions. These data highlight that different dosing strategies and co-administration with buffering agents can affect the availability and potential oxidation of ketones and other metabolites. Inducing acute metabolic acidosis as the consequent ketosis affects respiration and the affinity of oxygen to hemoglobin (Coleman et al., 2021; Poffe et al., 2021) and may be used as a countermeasures to acute severe hypoxic exposure. However, inducing acute metabolic acidosis and decreasing the ability to buffer acid prior to intense exercise has repeatedly shown to have little ergogenic effect for physical performance (Evans et al., 2022). This has led to multiple investigations into KME+BIC to 'unlock' potential ergogenic effects of KME in sports performance with mixed results (Poffé et al., 2020, (Poffé et al., 2020a, 2020b). The increase in resting HR (~8 to 16 bpm) and alterations in PNS/SNS indexes in the present study is less than optimal for maximizing physical performance if the elevated HR persists during exercise. It is important to understand how each form of EKS modulates metabolism, HRV, pH balance, respiration, and level of ketosis in a resting state (and latterly, studies during exercise) to best apply to sports performance or therapeutic applications.

In conclusion, exogenous ketosis produced by the ingestion of KS or KME alone (~2-4 mM) does not affect indirect calorimetry, HRV, hemodynamics, or cognitive performance at

rest in young healthy males and females. Co-administration of KME+BIC increased the degree of ketosis (~0.5 mM), altered HRV, and increased pH but had no effects on indirect calorimetry, hemodynamics, or cognitive performance. The non-racemic KS produced a more rapid and higher peak concentration compared to previous studies. This novel finding warrants future work into comparing KME and non-racemic KS at similar concentrations of βHB to elucidate any further differences or best application for either form of EKS. Lastly, this study also included a 50% female population but was not powered to study between-sex differences and future work appropriately powered for comparison of between-sex differences is required.

Chapter 3
Exogenous ketone monoester ingestion attenuates declines in cognitive performance (code substitution task) and oxygen saturation during severe hypoxia

3.1. ABSTRACT

EKS are emerging as a new potential augmentation strategy for cognitive resilience during acute hypoxic exposure due to their capacity to attenuate the decline in O_2 availability, and potentially by providing an alternative substrate for cerebral metabolism. Utilizing a single-blind randomized crossover design, sixteen male military personnel performed multiple cognitive tests in three different breathing environments: room air (baseline), and normoxia (20 min; 0 m; 20.9%O_2) and hypoxia (20 min; 6096 m, 9.7%O_2) using a reduced O_2 breathing device (ROBD). The R-BD R-βHB ketone monoester (KME; 650 mg/kg, split dose) or taste-matched placebo (PLA) was ingested prior to normoxia and hypoxia exposures with ROBD. Blood R-βHB and glucose concentrations, cognitive performance, O_2 saturation (SpO_2), and heart rate were collected throughout. KME ingestion accomplished the expected rise in blood R-βHB concentration, which was rapid and sustained (>4 mM 30 min post; $P<0.001$) and accompanied by reduced blood glucose concentration (-21.3±12.4 mg/dL; $P<0.001$) vs. PLA. Declines in cognitive performance during a Defense Automated Neurobehavioral Assessment (DANA) code substitution task during hypoxic exposure were attenuated with KME ($P=0.047$). The decline in SpO_2 during hypoxic exposure was attenuated with KME vs. PLA (KME, 79.6±4.1%SpO_2; PLA, 75.3±5.0%SpO_2; $P=0.002$). KME elevated resting heart rate during normoxia (KME, 75.0±7.2 bpm; PLA, 66.9±9.8 bpm; $P=0.007$) and hypoxia (KME, 87.3±8.6 bpm; PLA, 82.1±9.1 bpm). Acute nutritional ketosis after ingestion of KME attenuated the decline in cognitive performance during severe hypoxic exposure, which coincided with attenuation of declines in O_2 saturation.

3.2 INTRODUCTION

Hypoxia can occur during altitude exposure or pathologically through cardiovascular disease, asthma, chronic obstructive pulmonary disease (COPD), or respiratory viral infections. The associated reduction in oxygen (O_2) availability diminishes mitochondrial respiratory rate and increases oxidative stress and inflammation (Pasiakos et al., 2021), and negatively impacts on multiple organ and tissue systems, including the brain and musculoskeletal system (Chaillou, 2018, Goodall et al., 2014, Michiels, 2004). Acute hypoxic exposure can lead to a decline in circulating hemoglobin O_2 saturation (SpO_2), affecting cerebral metabolism which can result in impaired cognitive function (Williams et al., 2019). Moderate-to-severe hypoxic exposure has been linked to acute disruptions in working memory (Legg et al., 2016, Malle et al., 2013), cognitive flexibility (Asmaro et al., 2013, Turner et al., 2015), attention (Asmaro et al., 2013, Stepanek et al., 2014, Turner et al., 2015), executive function (Turner et al., 2015), and auditory processing(Beer et al., 2017).

Countermeasures that attenuate or eliminate the effects of hypoxia may have far-reaching implications for those affected by, or at risk for, hypoxia. Attenuating declines in circulating and tissue O_2 concentrations is a promising target to alleviate the symptoms of acute hypoxia and mitigate the negative impacts of long-term hypoxia. For example, descending to a lower altitude and increasing breathing rate through hyperventilation during environmental hypoxia can alleviate acute altitude-related hypoxia symptoms (Shaw et al., 2021). Daily O_2 therapy has been demonstrated to improve cognitive performance and mortality rates in COPD patients with chronic pathological hypoxia (Karamanli et al., 2015, Pavlov et al., 2018). Thus, whether acute or chronic, alleviating hypoxia symptoms are most effectively achieved by increasing oxygen availability. Furthermore, the use of the carbonic anhydrase inhibitor acetazolamide, which induces mild acidosis and stimulates respiratory rate, has been demonstrated to raise O_2 saturation and attenuate acute mountain sickness (AMS) symptoms

(Wang et al., 2015). Although acetazolamide is the standard of care for treating AMS, and has been shown to decrease the incidence of AMS at high altitude, studies have also demonstrated negative effects of carbon anhydrase inhibitors on sub-maximal and maximal physical performance (Posch et al., 2018) and learning, memory, and attention (Sun and Alkon, 2002, Wang et al., 2013b). Treatments that are effective at low-to-moderate altitudes are also often ineffective in more severe hypoxic environments (Robach et al., 2008). These limitations demonstrate a clear gap for special populations, such as military aviation pilots, mountain warfare operators, rescue operational personnel, amongst others who must perform cognitively demanding missions at altitudes up to and, on occasion exceeding 6096 m (Shaw et al., 2021). Even minor degradation to these processes can meaningfully decrease the likelihood of success under these extreme conditions and can have severe consequences. Given these factors, there is a pressing need to find more effective and practical countermeasures for hypoxic exposure.

A ketogenic diet (KD), hallmarked by an elevation in circulating ketone bodies (KB) referred to as ketosis, has demonstrated the potential to function as a countermeasure for hypoxic conditions in preclinical models (Brownlow et al., 2017, Gom et al., 2021). However, the KD requires a multiweek adaptation timeline and has the potential for reduced compliance over time (Burke, 2021). Acute ingestion of KBs to induce ketosis through EKS may demonstrate a more practical option as they have been demonstrated to rapidly elevate blood *R*-β-hydroxybutyrate (*R*-βHB) without requiring lifestyle or dietary manipulations (Evans et al., 2022, Poff et al., 2020). My collaborators conducted an initial pilot evaluation which demonstrated the potential ability for the R-BD R-βHB KME to mitigate the effects of acute moderate hypoxic exposure (5029 m) (Coleman et al., 2021). However, military operators are often required to maintain high levels of cognitive performance across diverse tasks when rapidly deployed into severely hypoxic environments. Thus, the aim of the present study was to investigate the impact of acute ingestion of KME on cognitive resilience during severe

hypoxia (simulated 6096 m altitude; 9.7%O_2), coupled by assessments of blood oxygen saturation and metabolic, cardiac, and autonomic function.

3.3 METHODS

3.3.1 Participants

Twenty-three male military aviation students aged 18 to 35 y stationed at Naval Air Station Pensacola were recruited to participate in the study (25.3±2.4 y, 86.2±9.3 kg). Each participant provided written informed consent to participate after written and verbal explanations of the procedures in accordance with the protocol approved by the Florida Institute for Human and Machine Cognition Institutional Review Board. Inclusion criteria: (1) 18 to 35 years of age, (2) regular consumption of a typical American diet. Exclusion criteria: (1) history of smoking, (2) metabolic or cardiovascular disease, (3) orthopedic, musculoskeletal, neurological, psychiatric disorder, and/or any other medical condition that prohibited exercise, (4) habitual prescription medications, (5) EKS or following a low-carbohydrate or ketogenic diet, (6) ergogenic aids within one month of study participation.

Participants were instructed to refrain from caffeine for 12 h, alcohol consumption and training for 24 h, and arrive in an overnight fasted state before each experimental trial. Participants were instructed to maintain their usual training frequency throughout the study intervention. During the study, N=7 participants did not complete the protocol: three volitionally withdrew and four were administratively withdrawn for safety reasons (during severe hypoxia exposure, two dropped below pre-determined SpO_2 safety threshold (SpO_2 ≤58%) and two had attenuated responsiveness verified via verbal communication and eye responsiveness). All data from these participants were excluded from the final analysis. Data analyses were thus performed based on the N=16 participants who completed all experimental protocols.

3.3.2 Experimental Design

Utilizing a single-blind, placebo-controlled, randomized crossover design, participants visited the laboratory on 3 separate occasions over 10- to 16-d period, comprising one familiarization and two main experimental sessions with ≥48-h wash-out period between experimental sessions. During the first visit, participants were familiarized with the protocol, equipment, and all cognitive tests (code substitution task, RightEye oculometric test). The two, experimental sessions (visits 2 and 3) consisted of cognitive assessments during normoxia (Norm 1 and Norm 2) and hypoxia (Hypo 1 and Hypo 2)) on a reduced oxygen breathing device (ROBD) at rest (Figure 3.1). Visits 2 and 3 were identical in terms of pretest preparation for 24 h (no physical activity and participants instructed to replicate diet) and the experimental sessions. The visits differed only in the randomly-assigned drink consumed before undergoing cognitive testing during hypoxia, in the form of a volume, color and flavor-matched placebo (PLA) or R-BD R-βHB KME. Within participants, PLA vs KME were randomized for sessions 2 and 3. The primary outcome was cognitive performance with secondary outcomes including blood oxygen saturation, heart rate (HR), heart rate variability (HRV), acute mountain sickness (AMS) symptoms, and circulating βHB and glucose concentrations.

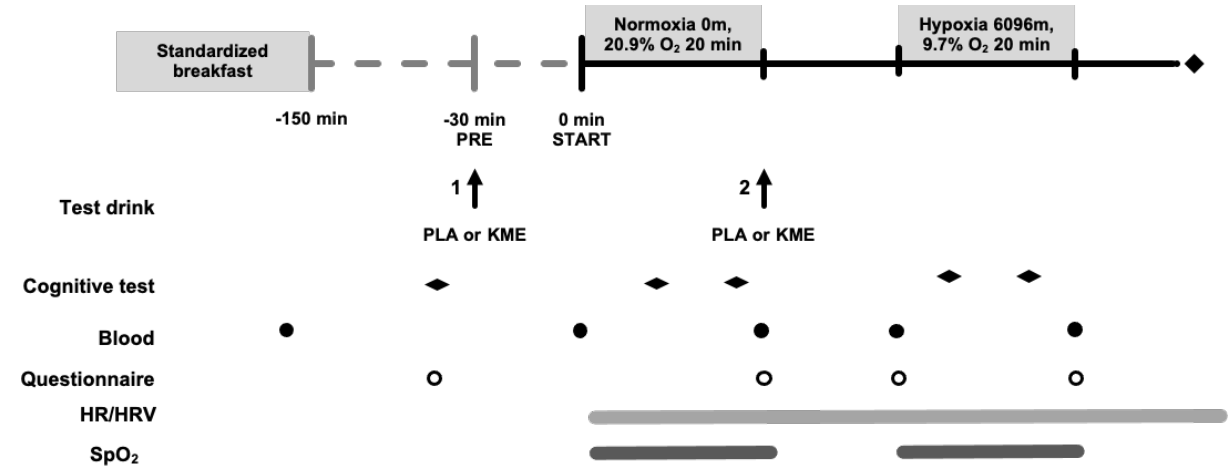

Figure 3.1. Study schematic

3.3.3 Cognitive assessments

At baseline, normoxia, and hypoxia, participants performed cognitive testing to assess cognitive performance. A familiarization test was performed during the first laboratory visit to reduce the possibility of a learning effect. All tests were performed in a sound-insulated room under controlled conditions (i.e., appropriate lighting, as quiet as possible, and isolation from unnecessary stimuli and timing feedback). Participants were instructed to complete the battery as quickly and accurately as possible. Each trial was administered identically.

Defense Automated Neurobehavioral Assessment (DANA)

Participants completed a simultaneous and delayed code substitution task on a self-directed tablet-based Defense Automated Neurobehavioral Assessment (DANA, AnthroTronix, inc, US) once at baseline and twice during each exposure (Norm 1 and Norm 2, Hypo 1 and Hypo 2) for five total timepoints during each experimental session. The simultaneous code substitution (CSS) was first, followed by delayed (CSD). For CSS, the participant refers to a code of 9 symbol-digit pairs displayed across the upper portion of the screen. A sequence of single symbol-digit pairs is shown below the key, and the participant indicates whether or not the single pair matches the code by pressing Yes or No. For CSD, the nine symbol-digit pairs are not displayed on the screen. However, the participant is still shown a sequence of single symbol-digit pairs and is asked to recall from memory if these are the correct pairs from the CSS session that just occurred, as the symbol-digit pairs change each timepoint. During this time, accuracy, reaction time (RT), correct reaction time (CRT) and cognitive efficiency (CE, correct responses per minute) were recorded for later analysis.

RightEye oculometric measurement

Participants completed an eye-tracking cognitive assessment (RightEye, Bethesda, MD, USA) once at baseline and twice during each experimental trial (Norm 1 and Norm 2, Hypo 1 and Hypo 2) for five total timepoints each session. At each timepoint participants were aligned

with the RightEye reading device and instructed to read a short prompt and answer Yes or No comprehension questions based on what they just read. During this time, accuracy, reading rate, blink rate and duration were all recorded for later analysis.

3.3.4 Experimental trials

After consent, participants underwent a familiarization visit involving practice of the cognitive tests and familiarization with the equipment. Dietary intake data for 24-h (recall) were collected and analyzed using the automated self-administered 24-hour (ASA24) Dietary Assessment Tool, version (2021), developed by the National Cancer Institute, Bethesda, MD, to ensure subjects were not currently consuming a ketogenic diet.

Participants arrived in the morning (0700-0800 h) in a fasted state, completed a behavioural compliance questionnaire, and were weighed. After a baseline blood sample was collected, participants were fed a standardized breakfast of a bagel (Kcal: 260, F:3g, C:48g, P:11g) with a single serving of peanut butter (Kcal: 250, F:21g, C:11g, P:9g). After consumption, participants rested for two hours before ingesting KME (Pure (Δ)G Ketone Ester H.V.M.N^{TM} Ketone; H.V.M.N, Inc., San Francisco, CA, United States) or PLA. During the rest period, participants completed a baseline cognitive testing session (DANA, RightEye). Participants were given five minutes at each timepoint to consume two separate drinks of KME or PLA each session. The first drink was administered 30 minutes prior to beginning the normoxia session. The second drink was administered after completing the normoxia session and 30 min prior to the hypoxia session. Doses of KME were based on body weight (drink 1: 500 mg/kg; drink 2: 125 mg/kg) while PLA was a taste, volume, and color matched non-caloric drink containing 2.6 mL of matched flavoring (HVMN Inc., San Francisco, CA, US), 0.87 mL of a bitter flavor stock (Bitrex, Edinburgh, Scotland), 0.13 mL of artificial sweetener (Truvia^{TM}, San Diego, CA, US), and 26 mL of water. This formulation was piloted to ensure effective blinding. Immediately after ingesting the study drink participants consumed approximately 20

mL of a calorie-free liquid water enhancer (Mio^{TM}, Kraft Heinz, Chicago, US) to remove any lingering flavor.

To ensure the participants and the researcher conducting the cognitive testing were blinded to the condition, the study drinks were prepared by the researcher who collected blood samples and served it in an unmarked paper cup. The participants and researcher assigned to collect cognitive data were blinded to the assigned condition. Only the researcher completing the ketone and glucose blood tests was aware of the participant's blinded drink assignment due to inability to blind blood ketone concentrations. KME and PLA data sets were coded during all data entry, QC, and statistical analyses.

Thirty minutes after the consumption of drink 1, a second blood sample was taken, after which the participants were attached to the ROBD and began the normoxia session (20 min at 20.9%O_2). Wearing the ROBD during the normoxia testing reduced any possible confounding influence of additional work of breathing due to the ROBD or physical distraction of the device. After 5 min of exposure, cognitive testing began (two sessions of RightEye followed by DANA). Once completed, the ROBD was removed, the second drink was administered followed by a 30 min rest period. A third blood sample was taken prior to beginning the hypoxic session (20 min at 9.7%O_2) on the ROBD. After 5 min of exposure, the cognitive testing began (two sessions of RightEye followed by DANA). Once completed, the ROBD was removed from the participant and blood sample 4 was recorded.

HR and HRV were measured (V800 polar, Polar, Kempele, Finland) throughout the normoxia and ROBD hypoxia sessions. Variables measured for future analysis included: high frequency (HF) power, low frequency (LF) power, very low frequency (VLF) power, LF/HF ratio, root mean square of successive RR interval differences (RMSSD), and mean R-R. Systemic oxygen saturation (SpO_2) was measured each second by pulse oximetry with the Nellcor Bedside Respiratory Patient Monitoring System (Covidien, Dublin, Ireland) placed

upon the forehead over the supraorbital artery during the normoxia and hypoxia ROBD sessions.

3.3.5 Blood sample analysis

Finger capillary blood was analyzed for R-βHB and glucose concentrations using a clinical grade point of care device (Precision Xtra, Abbott Diabetes Care Inc., Almeda, CA, United States). Blood samples were measured before KME supplementation (baseline), 30 minutes after supplementation (30 min), at the end of the normoxia session (normoxia), immediately before hypoxia (pre hypoxia), and after hypoxia (post hypoxia). Fingertip blood samples were collected using a lancet following alcohol cleaning. The first droplet was wiped away with a cotton swab, and the subsequent droplets were used for analysis.

3.3.6 Subjective measures of gastrointestinal symptoms and acute mountain sickness

At baseline, 30 min, post-normoxia, pre-hypoxia, and post-hypoxia, participants were asked to rate on a Likert scale (0-8, 0: no symptoms, 8: unbearable symptoms) gastrointestinal symptoms (heartburn, bloating, nausea, vomiting, intestinal cramps, abdominal pain, flatulence, diarrhea) as well as symptoms of acute mountain sickness (dizziness, headache, muscle cramp, urge to urinate).

3.3.6 Statistical analysis

Statistical analysis was performed using SAS (Statistical Software Suite, USA). This cross-over trial used descriptive statistics and paired differences within participants to assess evaluation components across the entire set of outcome measures. Mean, standard deviation (SD), missing values and/or dropouts were calculated. For key endpoints, analyses examined the order of presentation effects. Primary analyses involved testing the paired differences against a null hypothesis of no difference using a paired t-test with no adjustment for multiplicity. Additionally, delta change scores within treatment (Norm2 – hypo2) were compared across treatments utilizing paired t-tests. Repeated measures models were used to

assess the results, including partial results for participants dropping out during the study. Results were effectively unchanged when missing at random assumptions were employed, and no evidence of order effects was found; thus, these results are not presented. The threshold for statistical significance was set at $P \leq 0.05$ for all tests.

3.4 RESULTS

3.4.1 Blood concentrations of *R*-βHB and glucose

For baseline values prior to consuming study drinks, blood *R*-βHB and glucose concentration did not differ between the two experimental trial days (Figure 3.2). KME significantly increased blood *R*-βHB 30 min after ingestion (4.5±1.0 mM, P<0.001) compared to baseline and concentration remained elevated for all normoxia and hypoxia timepoints compared to PLA (P<0.001). KME ingestion significantly reduced blood glucose concentration 30 min after ingestion (-15.2±16.6 mg/dL, P<0.001) and throughout the normoxia and hypoxia exposures (P<0.001) compared to PLA, with the lowest value occurring just prior to hypoxia exposure (-21.4±12.4 mg/dL, P<0.001) (Figure 3.2).

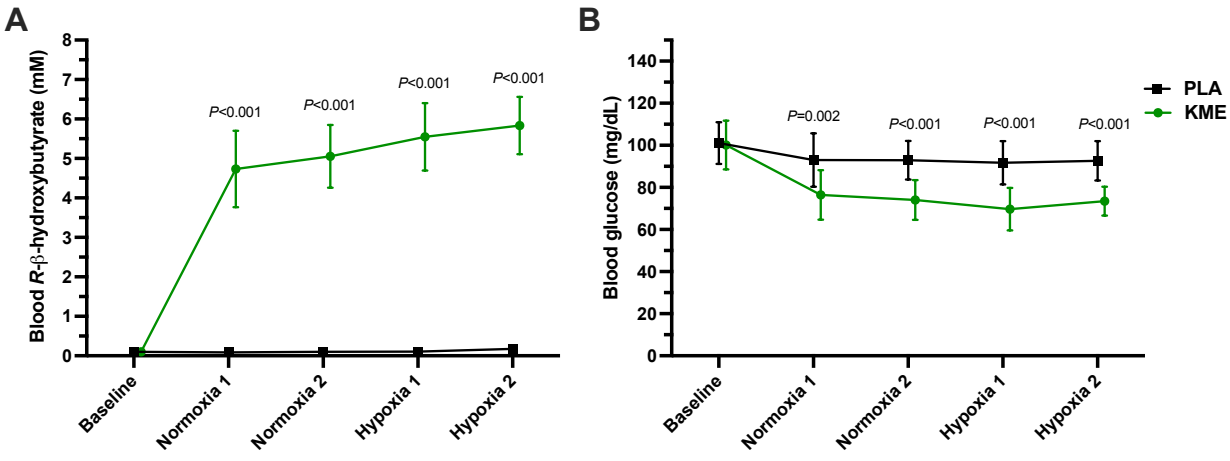

Figure 3.2. Blood *R*-β-hydroxybutyrate **(A)** and glucose **(B)** throughout baseline, normoxia, and hypoxia with exogenous ketone monoester (KME) or non-caloric taste matched placebo (PLA). n=16. Data are mean±SD.

3.4.2 Cognitive performance

DANA

No significant differences between treatments were found at baseline, Norm 1, Norm 2, or Hypo 1 for RT, CRT, CE, or accuracy for both CSS and CSD (Figure 3.3; Table 3.1). During Hypo 2, CE during CSD was significantly improved with KME leading to 4.1± 7.7 more correct responses per min compared to PLA (P=0.047) (Figure 3.3). From Norm 2 to Hypo 2, KME significantly attenuated the decline in CSD CE (+7.7±13.1 correct responses per min, P=0.031), and improved CSD CRT (-155.3±246.9 ms; P=0.023) and CSD RT (-146.7±248.6 ms; P=0.032) when compared to PLA across these timepoints (Figure 3.3; Table 3.1).

RightEye oculometric assessment

No significant differences occurred between treatments at baseline, normoxia, hypoxia, or from change from Norm 2 to Hypo 2 for accuracy, blink duration, blink rate and reading rate (Table 3.1).

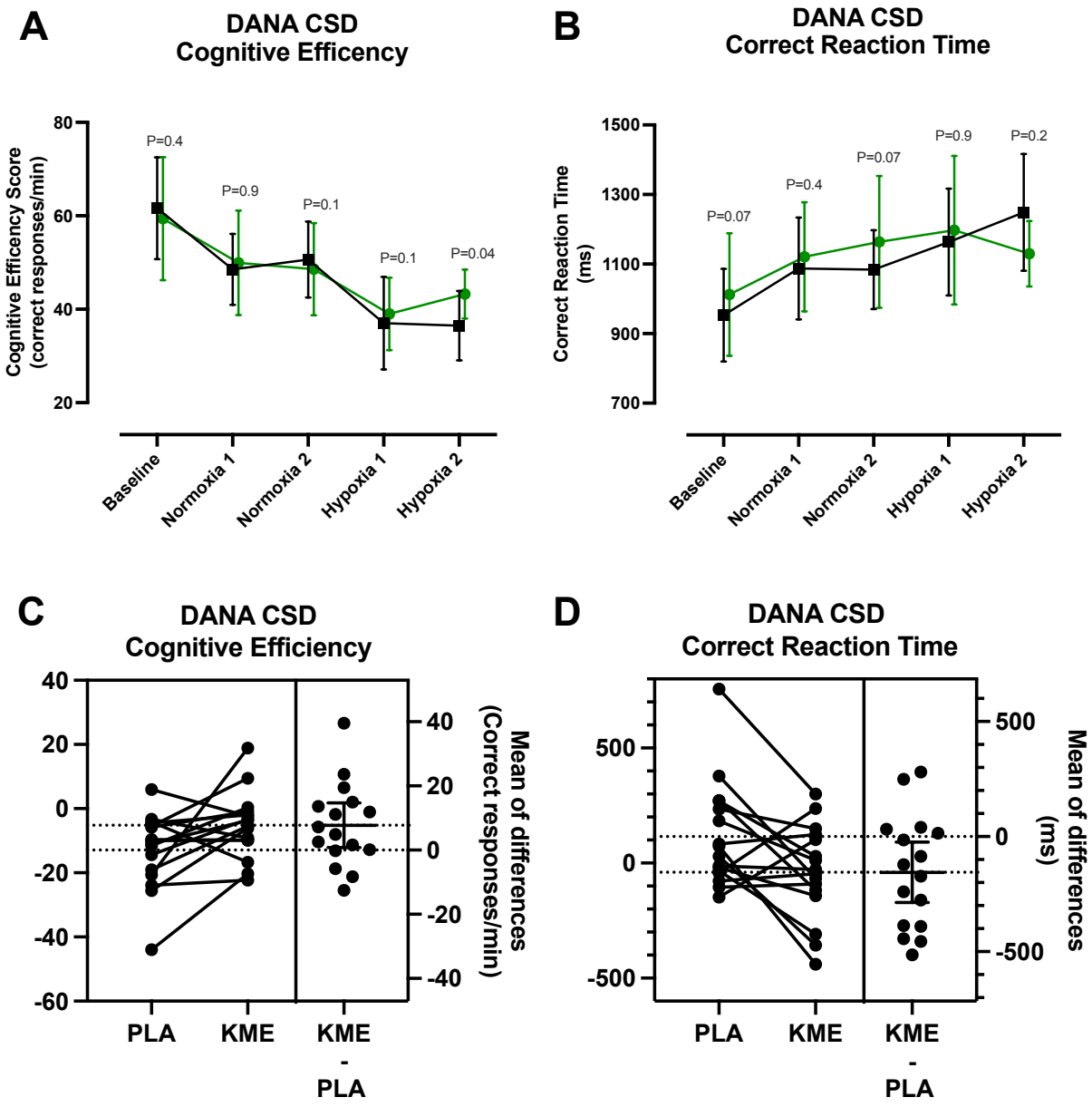

Figure 3.3. Cognitive performance. (A) DANA code substitution delayed (CSD) cognitive efficiency, and **(B)** DANA CSD correct reaction time, throughout baseline, normoxia 1-2, and hypoxia 1-2 with exogenous ketone monoester (KME) or non-caloric taste matched placebo (PLA)**.** Individual responses for Normoxia2-Hypoxia2 for **(C)** DANA code substitution delayed (CSD) cognitive efficiency (CE), and **(D)** DANA code substitution delayed (CSD) correct reaction time (CRT) individual responses (Normoxia2-Hypoxia2). n=16. Data are mean±SD.

Table 3.1. Cognitive outcomes

	Time point				
	Baseline	**Normoxia 1**	**Normoxia 2**	**Hypoxia 1**	**Hypoxia 2**
DANA CSD efficiency					

(Correct response/min)					
PLA	61.5±10.9	49.8±9.5	50.6±8.1	37.0±9.9	37.7±9.1
KME	59.4±13.1	49.9±11.1	47.0±11.6	40.2±9.3	41.8±7.7*
DANA CSD correct RT (ms)					
PLA	952.8±133.6	1087.2±146.4	1102.9±139.2	1203.6±230.8	1218.1±212.7
KME	1012.4±176.1	1120.7±157.1	1195.3±231.8	1197.2±213.8	1155.2±144.1
DANA CSS efficiency (Correct response/min)					
PLA	51.2±7.0	47.2±5.3	45.0±5.7	39.7±6.7	40.5±6.3
KME	50.7±9.8	46.7±7.3	43.7±7.8	42.4±7.8	41.3±7.0
DANA CSS correct RT (ms)					
PLA	1177.5±172.6	1261.0±140.6	1310.8±172.3	1454.3±252.6	1426.5±220.0
KME	1214.3±234.7	1288.6±191.2	1376.8±210.9	1382.5±225.5	1426.7±230.1
RightEye accuracy (%)					
PLA	87.5±13.4	95±7.3	96.2±5.0	83.7±14.5	94.3±12.6
KME	86.8±7.9	95±7.3	96.6±4.8	87.5±10	93.7±8.0
RightEye blink rate (blink/min)					
PLA	6.5±4.3	4.5±2.3	4.9±3.1	6.9±5.3	5.0±3.8
KME	6.5±6.5	5.0±3.5	5.4±3.5	5.3±5.9	4.0±2.7
RightEye blink duration (ms)					
PLA	8.0±5.9	13.6±12.5	11.3±10.3	13.8±11.8	12.6±9.1
KME	7.7±6.8	12.3±13.3	13.8±11.8	12.3±10.3	18.4±15.2
RightEye reading rate (words/min)					
PLA	179.6±50.8	197.8±52.4	199.5±62.1	160.5±57.5	166.4±55.4
KME	157.3±35.5	197.8±84.7	196±68.7	158.4±55.4	191.5±66.5

Data are presented as mean±SD, n=16; *P<0.05. CSD, Code Substitution Delayed; CSS, Code Substitution Simultaneous; DANA, Defense Automated Neurobehavioral Assessment; RT, reaction time.

3.4.3 Oxygen saturation, HR, and HRV

SpO_2 was not significantly different between treatments during normoxia. KME significantly attenuated the decline in SpO_2 during the hypoxic exposure compared to PLA (+4.2±3.4%SpO_2, P=0.002) (Figure 3.4). Heart rate was significantly elevated in KME during the normoxia session (+8.0±7.8 bpm, P=0.007) compared to PLA and remained directionally elevated during hypoxia exposure compared to PLA (+5.2 ±11.1 bpm, NS) (Figure 3.4). Mean

R-R was significantly lower in KME treatment across both normoxia (P<0.001) and hypoxia sessions (P<0.001) (Table 3.2). Of note, for SpO_2 and HR measurements, technical difficulties during data acquisition resulted in only n=12 in the final analysis of these secondary outcomes.

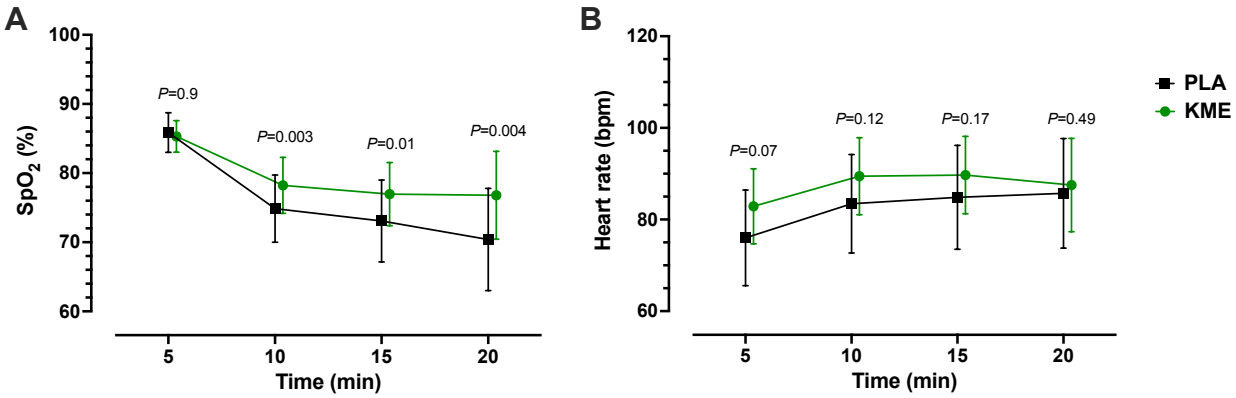

Figure 3.4. Cardiorespiratory responses. (A) Oxygen saturation and **(B)** heart rate during hypoxic exposure broken into 5 minute segments with exogenous ketone monoester (KME) or non-caloric taste matched placebo (PLA). n=12. Data are mean±SD.

Table 3.2. HRV outcomes

	Normoxia	Hypoxia
R-R interval (ms)		
PLA	945.3.9±145.8	765.7±99.9
KME	831.9±110.5*	691.1±61.0***
RMSSD (ms)		
PLA	79.1±35.4	58.3±46.7
KME	98.0±96.3	45.1±29.8

Data are presented as mean±SD, n=16. *P<0.05; ***P<0.001. RMSSD, root mean square of successive differences between heartbeats; R-R interval, the time elapsed between two successive R waves of the QRS signal.

3.4.4 Gastrointestinal and AMS symptoms

Subjective ratings of heartburn, bloating, nausea, vomiting, intestinal cramps, abdominal pain, flatulence, diarrhoea, dizziness, headache, and muscle cramps were not different across treatments in normoxia and hypoxia(Table 3.3).

Table 3.3. Gastrointestinal and Acute Mountain Sickness Symptom questionnaire

	Time point			
	Baseline	**30 min after first dose**	**30 min after second dose**	**After hypoxia**
Heartburn				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0±0.0	0.1±0.2	0±0.0	0±0.0
Bloating				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0.1±0.5	0.1±0.5	0.1±0.2	0±0.0
Nausea				
PLA	0±0.0	0±0.0	0±0.0	0.1±0.2
KME	0±0.0	0.1±0.2	0.1±0.7	0.2±0.7
Vomiting				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0	0±0.0
Intestinal Cramps				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0	0±0.0
Abdominal pain				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0	0±0.0
Flatulence				
PLA	0.1±0.2	0.1±0.8	0.1±0.2	0.1±0.2
KME	0.1±0.2	0±0.0	0±0.0	0±0.0
Diarrhea				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0	0±0.0
Dizziness				
PLA	0±0.0	0±0.0	0±0.0	0.7±1.2
KME	0±0.0	0.2±0.5	0.2±0.5	0.4±0.7
Headache				
PLA	0±0.0	0±0.0	0.1±0.5	0.8±1.3
KME	0±0.0	0.2±0.5	0.3±0.6	0.4±0.7
Muscle cramp				
PLA	0±0.0	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0	0±0.0
Urge to urinate				
PLA	1.1±1.3	0.3±0.8	0.5±0.9	0.5±0.9
KME	0.5±1.0	0.3±0.7	0.3±0.6	0.2±0.5

Data are presented as mean±SD, n=16.

3.5 DISCUSSION

The present study investigated whether acute ingestion of an EKS in the form of the R-BD R-βHB KME could affect cognitive performance in young men exposed to severe hypoxia (simulated 6096 m altitude; 9.7%O_2). KME rapidly elevated *R*-βHB and reduced blood glucose

concentrations throughout the testing day. KME induced a parasympathetic withdrawal in both normoxia and hypoxia exposures resulting in elevated HR and an attenuation in the decline in SpO_2 during hypoxic exposure. These physiologic changes induced by KME were associated with attenuation in the decline in cognitive performance in the latter stages of hypoxia exposure.

The pattern of cognitive performance parameters across conditions with and without KME shows a consistent pattern of effects. When participants were at the maximum level of hypoxic stress (hypoxia 2) cognitive performance across treatment conditions showed clear divergence inferring a prophylactic effect against declines in cognitive performance resulting from acute hypoxic exposure. In fact, while in the KME condition, CSD reaction time for correct responses demonstrated a 155 ms advantage during the hypoxia 2 session when compared to their performance in the PLA condition leading to 7.7 more correct responses per minute when the hypoxia stress was the highest. These results are consistent with observations of cognitive resilience during or following acute stress with KME (Coleman et al., 2021, Evans and Egan, 2018, Murray et al., 2016, Poffé et al., 2023) and at rest in obese subjects (Walsh et al., 2021a).

KME did not confer an improvement in cognitive performance in normoxic conditions suggesting an acute stress was required to observe any effect of KME on cognitive performance, which was also previously observed (Prins et al., 2021). Other supplemental ketone forms have noted mixed results. Prins et al., observed cognitive enhancement effects at rest using an exogenous ketone formulation of βHB salts and medium chain triglyceride (MCT) (Prins et al., 2020a); whereas another study of βHB salts showed no effect during or after exercise (Waldman et al., 2018). The results of the present study along with study findings on EKS across rest and stress, as well as diverse ketone formulations, suggests a potential interaction between exogenous ketone composition and stress/pathological conditions, as well as divergent mechanisms on cognitive performance across different exogenous ketone formulations (Poff et

al., 2020, Poff et al., 2021, Stubbs et al., 2020). The proposed mechanism for ergogenic effects of KME on cognitive performance have been suggested to be via altered substrate availability (Cunnane et al., 2020), elevated BDNF (Walsh et al., 2020a), heightened cerebral blood flow (Walsh et al., 2021a) and/or enhanced brain network stability (Mujica-Parodi et al., 2020).

The effect of KME to maintain higher cognitive efficiency and faster reaction time during hypoxic exposure is likely to be elicited through maintaining a higher O_2 saturation, given that cognitive performance during hypoxia exposure is strongly correlated to SpO_2 saturation (Ochi et al., 2018) and cerebral metabolic rate (Subudhi et al., 2007). The present study adds to the growing body of literature supporting the ability of acute nutritional ketosis to attenuate the decline in SpO_2 saturation by ~3-6% during hypoxic exposures at varying altitudes in populations at rest and during exercise (Coleman et al., 2021, Poffé et al., 2021b, Prins et al., 2021). We postulate that this enhanced ability to maintain SpO_2 during hypoxic exposure is mechanistically driven by the acid-base balance response to a rapid influx of KBs. An acute increase in circulating KBs has been previously demonstrated to increase HR and hydrogen ion load, leading to decreases in circulating bicarbonate and ultimately pH (Poffé et al., 2021b, Prins et al., 2021). Decreases in pH and O_2 availability stimulate pulmonary vasoconstriction and lead to hyperventilation (Dunham-Snary et al., 2017, Leaf and Goldfarb, 2007). This response allows for greater O_2 uptake and offloading of additional CO_2 formed from the bicarbonate buffering system (Dunham-Snary et al., 2017).

Interestingly, the leading treatment of hypoxic exposure is acetazolamide, which also elicits acute metabolic acidosis, although this treatment leads to lower cognitive performance when compared to PLA (Wang et al., 2013b). While both induce acute metabolic acidosis to counteract declines in SpO_2, acute ingestion of KME provides a useable metabolic substrate that has been reported to be more energy-efficient than glucose, potentially allowing for a greater O_2 “sparing” effect (Evans et al., 2022). In contrast, acetazolamide uses a carbonic

anhydrase inhibitor to shift pH into metabolic acidosis without the benefit of an alternative fuel substrate such as KBs that may help to maintain a high rate of cerebral metabolism (Leaf and Goldfarb, 2007). A recent evaluation of KME during altitude ascension combined with exercise suggests greater systemic and muscular O_2 uptake but no impact on physical performance (Poffé et al., 2021b). In contrast, the present suggest the SpO_2 advantage does translate to augmenting cognitive performance in the resting state. However, this SpO_2 advantage did not translate into reduced acute mountain sickness symptoms as seen with acetazolamide administration during rapid ascents (Toussaint et al., 2021). Future studies should consider addressing whether KME may be a superior treatment strategy to acetazolamide or alternative countermeasures at offsetting hypoxia-induced performance declines without negatively impacting cognitive and physical performance domains during altitude exposure.

While hypoxia occurs most prominently during altitude exposure, asthma, COPD (Sarkar et al., 2017), and respiratory viral infections (Huang et al., 2021), all can result in hypoxia and associated symptoms. Interestingly, both diet- and EKS-induced ketosis have been found to relieve asthma in pre-clinical models (Mank et al., 2022). A recent case report demonstrated remarkable improvements in COPD phenotype following diet-induced ketosis (Norwitz et al., 2021). Hypothetical benefits of KBs have been proposed across multiple domains of respiratory viral infections (Stubbs et al., 2020); some of which were subsequently demonstrated using diet-induced ketosis in pre-clinical models of coronavirus (Ryu et al., 2021). Inflammation, oxidative stress, and subsequent cognitive decline occur in conditions like asthma and COPD, yet emergent preclinical evidence (Mank et al., 2022) and case reports (Norwitz et al., 2021) suggest some utility of KBs in such conditions. The effects are suggested to be a consequence of KBs acting as anti-inflammatory, antioxidant, and epigenetic modifiers (Poff et al., 2020, Stubbs et al., 2020); although further investigations are required to understand these potential therapeutic applications for KBs.

There are a few limitations in the present study. While we observed metabolic, cognitive, oxygen saturation, autonomic, and heart rate differences in military personnel exposed to severe hypoxia with KME administration, whether these results will translate to females or to individuals who are less active is not known. Capillary blood samples were conducted with point-of-care devices and may not accurately reflect venous circulation values by laboratory-based measures (Evans et al., 2022). Collection of blood gases to confirm acidic state would be necessary in order to confirm the hypothesized mechanism of action, and unfortunately due to technical failures, the SpO_2 results are in a subset (12 of 16, or 75%) of participants. While we assessed normobaric hypoxia, hypobaric hypoxia may also result in physiological differences, which we were not able to assess in the present design (Coppel et al., 2015). Furthermore, due to the length and severity of the hypoxia exposure it was deemed best practice to remove the ROBD prior to administering the final GI/AMS questionnaire, which may have affected the self-reported hypoxia symptoms at that timepoint.

In conclusion, the attenuated decline in oxygen saturation and apparent parasympathetic withdrawal during severe hypoxia for KME compared to PLA were associated with attenuation of declines in cognitive performance associated with hypoxic exposure. These data suggest an effect of KME to attenuate declines in SpO_2 and improve cognitive resilience, and therefore that KME may be a viable option to mitigate both environmental and pathological hypoxia exposure (Stubbs et al., 2020, Shaw et al., 2021).

Chapter 4
Exogenous ketone monoester ingestion attenuates declines in cognitive performance (psychomotor vigilance test) during severe hypoxia

4.1 ABSTRACT

EKS are emerging as a new potential augmentation strategy for cognitive resilience during acute hypoxic exposure due to their capacity to attenuate the decline in O_2 availability, and potentially by providing an alternative substrate for cerebral metabolism. Utilizing a single-blind randomized crossover design, sixteen healthy males (age 24.6±2.8 y, height 1.78±0.08 m, body mass 82.3±11.2 kg) performed a cognitive tests in the form of the psychomotor vigilance test (PVT*)* in three different breathing environments: room air (baseline), and normoxia (15 min; 0 m; 20.9%O_2) and hypoxia (15 min; 6096 m, 9.7%O_2) using a reduced O_2 breathing device (ROBD). The R-BD R-βHB ketone monoester (KME; 500 mg/kg, split dose) or taste-matched placebo (PLA) was ingested prior to normoxia and hypoxia exposures with ROBD. Blood R-βHB and glucose concentrations, cognitive performance, O_2 saturation (SpO_2), and heart rate were collected throughout. KME ingestion accomplished the expected rise in blood R-βHB concentration, which was rapid and sustained (>3 mM; P<0.001) and accompanied by reduced blood glucose concentration (-22.1±13.7 mg/dL; P<0.001) compared to PLA. During hypoxia exposure, KME significantly attenuated the hypoxia-induced effects on PVT in mean RT (-27.8±29.5 ms; P<0.01), lapses over 500 ms (-2.4± 4.1 lapses; P<0.05), mean reciprocal RT of slowest 10% (-27.9±37.0 1 ms; P<0.01), mean RT of fastest 10% (-13.7±13.3 ms; P<0.01), and total errors (1.0±1.7 errors; P<0.05) when compared to PLA. KME induce parasympathetic withdrawal and increased heart rate during the normoxia session (+6.6±7.4 bpm, P<0.01) and hypoxia session (+10.6±7.4 bpm, P<0.001) compared to PLA. Acute nutritional ketosis after the ingestion of the R-BD R-βHB KME attenuated the decline in cognitive performance in the form of attention assessed by psychomotor vigilance test induced by acute severe hypoxic exposure.

4.2 INTRODUCTION

In addition to the cognitive domains studied in Chapter 3 (working memory and executive function), attention is another cognitive domain on which hypoxic exposure may have a beneficial effect (Bliemsrieder et al., 2022). The psychomotor vigilance test (PVT) is a commonly used attention and vigilance test that is often used in studies of sleep deprivation (Lim & Dinges, 2008). While it is administrable at different lengths (3 min, 5 min and 10 min), the 10 min PVT test is most sensitive to disturbances in cognitive performance and therefore was chosen for this study (Basner & Dinges, 2011). Additionally, the PVT has been used in previous studies of real world hypoxic exposures that allowed for acclimatization, demonstrating impairments at altitudes of 3800 m (Frost et al., 2021) and 5050 m (Pun et al., 2018). It has not previously been evaluated during a laboratory-based study using NH.

Chapter 3 investigated the impact of severe normobaric hypoxia on working memory (code substitution task) and executive function (Stroop). This Chapter is a follow-up to Chapter 3. A randomized cross-over trial in a entirely separate participant cohort utilizing a reduced KME dosing strategy (drink 1: 350 mg/kg; drink 2: 150 mg/kg) and a 25% shorter hypoxic exposure (15 min vs. 20 min at 6096 m) was undertaken to assess neurobehavioral performance and sustained vigilance attention at baseline, normoxia and hypoxia and the utility of R-BD R-βHB KME to mitigate any declines, if present. The aims of this investigation were: (i) to evaluate the effects of KME on PVT at rest, prior to exposure to NH, (ii) to evaluate PVT performance during severe NH and (iii) to investigate if a lower dose of KME can attenuate declines oxygen saturation (SpO_2) and PVT performance, if declines are observed.

4.3 METHODS

4.3.1 Participants

Twenty healthy males aged 18 to 35 y were recruited to participate in the study (age 24.6±2.8 y, height 1.78±0.08 m, body mass 82.3±11.2 kg). Each participant provided written informed consent to participate after written and verbal explanations of the procedures in accordance with the protocol approved by the Florida Institute for Human and Machine Cognition Institutional Review Board aligned with the Declaration of Helsinki. Inclusion criteria: (1) 18 to 35 years of age, (2) regular consumption of a typical American diet. Exclusion criteria: (1) history of smoking, (2) metabolic or cardiovascular disease, (3) orthopedic, musculoskeletal, neurological, psychiatric disorder, and/or any other medical condition that prohibited exercise, (4) habitual prescription medications, (5) use of EKS or following a low-carbohydrate or ketogenic diet, (6) taking ergogenic aids within one month of study participation.

Participants were instructed to refrain from caffeine for 12 h, alcohol consumption and training for 24 h, and arrive in an overnight fasted state before each experimental trial. Participants were instructed to maintain their usual training frequency throughout the study intervention. During the study, n=4 participants did not complete the protocol: two volitionally withdrew and two were administratively withdrawn for safety reasons (during severe hypoxia exposure, two dropped below pre-determined SpO_2 safety threshold of ≤58% and two had attenuated responsiveness verified via verbal communication and eye responsiveness. All data from these participants were excluded from the final analysis. Data analyses were thus performed based on n=16 participants who completed both hypoxia experimental protocols.

4.3.2 Experimental design

This study followed the same experimental design and statistical approach as the previous investigation (Chapter 3) except for differences in R-BD R-βHB KME administered (1^{st} drink: 350 mg/kg; 2^{nd} drink: 150 mg/kg), and exposure time (15 min per condition), and was performed in a cohort of participant with similar profile, but entirely distinct from Chapter

3. Additionally, the cognitive test was changed to a 10 min PVT administered once during baseline, normoxia and hypoxia (Figure 4.1).

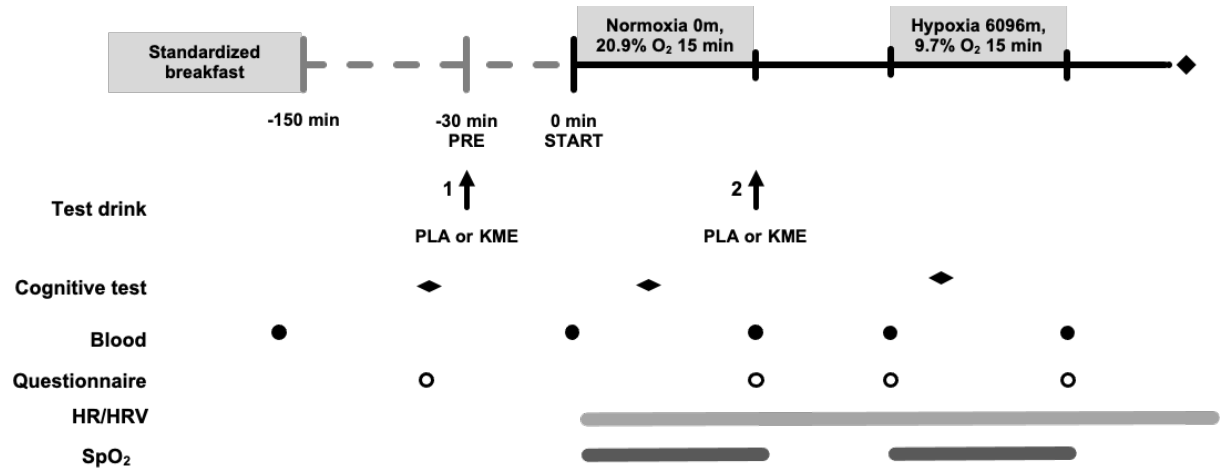

Figure 4.1. Experimental design schematic

The PVT is a simple and validated portable reaction time (RT) task to assess neurobehavioral performance and sustained vigilance attention (Dinges & Powell, 1985), an operator-relevant function not assessed in the previous study. The participant is instructed to press a button as soon as the stimulus appears (LED-digital counter). The duration of a single PVT was 10 min and the inter-stimulus interval varied randomly from 2 to 10 s. The PVT was assessed for mean RT, lapses over 500 ms, mean reciprocal reaction time (RRT) of slowest 10%, mean RT of fastest 10%, and total errors.

All other experimental design features (e.g. standardisation of preparation), measurements (e.g. anthropometry) and analysis (blood concentrations) were identical to Chapter 3).

4.3.3 Statistical analysis

Statistical analysis was performed using SAS (Statistical Software Suite, USA). This cross-over trial used descriptive statistics and paired differences within participants to assess

evaluation components across the entire set of outcome measures. Mean, standard deviation (SD), missing values and/or dropouts were calculated. For key endpoints, analyses examined the order of presentation effects. Primary analyses involved testing the paired differences against a null hypothesis of no difference using a paired t-test with no adjustment for multiplicity. Additionally, delta change scores within treatment (Norm2 – Hypo2) were compared across treatments utilizing paired t-tests. Repeated measures models were used to assess the results, including partial results for participants dropping out during the study. Results were effectively unchanged when missing at random assumptions were employed, and no evidence of order effects was found; thus, these results are not presented. The threshold for statistical significance was set at $P \leq 0.05$ for all tests.

4.4 RESULTS

4.4.1 Blood concentrations of *R*-βHB and glucose

For baseline values prior to consuming study drinks, blood *R*-βHB and glucose concentration did not differ between the two experimental trial days. KME significantly increased blood *R*-βHB 30 min after KME ingestion (3.4±0.8 mM, P<0.001) compared to baseline and remained elevated for all normoxia and hypoxia timepoints compared to PLA (P<0.001). KME ingestion significantly reduced blood glucose concentration 30 min after ingestion (-18.1±6.3 mg/dL, P<0.001) and throughout the normoxia and hypoxia exposures (P<0.001) compared to PLA, with the lowest value occurring just after the normoxia session (-22.1±13.7 mg/dL, P<0.001) (Figure 4.2).

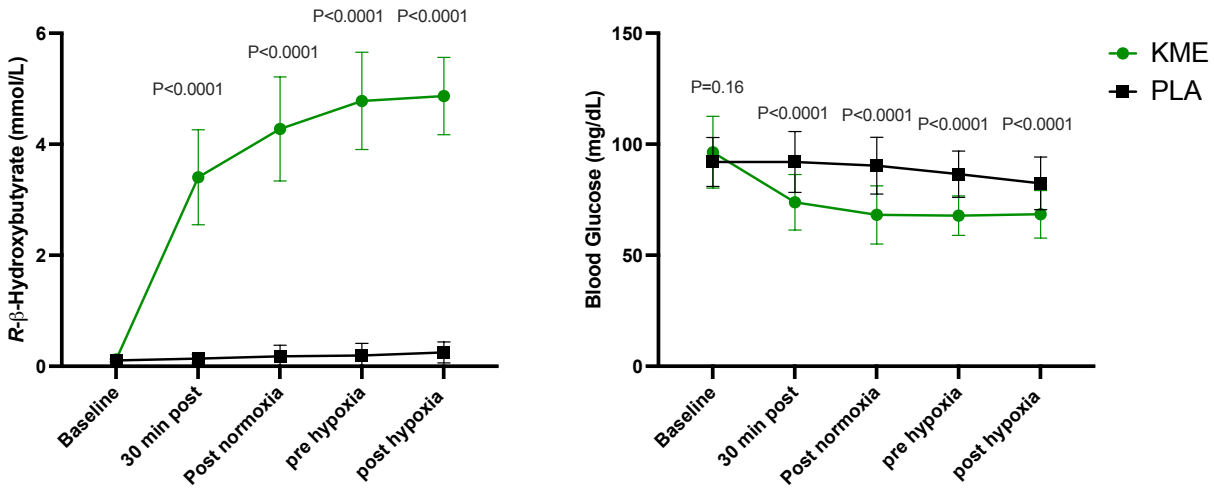

Figure 4.2. Blood metabolites. Blood *R*-βHB (A) and glucose (B) concentration throughout baseline, normoxia, and hypoxia with exogenous ketone monoester (KME) or non-caloric taste matched placebo (PLA). n=16. Data are mean±SD.

4.4.2 Cognitive performance assessed via PVT

No significant differences between treatments were found at baseline, normoxia, or hypoxia for mean RT, lapses over 500 ms, mean reciprocal RT of slowest 10%, mean RT of fastest 10%, and total errors. During hypoxia exposure, KME significantly attenuated the hypoxia-induced effects on mean RT (-27.8±29.5 ms; P<0.01), lapses over 500 ms (-2.4± 4.1 lapses; P<0.05), mean reciprocal RT of slowest 10% (-27.9±37.0 1 ms; P<0.01), mean RT of fastest 10% (-13.7±13.3 ms; P<0.01), and total errors (1.0±1.7 errors; P<0.05) when compared to PLA (Figure 4.2; Table 4.1).

Table 4.1. Psychomotor Vigilance Test outcomes

	Time point		
	Baseline	**Normoxia**	**Hypoxia**
Mean RT (ms)			
PLA	249.5±31.2	279.6±38.6	343.9±78.6
KME	257.0±34.6	277.3±37.7	316.0±79.9**
Lapse > 500ms (ms)			
PLA	0.6±1.4	1.5±1.9	7.0±8.6
KME	1.1±1.7	0.9±1.2	4.6±8.1*
Mean reciprocal RT (slowest 10%) (1/ms)			
PLA	2.9±0.4	2.4±0.4	1.9±0.5
KME	2.7±0.5	2.5±0.4	2.2±0.5**
Mean RT (fastest 10%) (ms)			
PLA	196.3±18.2	204.6±25.0	225.1±35.3
KME	194.4±14.4	204.5±18.6	211.4±37.6***
Total error (#)			
PLA	1.3±1.4	1.0±1.0	2.9±2.2
KME	1.5±1.4	0.5±0.8	1.9±1.4*

Data are presented as mean±SD, n=16. *P<0.05; **P<0.01; ***P<0.001. RT = reaction time

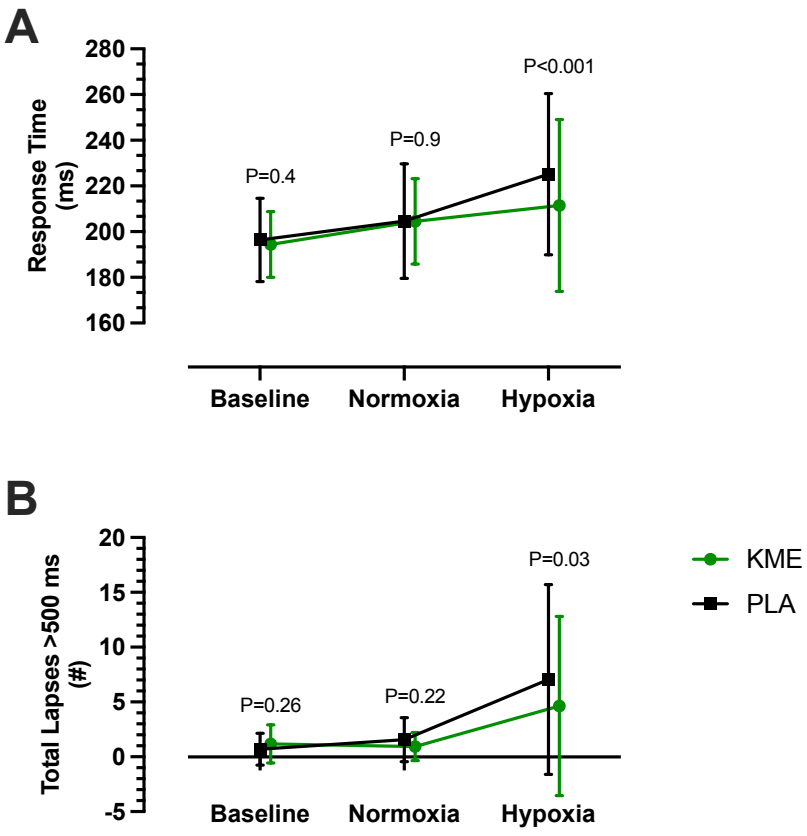

Figure 4.3. Cognitive Performance During Psychomotor Vigilance Test (PVT). (A) PVT fastest 10% reaction time **(B)** PVT lapses >500 ms throughout baseline, normoxia, and hypoxia with exogenous ketone monoester (KME) or non-caloric taste matched placebo (PLA). n=16. Data are mean±SD.

4.4.3. Oxygen saturation, HR, and HRV

While SpO_2 was measured during both sessions, there were technical difficulties with the post-hoc analysis. Heart rate was significantly elevated in KME during both the normoxia session (+6.6±7.4 bpm, P<0.01) and hypoxia sessions (+10.6±7.4 bpm, P<0.001) compared to PLA (Figure 4.4). During normoxia, mean RR (P<0.001), RMSSD (P<0.05), SNS (P<0.05) and PNS (P<0.01) indexes were all significantly different compared to PLA. During hypoxia, mean RR (P<0.001), RMSSD (P<0.05), SNS (P<0.001) and PNS (P<0.001) indexes were all significantly different than PLA(Table 4.2).

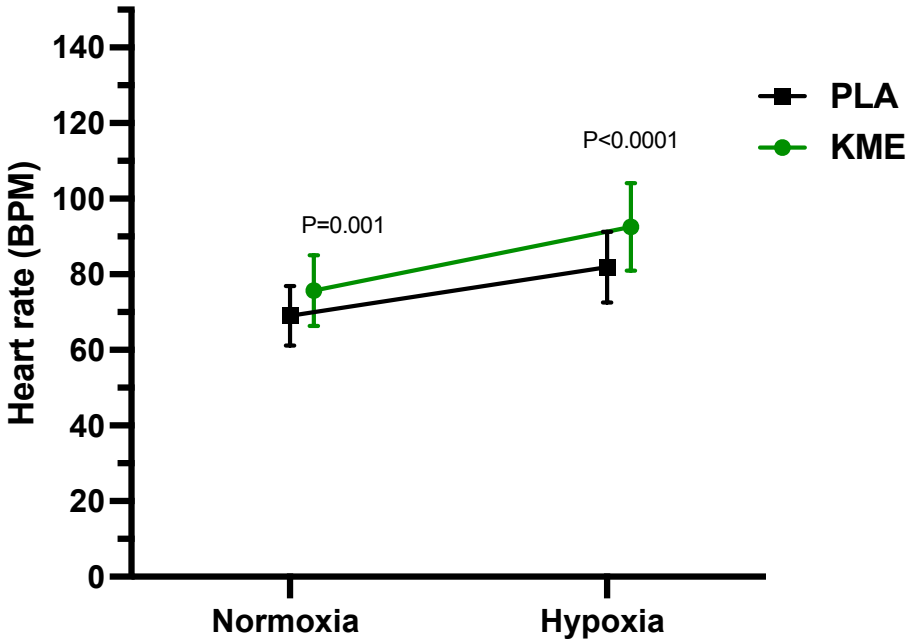

Figure 4.4. Heart rate (HR) during normoxia and hypoxia at rest. with exogenous ketone monoester (KME) or non-caloric taste matched placebo (PLA). n=16. Data are mean±SD.

Table 4.2. Heart rate variability (HRV) outcomes

	Time	
	Normoxia	**Hypoxia**
R-R interval		

(ms)		
PLA	880.1±101.6	742.1±80.9
KME	804.2±96.4***	657.75±79.7***
RMSSD (ms)		
PLA	69.1±24.5	42.8±19.6
KME	58.3±24.5*	31.7±22.4*
Stress index (AU)		
PLA	5.9±1.8	9.2±2.9
KME	7.2±3.0*	13.0±6.3*
SNS Index (AU)		
PLA	-0.3±0.6	1.0±0.9
KME	0.3±0.9**	2.4±1.6**
PNS Index (AU)		
PLA	0.5±0.9	-0.8±0.8
KME	-0.1±0.9**	-1.5±0.9***

Data are presented as mean±SD, n=16..***P<0.001, **P<0.01, *P<0.05 for KME vs PLA

4.4.4. Gastrointestinal and AMS symptoms

Subjective ratings of heartburn, bloating, nausea, vomiting, intestinal cramps, abdominal pain, flatulence, diarrhoea, dizziness, headache, and muscle cramps were not different across treatments.

4.4 DISCUSSION

The present study investigated the effect of acute ingestion of an EKS in the form of the R-BD R-βHB KME on performance during a PVT in young men during acute exposure to severe hypoxia (simulated 6096 m altitude; 9.7%O_2). KME rapidly elevated *R*-βHB and reduced blood glucose concentrations throughout the test period. KME induced a parasympathetic withdrawal in both normoxia and hypoxia exposures resulting in elevated heart rate. The physiologic changes induced by KME were associated with attenuation in the decline in cognitive performance in the latter stages of hypoxic exposure.

This study extends the previous findings of Chapter 3 on the utility of KME as a countermeasure to cognitive declines during hypoxic exposure. Additionally, the study is the first to demonstrate a negative impact of NH on vigilance in a laboratory based trial. Previous studies have been conducted only in-field (Falla et al., 2021b, Latshang et al., 2013, Frost et al., 2021, Pun et al., 2018). Moreover, the "altitude" exposure was 1000 m higher than previous studies. Unfortunately, technical difficulties precluded analysis of the SpO_2 values and it was therefore not possible to determine if the same mitigation of decline in SpO2 as observed in Chapter 3 and Coleman et al. (2021) was evident. Nonetheless, KME mitigated declines in vigilance attention during acute severe hypoxic exposure compared to PLA. The level of ketosis achieved in the present study has been found to induce acidosis at rest in other investigations (Chapter 2; McCarthy et al., 2023). It is therefore possible that the mitigation in the decline in cognitive performance may have been due in part to a greater oxygen availability and higher partial pressure of oxygen (PaO_2) secondary to ketosis induced acidosis. (McMorris et al., 2017). Besides the level of ketosis and mitigation in cognitive declines, the present study did not provide mechanistic data and therefore, the precise mechanisms explaining these effects remain to be established.

In conclusion, the rapid rise in blood R-βHB concentration, decreases in blood glucose concentration, and apparent parasympathetic withdrawal during severe hypoxia in response to KME compared to PLA attenuated of the declines in cognitive performance associated with hypoxic exposure. These data suggest an effect of KME to enhance cognitive resilience under acute hypoxic exposure. KME may be viable option to mitigate environmental hypoxia as previously suggested (Shaw et al., 2021), and indicated in Chapter 3.

Chapter 5
Exogenous ketone monoester attenuates declining oxygen saturation during weighted ruck exercise at simulated high altitude, but does not impact cognitive performance

5.1. ABSTRACT

Acute ingestion of EKS in the form of a R-BD R-βHB KME can attenuate declines in oxygen availability during hypoxic exposure, and may impact cognitive performance at rest and in response to moderate intensity exercise. In a single-blind randomized crossover design, sixteen males performed cognitive performance assessments before and during hypoxic exposure with moderate exercise (2x20 min weighted ruck (~24 kg) at 3.2 km/h at 10% incline) in a normobaric altitude chamber (4572 m, 11.8%O_2). The R-BD R-βHB ketone monoester (573 mg/kg; KME) or a calorie- and taste-matched placebo (PLA; ~50 g maltodextrin) were co-ingested with 40 g dextrose prior to hypoxia exposure. R-βHB concentrations were rapidly elevated and sustained (>3 mM; $P<0.001$) by KME, and the rise in blood glucose concentrations was attenuated compared to PLA throughout the ~90 min of hypoxic exposure. The decline in oxygen saturation (SpO_2) during hypoxic exposure was attenuated in KME by 2.4% to 4.2% ($P<0.05$) compared to PLA. Defense Automated Neurobehavioral Assessment (DANA) code substitution task, Stroop color and word task, or shooting simulation cognitive performance tasks did not differ between trials before and during hypoxic exposure. These data suggest that the acute nutritional ketosis induced by KME ingestion can attenuate declining O_2 saturation during severe hypoxia both at rest and during moderate-intensity exercise, but this SpO_2 "advantage" did not translate into differences in cognitive performance before or after exercise in the conditions investigated.

5.2 INTRODUCTION

Hypoxia occurs when oxygen availability does not match the demand required to maintain homeostasis, most often due to insufficient oxygen delivery to a tissue because of reduced blood oxygen saturation (SpO_2) or reduced tissue perfusion (Sarkar et al., 2017). During high altitude exposure, the partial pressure of oxygen declines and reduces the driving force for diffusion of oxygen across alveolar membranes, which decreases SpO_2. One consequence can be acute mountain sickness (AMS) which predominately include headache, weakness, fatigue, nausea, insomnia, and decreased appetite symptoms (Peacock, 1998). These result from local tissue hypoxia with decreases in oxidative capacity and cerebral metabolic rate (CMR) (Williams et al., 2019).

Blunted oxidative capacity during hypoxia impairs physical performance as relative workload intensities are higher (Deb et al., 2018). As the severity of hypoxia increases, reliance on anaerobic metabolism rises, elevating the relative difficulty of performing and maintaining performance in a given physical task (Deb et al., 2018). Moreover, reduced CMR during hypoxic exposure is detrimental to cognitive performance by negatively impacting working memory (Malle et al., 2016, Legg et al., 2016), cognitive flexibility (Asmaro et al., 2013, Turner et al., 2015), attention (Asmaro et al., 2013, Turner et al., 2015, Stepanek et al., 2014), executive function (Turner et al., 2015), and auditory processing (Beer et al., 2017).

Military personnel are often required to simultaneously perform high-level cognitive and physical tasks in hypoxic environments without a prior period of adaptation, either acute or chronic, to hypobaric hypoxia. Therefore, any therapeutic strategy to mitigate the effects of hypoxic exposure may need to consider potential interaction effects of physical work or exercise and whether this influences countermeasure utility. Acute bouts of moderate intensity-exercise lasting ≥20 min have been found to improve cognitive performance during hypoxic exposure (Chang et al., 2012). The cardiorespiratory and metabolic demands of exercise

enhance the ability to maintain a higher level of CMR, therefore mitigating the negative consequences of acute hypoxic exposure (Ando et al., 2020). These ergogenic effects likely result from increases in cerebral blood flow (CBF), catecholamine concentrations (dopamine, adrenaline, and noradrenaline), and brain-derived neurotrophic factor (BDNF) (McMorris et al., 2017, McMorris et al., 2016, Chang et al., 2012). However, exercise as a countermeasure to combat hypoxic exposure is only effective *during* exercise; thus requiring sustained moderate-intensity exercise, which limits its practical use when hypoxia exposure is prolonged.

Attenuating the decline in SpO_2 is the most promising countermeasure for mitigating the negative consequences of acute hypoxic exposure by maintaining CMR and a higher rate of aerobic metabolism during exercise (Ando et al., 2020; Scott et al., 2016). Acetazolamide is the gold standard of treatment for AMS primarily by increasing SpO_2 and reducing symptoms of hypoxic exposure, but compared to placebo during hypoxic exposure, acetazolamide can impair exercise capacity (Bradwell et al., 2018) and neuropsychological measures of concentration, cognitive processing speed, reaction time, short-term memory, and working memory (Wang et al., 2013a). Therefore, a hypoxia countermeasure that mitigates SpO_2 declines, decreases symptoms of AMS, and maintains or attenuates declines in physical and/or cognitive performance during exercise or other physically demanding tasks is needed.

EKS, particularly in the form of ketone esters, are an emerging therapeutic modality that may counter hypoxia (Poffe et al., 2021; Evans et al., 2022). Ingestion of EKS elevates circulating concentrations of the ketone bodies β-hydroxybutyrate (βHB) and acetoacetate (AcAc) within minutes of ingestion, and these increases can be maintained for several hours (Stubbs et al., 2017; Vandoorne et al., 2017), compared to dietary ketosis which needs a multiple week adaptation period to achieve similar benefits (Burke, 2021). This acute transient increase has been termed “acute nutritional ketosis” and has been consistently observed to have effects on metabolism both at rest, and during and after exercise (Evans et al., 2022). Acute ingestion

of a R-BD R-βHB KME has been shown to attenuate declines in cognitive performance under hypoxic conditions at rest (Coleman et al., 2021; and Chapters 3 and 4). Both at rest (Coleman et al., 2021; and Chapter 3) and during exercise (Poffe et al., 2021; Prins et al., 2021), acute ingestion of KME attenuates the decline in SpO_2 under hypoxic conditions. Indeed, there may be overlapping mechanisms of action for acute nutritional ketosis and exercise to improve cognitive performance during hypoxic exposure (Prins et al., 2020). The aim of the present study was to investigate whether acute ingestion of R-BD R-βHB KME impacts cognitive performance and SpO_2 in young, healthy males experiencing short-duration simulated high-altitude (simulated 15,000 ft/4572 m, 11.8%O_2) at rest and during moderate-to-vigorous intensity exercise in the form of a weighted treadmill ruck.

5.3 METHODS

5.3.1 Participants

Sixteen healthy males (1.81± 0.06 m, 82.0±8.3 kg, 24.4±4.3 y) provided written informed consent to participate after written and verbal explanations of the procedures. An *a priori* sample size calculation was performed based on two conditions with up to eight serial measurements in a repeated measures within-between interaction design. To detect an effect size *f* of 0.253 [based on a partial eta squared (η^2_p) = 0.06; '*moderate*' effect] for a given parameter at a Type I error rate (α) of 0.05 and a power (1−β) of 0.8 required a sample size of *n*=16 (G*Power v3.1).

Each participant provided written informed consent to participate after receiving written and verbal explanations of the procedures in accordance with the protocol approved by the Florida Institute for Human and Machine Cognition Institutional Review Board and Office of Human Research Oversight, U.S. Army Medical Research and Development Command, and followed DoD Instruction 3216.02, "Protection of Human Subjects and Adherence to Ethical

Standards in DoD-Conducted and Supported Research". Inclusion criteria: (1) exercised 150 min per week at moderate to high intensity for ≥1 year; (2) 18 to 35 years of age; and (3) consumed a standard American diet. Exclusion criteria: (1) history of smoking; (2) metabolic or cardiovascular disease; (3) orthopedic, musculoskeletal, neurological, psychiatric disorder, and/or any medical conditions that prohibit exercise; (4) habitual prescription medications; (5) taking EKS or following a low-carbohydrate or ketogenic diet; or (6) use of ergogenic aids affecting cognitive or physical performance within one month of study participation. Participants were instructed to refrain from caffeine for 12 h, alcohol consumption and exercise training for 24 h, and arrive in an overnight fasted state before each experimental trial. Participants were instructed to maintain their usual exercise training frequency throughout the study intervention. One participant withdrew due to volitional exhaustion during one of the trials.

5.3.2 Experimental design

Utilizing a single-blind, randomized crossover design, participants visited the laboratory on three separate occasions over a 10 to 16-day period, comprising one familiarization and two main experimental trials separated by a washout period >48 h. During the first visit, participants were familiarized with the protocol, equipment, weighted ruck, subjective measurement scales, and all tests of cognitive performance. Dietary intake data (24 h recall) were collected and analyzed using the automated self-administered 24 h (ASA24) dietary assessment tool (version 2021), developed by the National Cancer Institute, Bethesda, MD, to ensure participants were not currently consuming a ketogenic diet.

The two main experimental trials (visits 2 and 3) were comprised of an exercise protocol consisting of 2x20 min weighted rucks (~24 kg) at 3.2 km/h on a 10% incline, with the battery of cognitive performance tests administered before, within, and immediately after exercise during hypoxic exposure (Figure 5.1). The primary outcome was cognitive performance

between KME and a calorie and taste-matched placebo (PLA) condition, with secondary outcomes SpO_2, circulating βHB, glucose, and lactate concentrations, heart rate (HR), parameters of heart rate variability (HRV), rating of perceived exertion (RPE), symptoms of AMS, and symptoms of gastrointestinal (GI) disturbances.

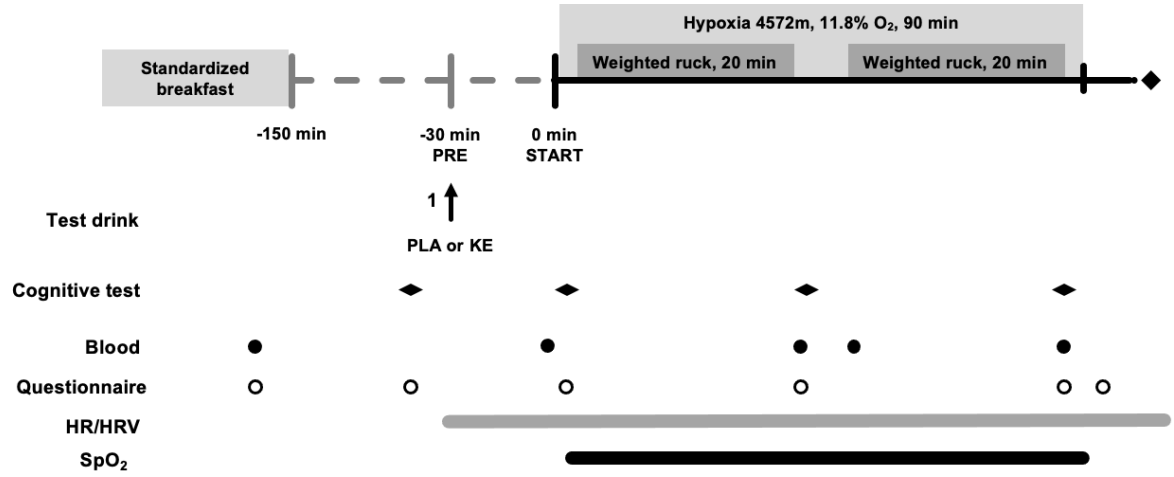

Figure 5.1. Experimental design schematic

5.3.3 Assessments of cognitive performance

At baseline, pre-exercise (R1 Pre), after the first ruck (R1 Post), and after the second ruck (R2 Post), cognitive performance including processing speed, selective attention, interference, and executive functioning was assessed using a self-directed computerized Automated Neuropsychological Assessment Metric (ANAM^R) test with established test-retest reliability for the Stroop Color and Word Task (ANAM-4, Vista Life Sciences, USA), a self-directed tablet-based Defense Automated Neurobehavioral Assessment (DANA; AnthroTronix Inc, USA) for the Code Substitution Task, and a Shoot Simulation Task (VirTra Inc., USA). All tests were performed in a sound-insulated room under controlled conditions (i.e., appropriate lighting, as quiet as possible, and isolation from unnecessary stimuli and timing feedback). Participants were instructed to complete the battery of tests as quickly and accurately as possible.

Stroop Colour and Word Task

The Stroop Colour and Word Task measures cognitive flexibility, processing speed, and executive function (Periáñez et al., 2021) and comprises of three “blocks”. In the first block (“Neutral”), the words RED, GREEN, and BLUE are presented individually in black text on the display. The user is instructed to read each word aloud and to press a corresponding key for each word (1 for RED, 2 for GREEN, 3 for BLUE). In the second block (“Congruent”), a series of XXXX's is presented on the display in one of three colors (red, green, or blue). The user is instructed to say the color of the XXXX's aloud and press the corresponding key based on color. In the third block (“Incongruent”), a series of individual words (RED, GREEN, BLUE) are presented in a color that does not match the name of the color depicted by the word. The user is instructed to press the response key assigned to that color. Outcome measures are correct answers (*n* and %), reaction time in milliseconds (ms), reaction time for correct responses (CRT), and the interference score.

Code Substitution Task

The Code Substitution Task was performed in the form of a simultaneous (CSS) and a delayed (CSD) code substitution task at each time point. For CSS, the participant refers to a code of 9 symbol-digit pairs displayed across the upper portion of the screen. A sequence of single symbol-digit pairs is shown below the key, and the participant indicates whether or not the single pair matches the code by pressing "Yes" or "No." For CSD, the nine symbol-digit pairs are not displayed on the screen. However, the participant is still shown a sequence of single symbol-digit pairs and is asked to recall from memory if these are the correct pairs from the CSS test that just occurred, with importantly, the symbol-digit pairs changing at each time point. Outcome measures in both CSS and CSD are cognitive efficiency (correct answers per min), incorrect scores, reaction time, and reaction time for correct responses.

Shoot Simulation Task

The Shoot Simulation Task was a vigilance task with a real M4 rifle modified with a laser pin and CO_2 cartridge (VirTra inc., AZ, US). Participants shot at a projector screen at a set distance displaying targets. At each time point (Baseline, R1 Post, R2 Post), participants were shown three different targets described as "shoots" (all bullseyes of different colors, six total), and were instructed to shoot only these targets as fast and as accurately as possible. Thirty total targets were shown during each time point, one at a time for 1.5 s (15 “shoots”, 15 “no shoots”) to ensure accurate shot capture via internal piloting. Outcome measures are a total score based on where the hit is relative to the bullseye (1, 2, 3 scoring system), reaction time, and numbers of hits, misses, and incorrect target hits.

5.3.4 Main experimental trials

The main experimental trials were performed in a single-blind, placebo-controlled, randomized crossover design. Visits 2 and 3 were identical in terms of pre-test preparation (standardized physical activity and instructed to replicate diet 24 h before each visit), the exercise challenge and the test battery. The visits differed only in the drinks consumed before undergoing exercise and cognitive testing during hypoxia (i.e., KME vs. PLA).

Participants arrived in the morning (0700 to 0800 h) in a fasted state, completed a behavioural compliance questionnaire, and were weighed to the nearest 0.05 kg using a calibrated digital weighing scale. After a resting (baseline) blood sample was collected, participants were fed a standardized breakfast consisting of a bagel (Dave’s Killer Bread, OR, USA; kcal: 260, F: 3 g, C: 48 g, P: 11 g) with a single serving of peanut butter (J.M. Smuckers Co.; kcal: 250, F: 21 g, C: 11 g, P: 9 g) to replicate the fed state. After consumption, participants rested for two hours, then they completed the baseline assessments of cognitive performance, before ingesting the respective test drinks for KME or PLA. The KME condition consisted of 573 $mg.kg^{-1}$ body mass of commercially-available R-BD R-βHB KME (120 kcal/25 g; Pure ΔG ketone monoester; HVMN, Inc., USA). The KME was mixed directly with a carbohydrate

solution providing 40 g dextrose. The PLA beverage was a ketone-free, isocaloric, taste-matched placebo that contained 668 mg.kg^{-1} body mass of maltodextrin combined with 40 g dextrose. The PLA drink was taste-matched using a bitterness additive (Bittrex, USA) and internally piloted to ensure participant blinding. Both drinks were dissolved in water to 500 mL and given to participants in an opaque sports bottle. All dry ingredients were measured to the nearest 0.001 g on a calibrated balance scale (Denver Instruments, Bohemia, NY, USA). Participants were given 20 mL of calorie-free flavored drink mix (MIO, Kraft Heinz, Chicago, IL, USA) immediately after ingesting the experimental drinks to remove any lingering taste.

Approximately 40 min after ingestion, participants entered the normobaric high altitude chamber (Hypoxico, New York, USA; 4572 m, 11.8%O_2) and rested in a seated position for 5 min before completing the pre-exercise cognitive performance assessments. Participants then completed the two x 20 min standardized weighted rucks (MOLLE II, USA; ~22 kg, 3.2 km/h, 10% incline) on a Woodway 4front treadmill (Woodway Inc., USA). Prior to implementation, the protocol was piloted (n=6) for feasibility (ruck weight, exercise tolerance), safety, and indices of cardiorespiratory demand (SpO_2, HR) (Supplemental Materials). Subjective measures were collected in the final minute of each ruck. Participants remained in the altitude chamber until the R2 Post time point at which cognitive performance was assessed, which resulted in ~90 min total exposure time.

5.3.5 SpO_2, HR and HRV

HR and HRV were measured continuously throughout the trials (V800 Polar, Polar, Kempele, Finland). Upon entering the chamber, SpO_2 was continuously measured with the Nellcor Bedside Respiratory Patient Monitoring System (Covidien, Dublin, Ireland) placed on the forehead over the supraorbital artery. Before entering the chamber and during the recovery period outside, systemic oxygen saturation (SpO_2) was measured by pulse oximetry with the Nellcor Bedside Respiratory Patient Monitoring System (Covidien, Dublin, Ireland)

5.3.6 Blood sample analysis

Concentrations of blood R-βHB (Precision Xtra, Abbott Diabetes Care Inc., USA), glucose (Precision Xtra), and lactate (Lactate Plus, Nova Biomedical, USA) were measured in finger stick capillary samples using commercially-available handheld devices. Blood samples were collected using a lancet following alcohol cleaning. The first droplet was wiped away with a cotton swab, and the subsequent droplets were used for analysis.

5.3.7 Subjective measures of exertion, symptoms of GI disturbances and AMS

Subjective measures of exertion including rating of perceived exertion (RPE), leg discomfort scale, breathlessness intensity scale, anxiety of breathing, and anxiety of leg discomfort were recorded on a Likert scale (1-10; 1: none to 10: maximal) (Faull et al., 2019). Similarly, participants were asked to rate GI symptoms (heartburn, bloating, nausea, vomiting, intestinal cramps, abdominal pain, flatulence, diarrhoea) as well as symptoms of AMS (dizziness, headache, muscle cramp, urge to urinate) on a Likert scale (0-8; 0: no symptoms to 8: unbearable symptoms) (Stubbs et al., 2019).

5.3.8 Statistical analysis

All statistical analyses and graphical representation of data were performed using Prism v9 (GraphPad Software Inc, San Diego, CA USA). Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. Data are presented as mean±SD, or mean difference (lower, higher 95% confidence limits of mean) where indicated. A two-way (Time*Condition) repeated measures ANOVA was used to identify differences if any between the conditions across time. When a Time*Condition interaction effect or a main effect of Condition was observed, *post-hoc* pairwise comparisons were performed with Bonferroni correction applied and multiplicity-adjusted *P* values are reported. Sphericity was not assumed, and the Greenhouse-Geiser correction was applied to all ANOVA analyses. The threshold for statistical significance was set at $P \leq 0.05$ for all tests.

5.4 RESULTS

5.4.1 Blood metabolites

Fasting concentrations of βHB (KME: 0.12±0.05 mM; PLA: 0.14±0.06 mM), glucose (KME: 87.62±15.34 mg/dL; PLA: 89.06±9.70 mg/dL), and lactate (KME: 1.92±0.61 mM; PLA: 1.73±0.84 mM) did not differ between trials at baseline (Figure 5.2). A main effect of time and condition (both $P<0.001$), as well as time*condition interaction effect ($P<0.001$) were observed for plasma R-βHB concentrations. Blood R-βHB was significantly elevated at all subsequent timepoints after consuming the KME compared to PLA ($P<0.001$). Ingestion of KME resulted in a rise in circulating R-βHB concentrations (2.99±0.96 mM, $P<0.001$) by the start of the first ruck (R1). R-βHB concentrations continued to rise and peaked at 4.93±0.82 mM prior to starting the second ruck (R2), where they remained elevated >4 mM for the remaining KME trial ($P<0.001$) (Figure 5.2A). A main effect of time and condition (both $P<0.001$) and time*condition interaction effect ($P<0.001$) were observed for plasma glucose concentrations. Blood glucose was significantly lower in the KME condition compared to PLA by 30 min after ingestion and before R2 (both $P<0.001$) (Figure 5.2B). Blood lactate concentration was not significantly different between conditions at any time point, although lactate concentrations increased throughout the experimental trials (main effect of time, $P<0.001$) such that values in both conditions at R1 Post, R2 Pre, and R2 Post were elevated above Baseline and Pre-chamber (Figure 5.2C).

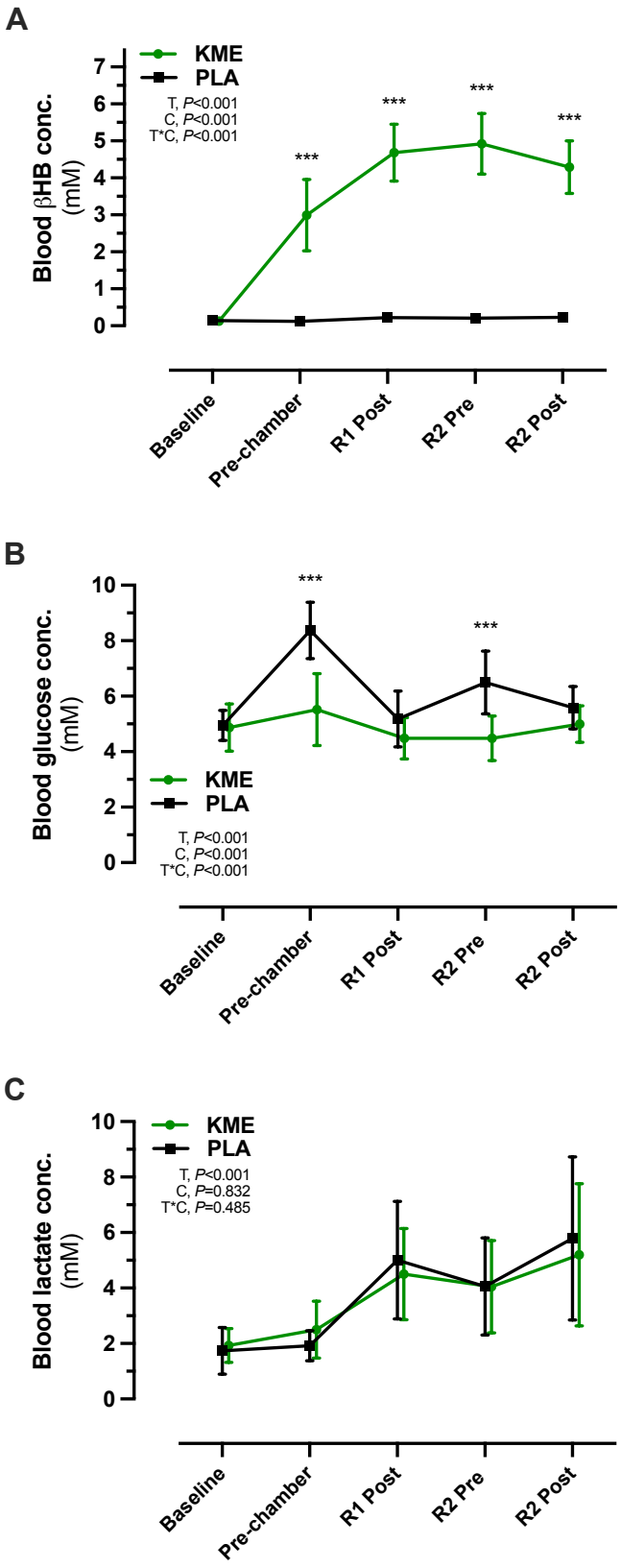

Figure 5.2. Blood R-βHB (A), glucose (B), and lactate (C) concentrations during ketone monoester (KME) and placebo (PLA) throughout baseline, pre-chamber rest, post ruck 1 (R1), pre and post ruck 2 (R2). Data are presented as mean±SD. T: time; C: condition (KME vs. PLA); T*C: time*condition interaction. ***P<0.001 for KME vs PLA.

5.4.2 Cognitive performance outcomes

All cognitive performance outcomes did not differ at baseline between conditions (Figure 5.3; Table 5.1). A main effect of time was observed for Code Substitution Task CSS correct RT, CE, and incorrect responses (P=0.01, P=0.04, P=0.03, respectively), Code Substitution Task CSD incorrect responses (P=0.03), Stroop Colour and Word Task neutral and incongruent CRT (P<0.001, P=0.002, respectively), as well as neutral and incongruent percent correct (P=0.001 and P=0.006, respectively) (Table 5.1). The total score in the Shoot Simulation Task increased during the trials (main effect of Time, P=0.003) (Figure 5.3; Table 5.1). The absence of time*condition interaction effects across all outcome measures indicates no differences between KME and PLA on cognitive performance for outcomes reported in Figure 5.3 and Table 5.1, with similar findings also evident for the Stroop Colour and Word Task for %correct Neutral, %correct Congruent, correct RT Neutral, correct RT Congruent, CSS incorrect, CSD incorrect, Shoot Simulation hit, Shoot Simulation miss (data not shown).

5.4.3 Oxygen saturation

Before entering the altitude chamber, there were no differences in SpO_2 saturation between conditions (KME: 98.80±0.40%; PLA: 98.75±0.44%). During high-altitude exposure, the decline in SpO_2 was attenuated in KME across the entire exposure time compared to PLA (main effect of condition, P<0.001) (Figure 5.4A). Pairwise comparisons revealed higher %SpO_2 at R1 Pre [2.4%, (0.3, 4.4); P=0.020], R1 20 min [2.8%, (0.4, 5.1); P=0.016], R2 10 min [4.2%, (0.9, 7.5); P=0.008], R2 20 min [3.5%, (1.0, 5.9); P=0.003], and R2 Post [3.6%, (2.2, 5.2); P<0.001] (Figure 5.4B).

TABLE 5.1. Cognitive outcomes

	Time				
	Baseline	**R1 Pre**	**R1 Post**	**R2 Post**	***P* value**
Stroop incongruent correct (%)					Time, *P*=0.006
PLA	93.9±5.1	91.2±4.6	89.5±6.0	91.1±4.7	Condition, *P*=0.471
KME	93.5±5.3	92.6±4.2	90.3±4.0	91.7±4.9	Interaction, *P*=0.660
Stroop incongruent correct RT (ms)					Time, *P*=0.002
PLA	741.7±146.9	683.5±108.4	718.9±111.2	700.7±112.3	Condition, *P*=0.409
KME	735.7±98.4	698.2±99.6	688.0±131.5	659.6±94.1	Interaction, *P*=0.288
Stroop Interference score					Time, *P*=0.19
PLA	24.05±6.1	25.51±5.8	22.06±6.3	24.03±7.2	Condition, *P*=0.63
KME	23.3±6.5	23.45±4.7	24.44±6.6	26.52±6.1	Interaction, *P*=0.06
DANA CSD correct RT (ms)					Time, *P*=0.26
PLA	969.8±241.2	978.7±245.0	985.6±152.9	933.1±205.6	Condition, *P*=0.68
KME	994.6±169.5	1008.3±276.7	932.0±200.7	948.6±118.7	Interaction, *P*=0.54
DANA CSD efficiency (Correct response/min)					Time, *P*=0.20
PLA	57.3±14.1	56.1±12.8	56.6±12.7	60.0±11.7	Condition, *P*=0.84
KME	60.6±11.0	54.3±13.6	55.7±15.2	60.1±9.2	Interaction, *P*=0.50
DANA CSS correct RT (ms)					Time, *P*=0.01
PLA	1146.4±197.4	1117.2±171.4	1140.2±201.6	1055.7±156.7	Condition, *P*=0.71
KME	1186.1±307.8	1134.0±197.2	1118.2±222.7	1074.8±176.1	Interaction, *P*=0.53
DANA CSS efficiency (correct response/min)					Time. *P*=0.04
PLA	53.05±9.0	53.30±7.2	52.71±8.7	56.18±7.2	Condition, *P*=0.96
KME	52.63±12.5	52.93±8.9	53.99±10.0	55.35±8.6	Interaction, *P*=0.72
VirTra correct RT (ms)					Time, *P*=0.08
PLA	1.29±0.07	-	1.27±0.07	1.30±0.05	Condition, *P*=0.26
KME	1.29±0.07	-	1.25±0.06	1.27±0.07	Interaction, *P*=0.19
VirTra correct RT (#)					Time, *P*=0.003
PLA	23.68±7.9	-	27.0±9.8	26.87±8.1	Condition, *P*=0.59
KME	22.12±6.3	-	27.75±7.5	25.06±9.0	Interaction, *P*=0.55

Data are presented as mean±SD, n=16. CSD, Code Substitution Delayed; CSS, Code Substitution Simultaneous; DANA, Defense Automated Neurobehavioral Assessment; RT, reaction time. *only 3 shoot timepoints

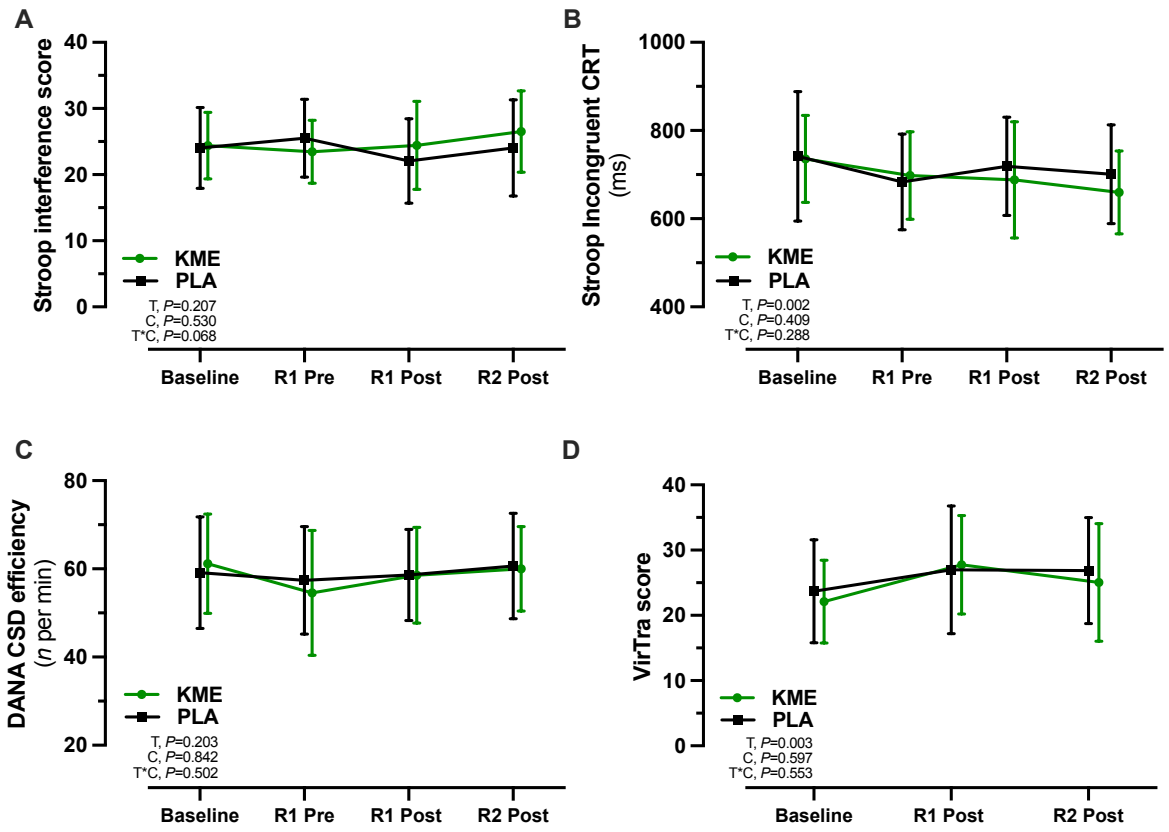

Figure 5.3. Cognitive performance assessed by Stroop interference score (A), Stroop incongruent correct reaction time (CRT) (B), DANA code substitution delayed efficiency (C), and Virtra shoot score (D) during ketone monoester (KME) and placebo (PLA) throughout baseline, pre (excluding VIRTRA) and post ruck 1 (R1), and post ruck 2 (R2). Data are presented as mean±SD. T: time; C: condition (KME vs. PLA); T*C: time*condition interaction.

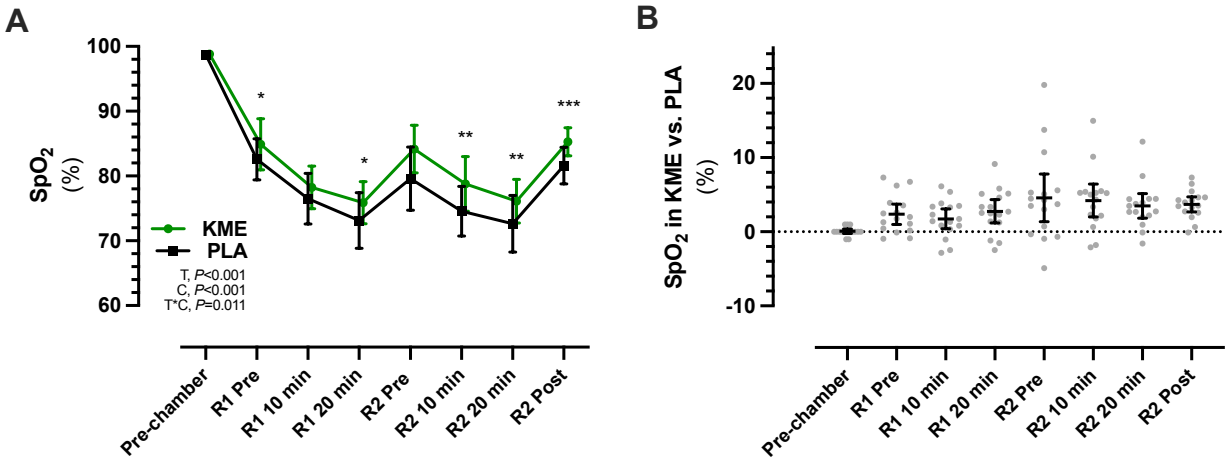

Figure 5.4. Average oxygen saturation (SpO_2) (A), and mean differences in SpO_2 between trials (B), during ketone monoester (KME) and placebo (PLA) throughout pre-chamber rest, ruck 1 (R1) and Ruck 2 (R2). Data are presented as mean±SD in A, and as mean±95% confidence limits in B. T: time; C: condition (KME vs. PLA); T*C: time condition interaction. *P<0.05, **P<0.01, and ***P<0.001 for KME vs PLA.

5.4.4 Heart rate and HRV parameters

A main effect for time was observed for root mean square of successive differences (RMSSD), R-R interval, stress index, sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) indexes (all $P<0.001$; Table 5.2). Condition*time interaction effects were observed in RMSSD ($P=0.002$) and R-R interval ($P<0.001$) indicating a difference in autonomic function between the KME and PLA conditions. Average HR was higher in KME compared to PLA before (Pre chamber) and after (R1 Pre) hypoxic exposure, and after completion of R2 (R2 Post), but did not differ between conditions (Figure 5.5A). As a result, the R-R interval was greater in KME compared to PLA at the same time points ($P<0.001$; Table 5.2). RMSSD was significantly lower in KME compared to PLA [-23.7 ms (-35.7, -11.7); $P<0.001$] 30 min after ingestion of the drinks (Pre-chamber) but was not different at any other time point (Figure 5.5B). PNS index was significantly lower in KME compared to PLA before (Pre chamber) and after (R1 Pre) hypoxic exposure ($P<0.001$ and $P=0.005$, respectively), whereas SNS index did not differ between conditions at any time point (Table 5.2), None of the other HR parameters measured were different between conditions (e.g. LF, HF, total power (data not shown)). RPE increased during the respective trials but did not differ between conditions (Figure 5.5D)

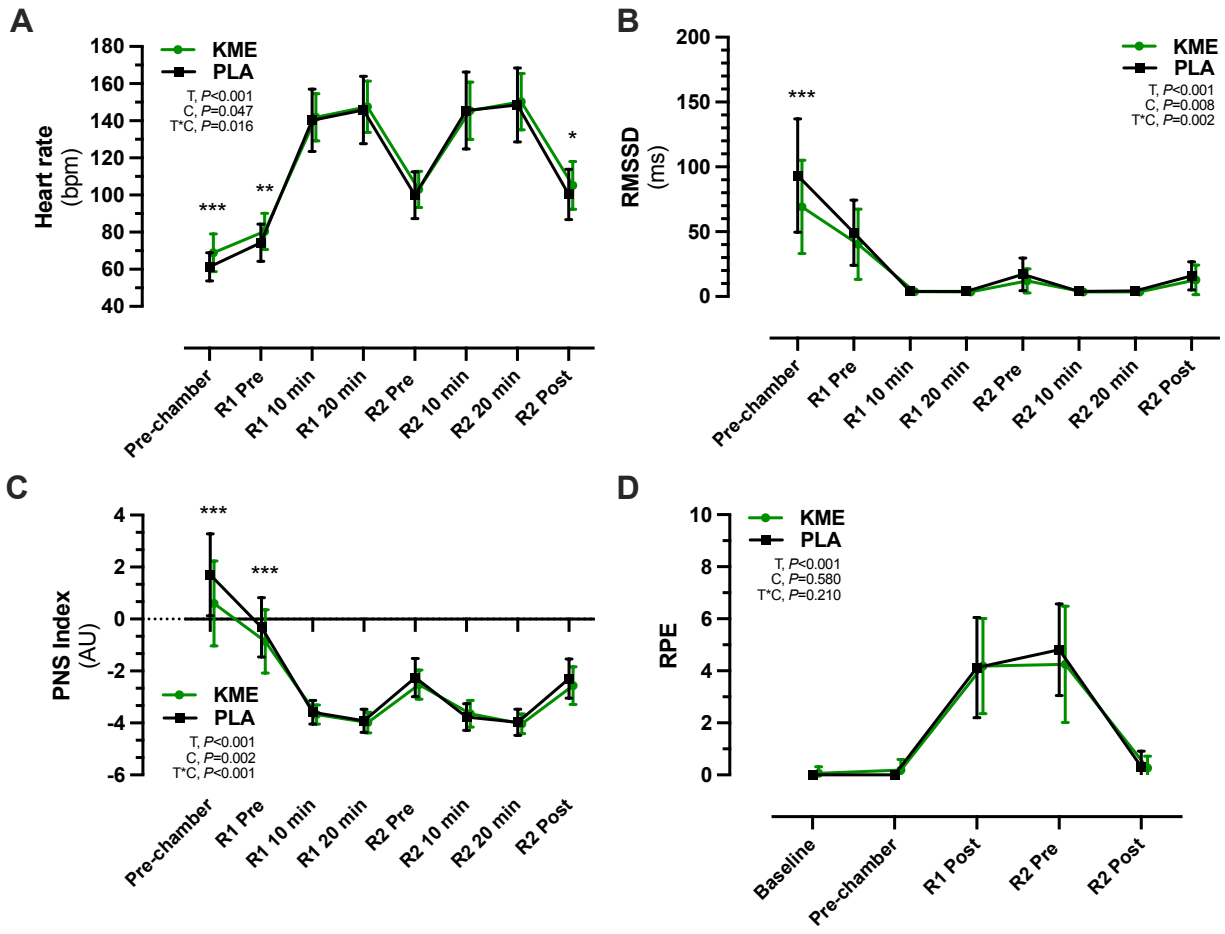

Figure 5.5. Heart rate (HR) (A), room mean square of successive differences (RMSSD) (B), parasympathetic (PNS) index (C) and rating of perceived exertion (RPE) (D) during ketone monoester (KME) and placebo (PLA) throughout baseline, pre-chamber rest, ruck 1 (R1), and ruck 2 (R2). Data are presented as mean±SD. T: time; C: condition (KME vs. PLA); T*C: time condition interaction. *P<0.05, **P<0.01, and ***P<0.001 for KME vs PLA.

Table 5.2. Heart rate variability (HRV) outcomes

	Time								
	Pre-chamber	**R1 Pre**	**R1 10 min**	**R1 20 min**	**R2 Pre**	**R2 10 min**	**R2 20 min**	**R2 Post**	***P* value**
R-R interval (ms)									Time, *P*<0.001
PLA	991.9±126.4	820.6±112.1	433.3±51.54	417.6±53.6	609.7±77.0	420.2±60.9	411.1±56.8	608.0±81.4	Condition, *P*<0.001
KME	889.9±144.9***	756.8±99.3***	426.5±41.6	410.2±40.6	587.2±56.0	416.9±45.8	403.0±41.3	578.8±71.7	Interaction, P<0.001
RMSSD (ms)									Time, *P*<0.001
PLA	93.2±43.8	49.1±25.1	3.8±2.3	3.9±2.7	17.0±12.5	4.0±2.3	4.2±2.9	16.0±10.8	Condition, *P*=0.009
KME	69.9±37.1***	40.1±28.1***	3.8±2.3	3.6±1.7	22.2±3.2	3.7±1.8	3.6±1.8	12.4±11.9	Interaction, *P*=0.002
Stress index (AU)									Time, *P*<0.001
PLA	5.4±2.3	8.0±2.9	60.1±30.2	71.8±25.8	18.0±7.1	57.7±28.9	71.9±28.2	18.8±8.9	Condition, *P*=0.043
KME	7.0±3.0	10.5±4.0	60.9±27.4	81.3±26.0	19.0±7.0	62.5±28.6	82.5±20.9	21.7±9.5	Interaction, *P*=0.213
SNS Index (AU)									Time, *P*<0.001
PLA	-0.89±0.8	0.39±1.0	14.37±6.1	17.73±6.3	3.78±2.0	14.59±6.3	18.10±6.9	3.97±2.4	Condition, *P*=0.09
KME	-0.15±1.1	1.19±1.2	14.69±4.0	19.73±5.9	4.17±1.8	14.76±5.3	20.17±5.3	4.81±2.5	Interaction, *P*=0.58
PNS Index (AU)									Time, *P*<0.001
PLA	1.70±1.5	-0.32±1.1	-3.58±0.4	-3.91±0.4	-2.24±0.7	-3.77±0.5	-3.97±0.5	-2.29±0.7	Condition, *P*=0.54
KME	0.59±1.6***	-0.86±1.2**	-3.67±0.3	-3.97±0.3	-2.52±0.5	-3.79±0.4	-4.02±0.3	-2.56±0.7	Interaction, *P*=0.87

Data are presented as mean±SD, n=16. RMSSD, root mean square of successive differences between normal heartbeats; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.***P<0.001, **P<0.01 for KME vs PLA

5.4.5 Symptoms of GI disturbance and AMS

Heartburn, bloating, nausea, vomiting, intestinal cramps, abdominal pain, flatulence, diarrhoea, dizziness, headache, and muscle cramps were not different across treatments in both studies (Table 5.3).

Table 5.3. Gastrointestinal and acute mountain sickness symptom questionnaire

	Time point		
	Baseline	**30 min after ingestion**	**In chamber rest**
Heartburn			
PLA	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.2	0.06±0.2
Bloating			
PLA	0±0.0	0±0.0	0±0.0
KME	0.06±0.2	0.06±0.2	0±0.0
Nausea			
PLA	0.06±0.2	0.06±0.2	0±0.0
KME	0±0.0	0±0.0	0±0.0
Vomiting			
PLA	0.12±0.5	0±0.0	0±0.0
KME	0±0.0	0.06±0.2	0.06±0.2
Intestinal Cramps			
PLA	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0
Abdominal pain			
PLA	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0
Flatulence			
PLA	0.12±0.5	0.06±0.2	0±0.0
KME	0.18±0.7	0±0.0	0±0.0
Diarrhea			
PLA	0±0.0	0±0.0	0±0.0
KME	0±0.0	0±0.0	0±0.0
Dizziness			
PLA	0±0.0	0±0.0	0.12±0.5
KME	0±0.0	0±0.0	0.12±0.3
Headache			
PLA	0.06±0.2	0±0.0	0±0.0
KME	0±0.0	0±0.0	0.06±0.2
Muscle cramp			
PLA	0.18±0.7	0.06±0.2	0.06±0.2
KME	0.06±0.2	0±0.0	0.06±0.2
Urge to urinate			
PLA	0.18±0.5	0.12±0.3	0.3±0.6
KME	0±0.0	0.12±0.3	0.12±0.3

Scores ranging from 0-8, 0: no symptoms; 8: unbearable symptoms. Data are presented as mean±SD, n=16

5.4.6 Assessment of blinding

After the experimental protocol in the third visit was completed, but before participants were informed of their results achieved during each trial, participants were asked to indicate which trial they believe to be KME and PLA. Participants answered correctly six times, incorrectly eight times, and "I don't know" twice, which suggests successful blinding in that participants were unable to detect treatment versus placebo outside of random chance.

5.5 DISCUSSION

The present study investigated whether acute ingestion of an EKS in the form of the R-BD R-βHB KME could acutely impact SpO_2 and cognitive performance during acute, short-duration high altitude exposure at rest and during moderate-intensity exercise. Ingestion of R-BD R-βHB KME resulted in circulating R-βHB concentrations of >3mM through the ~90 min of hypoxic exposure and resulted in an attenuation of the decline in SpO_2 of 2.4% in R1 and 4.2% in R2. However, this SpO_2 "advantage" compared to PLA did not translate into differences in the primary outcome of cognitive performance during the experimental protocol.

Ingesting 573 $mg.kg^{-1}$ of R-BD R-βHB KME in a postprandial state rapidly increased circulating R-βHB concentrations to ~3 mM at 20 min after ingestion, and concentrations remained elevated above 4 mM on average throughout the hypoxic exposure. These circulating R-βHB concentrations align with previous work using the same single dose of KME before exercise (Cox et al. 2016; Dearlove et al., 2021). Blood glucose concentrations were lower throughout KME compared to PLA, which is consistent with published literature on the effect of acute nutritional ketosis to attenuate the rise in postprandial blood glucose concentration (Falkenhain et al., 2022). It is important to note that in the present study, additional CHO (~50 g) was consumed during PLA in order for both conditions to be isocaloric, which varies from the previous hypoxia work that compared KME to a non-caloric PLA (Coleman et al., 2021;

Chapters 3 and 4). The addition of CHO likely influenced the biological impact of exogenous ketosis as prior studies have demonstrated that co-administration of KME with CHO alters substrate utilization (Cox et al., 2016; Brady et al. 2023) and could potentially impact CO_2 concentrations produced from CHO metabolism. Blood lactate concentrations did not differ between trials before or after exercise. This finding contradicts the previous finding of attenuated lactate response to exercise during hypoxia after KME ingestion (Poffe et al., 2021), but may reflect differences between the studies in the exercise type, intensity, and duration of the respective studies. The intensity of exercise in the present study was markedly lower than that previous study, which consisted of varied intensity cycling exercise between 60 to 90% of the power output at lactate threshold, a 15 min time trial, and an all-out sprint at 175% of the power output at lactate threshold (Poffe et al., 2021). In general, there remains no consistent pattern as to whether KME ingestion attenuates the increase in blood lactate concentrations in response to moderate to high-intensity exercise (Evans et al., 2022).

The present observation of an attenuation of the decline in SpO_2 of 2.4% to 4.2% during simulated high-altitude exposure aligns with previous investigations (including Chapters 3 and 4) that utilized acute nutritional ketosis as a countermeasure during severe hypoxic exposure at rest or during exercise (Coleman et al., 2021; Poffe et al., 2021). The proposed mechanism for attenuation of the decline in SpO_2 is the increased ventilatory response from declines in pH after R-BD R-βHB KME ingestion, which reduces the partial pressure of CO_2 (Prins et al., 2021) leading to a leftward shift in the oxyhemoglobin saturation curve. Although not measured in the present study, multiple studies have observed a decrease in pH of ~0.5 units during acute nutritional ketosis in normoxia (Dearlove et al., 2020; Poffé, Ramaekers, et al., 2020; Poffé, Wyns, et al., 2020) and hypoxia (Poffe et al., 2021). An increased ventilation rate leading to increased breathing frequency and tidal volume has also been observed (Dearlove et al., 2019; McCarthy et al., 2021; Poffé et al., 2020). Importantly, co-ingestion of R-BD R-βHB KME with

an acid buffer in the form of sodium bicarbonate reduced the effect on attenuating the decline in SpO_2, and increased ventilation during hypoxic exposure (Poffe et al., 2021), which supports the proposal that the increased rate of ventilation is a mechanism involved in attenuation of declines in SpO_2 in hypoxic environments. In the present study, the SpO_2 advantage during KME increased from 2.4% to 4.2% from Ruck 1 to Ruck 2, which suggests increased resiliency to the decline in SpO_2 with prolonged hypoxic exposure under acute nutritional ketosis. However, these differences in SpO_2 did not translate into differences in cognitive performance during the experimental protocol before or after exercise compared to PLA.

The rationale for why acute nutritional ketosis may produce enhanced resiliency to maintain cognitive performance during hypoxic exposure is proposed as a combination of greater O_2 availability (as observed), and effects on circulating BDNF (Walsh et al., 2020), and CBF (Walsh et al., 2021). Moreover, ketone bodies provide the brain with a complimentary or alternative substrate to glucose, which is potentially of utility in hypoxic environments given that CHO utilization may decrease in response to hypoxia-induced insulin resistance (Myette-Côté et al., 2018; Pasiakos et al., 2021). In two other studies (Coleman et al., 2021; and Chapters 3 and 4) – each using a randomized, cross-over, placebo-controlled design matching the design implemented here – the potential effects of KME on hypoxia-induced declines in cognitive performance in the *non-exercising, resting state were assessed.* In each, some attenuation of cognitive decline after ingesting KME was observed: (i) in code substitution tasks at rest at simulated 5029 m (Coleman et al., 2021); and (ii) in code substitution (Chapter 3) and psychomotor vigilance (Chapter 4) tests at rest at simulated 6096 m. In Chapter 3, the apparent cognitive performance benefits of KME were coupled with a 4% SpO2 advantage, which we presume played a major role in cognitive performance resiliency.

In the present study, no cognitive advantage of KME was noted, most likely due to the co-intervention with two bouts of moderate-to-vigorous exercise. Acute exercise can

temporally enhance cognitive performance (Chang et al., 2012), which could have the effect of superseding any potential benefits to cognitive performance induced by KME during hypoxic exposure. Other design factors potentially contributing to lack of cognitive benefit in this exercise trial vs. the two prior resting state trials include severity of hypoxic exposure (4572 m vs. 5029 m and 6096 m) and duration of hypoxic exposure time at rest prior to cognitive testing, as time of useful consciousness varies with severity and length of hypoxic exposure (Shaw et al., 2021). Acute hypoxic exposure is well-established to impair cognitive performance (Shaw et al., 2021; Chapters 3 and 4). Here, on the other hand, not all measures of cognitive performance declined during the hypoxic exposure in either PLA or KME. We think this reflects the potent effect of acute exercise on cognitive performance that likely trumped the declines expected under hypoxia. Skeletal muscle and cerebral oxygenation were not measured; thus, despite the effects found on blood SpO_2 further investigation would be required to determine whether oxygenation of specific tissues is influenced.

A novel finding in this study is the effect of KME on altering HRV at rest with and without the hypoxic exposure. Hypoxic exposure at similar simulated altitudes has shown to alter HRV at rest and during exercise by increasing heart rate, and lowering parasympathetic cardiac activation (Buchheit et al., 2004; Rupp et al., 2013). KME elicited a parasympathetic withdrawal both in normoxia and hypoxia when at rest, resulting in an elevated heart rate during rest (~8 bpm) compared to PLA. Interestingly, once exercise commenced during the hypoxic exposure, there were no differences in autonomic function or heart rate. KME ingestion further affected HRV by decreasing mean R-R intervals and RMSSD at rest compared to PLA, leading to an elevated heart rate and lower parasympathetic cardiac activation prior to exercise in the hypoxic environment. However, the addition of exercise to the hypoxic exposure eliminated any differences in heart rate or HRV between trials. Analogously, even just 30% of maximal voluntary concretion utilizing a handgrip exercise abolished differences in

parasympathetic/sympathetic tone induced by prolonged cold exposure, confirming the hierarchical impact of exercise on regulating autonomic function (Sanchez-Gonzalez & Figueroa 2013). These data are consistent with previous findings on heart rate during exercise of varying intensities during hypoxic exposure with KME ingestion being no different compared to PLA (Poffe et al., 2021). However, the effects of KME ingestion on HRV at rest and during exercise in normal and hypoxic conditions may warrant future investigation because the mechanisms underlying these relationships remain to be fully understood, and are in contrast to findings showing increases in mean R-R and parasympathetic cardiac activation after adherence to a ketogenic diet (Polito et al., 2022).

There are a few limitations in the present study. While we observed metabolic, oxygen saturation, autonomic, and heart rate differences in healthy males exercising under severe hypoxia with KME administration, whether these results will translate to females or to individuals who are less active is not known. Capillary blood samples were analyzed with point-of-care devices and may not accurately reflect venous circulation values by laboratory-based measures (Evans et al., 2022). Collection of blood gases to confirm acidic state would be necessary in order to confirm the hypothesized mechanism of action. While we assessed normobaric hypoxia, hypobaric hypoxia may also result in physiological differences, which we were not able to assess in the present design (Coppel et al., 2015).

In conclusion, acute ingestion of KME had not impact on cognitive performance during exercise in simulated high altitude despite attenuating the declines in systemic oxygen saturation. These results suggest that KME ingestion increased resilience to the SpO_2 decline associated with severe hypoxic exposure during exercise, while cognitive performance and physical capacity were maintained. Acute ingestion of KME may therefore be a potential countermeasure for hypoxic exposure by attenuating declines in oxygen availability, without decrements to cognitive performance or moderate exercise in severe hypoxic conditions. These

finds are relevant to high-altitude activities (e.g., mountaineering) or clinical conditions causing hypoxemia (e.g., asthma, respiratory viral infections, or COPD). However, further work is required to establish the ability of KME to maintain cerebral oxygenation and cerebral metabolic rate during severe hypoxic exposures, in both environmental and pathological contexts.

Chapter 6
General Discussion

6.1. MAIN RESEARCH FINDINGS

At the time of commencing my PhD studies, collaborators of my supervisor at the Institute for Human and Machine Cognition (Pensacola, Florida) had published preliminary work that supported the utility of acute ingestion of the R-BD R-βHB KME in severe hypoxic exposure (20 min; 5029 m) to mitigate declines in SpO_2 and cognitive performance (Colemen et al., 2021). Since then, two other research groups have built upon this novel approach to mitigate the adverse effects of acute hypoxic exposure with the R-BD R-βHB KME *during exercise conditions* (Poffe et al., 2021; Prins et al., 2021). Additional studies are required to better understand the cardiorespiratory and metabolic modulations at rest following acute ingestion of various EKS's before adding confounding variables such as exercise that alter heart rate, fuel selection/utilization and ventilation. This PhD thesis has made multiple contributions that have advanced the understanding of the R-BD R-βHB KME as a countermeasure for severe hypoxic exposure.

6.1.1. Study 1/Chapter 2

Study 1 investigated the effect of multiple forms of EKS (R-BD R-βHB KME, R-BD R-βHB KME+BIC; KS) compared to a non-caloric PLA condition on metabolism, blood gases, indirect calorimetry, oxygen saturation, automatic function, hemodynamics, and cognitive performance at rest under normoxic conditions. Significant differences between conditions were found for increases in R-βHB concentrations, decreases in glucose concentrations, and changes in blood pH. For the latter, R-BD R-βHB KME lowered pH by -0.07±0.03 units at 60 min after ingestion, whereas both KME+BIC and KS increased pH (0.05±0.05 units, 0.05±0.04 units; respectively) at 120 min after ingestion. Interestingly, none of these alterations in metabolism, autonomic function, or blood gases significantly affected any measures of indirect calorimetry, hemodynamics, or cognitive performance at rest.

6.1.2 Study 2/Chapter 3

Study 2 investigated the utility of an R-BD R-βHB KME [650 mg/kg split dose (500/150)] as a countermeasure to mitigate declines in SpO_2 and cognitive performance during a severe NH exposure (20 min at 6096 m). KME ingestion increased blood R-βHB concentration (5.6±0.8 mM) and decreased blood glucose concentration (-21.4±12.4 mg/dL) compared to PLA during hypoxic exposure. KME ingestion attenuated declines in SpO_2 (+4.2±3.4%) and cognitive efficiency (+4.1±7.7 correct response/min) during a code substitution task compared to PLA. Additionally, across time within condition (Norm 2 to Hypo 2), KME ingestion attenuated declines in CE (+7.7±13.1 CR/min), CRT (-155.3±246.9 ms), and RT (-146.7±248.6 ms) during hypoxic exposure compared to PLA.

6.1.3 Study 3/Chapter 4

Study 3 aimed to extend the findings of Study 2 by investigating the utility of R-BD R-βHB KME in a different domain of cognitive performance (attention/vigilance) and at a lower dose [(500 mg/kg split dose (350/150)] in a similar participant profile (but new cohort) during a severe NH exposure (15 min at 6096 m). KME ingestion increased blood R-βHB concentration (4.8±0.8 mM) and decreased blood glucose concentration (-18.6±8.3 mg/dL) compared to PLA during hypoxic exposure. KME significantly attenuated the hypoxia-induced effects on mean RT (-27.8±29.5 ms), lapses over 500 ms (-2.4± 4.1 lapses), mean reciprocal RT of slowest 10% (-27.9±37.0 1 ms), mean RT of fastest 10% (-13.7±13.3 ms), and total errors (1.0±1.7 errors) when compared to PLA, suggesting yet again, that KME ingestion attenuates the declines in cognitive performance during severe hypoxic exposure.

6.1.4 Study 4/Chapter 5

Study 4 investigated the utility of R-BD R-βHB KME (573 mg/kg + 40 g of CHO) as a countermeasure to mitigate declines in SpO_2 and cognitive performance with the addition of

moderate weighted exercise (2x20 min weighted ruck) during a severe NH exposure (~90 min at 4572 m). KME ingestion increased blood R-βHB concentrations, attenuated the rise in blood glucose concentration, and had no effect on lactate compared to PLA during hypoxic exposure. The decline in SpO_2 was attenuated between 2.4 to 4.2% in KME throughout the entire exposure time compared to PLA, and was greatest during Ruck 2 (+4.2 %). There were no differences in any cognitive tests at any time point during this study, yet there was an overall main effect of time (i.e., exercise *improved* cognitive performance in both conditions).

6.2 CURRENT RESEARCH ON THE R-BD R-BHB KETONE MONOESTER AS A COUNTERMEASURE DURING ACUTE HYPOXIC EXPOSURE

The central focus of my PhD was to evaluate the utility of the R-BD R-βHB KME as a countermeasure to attenuate declines in SpO_2 and cognitive performance during an acute exposure to severe NH. This series of studies provides justification for the potential utility of R-BD R-βHB KME as a countermeasure for acute hypoxic exposure due to its ability to mitigate declines in SpO_2 at rest and during exercise. Moreover, this thesis demonstrated potential utility of R-BD R-βHB KME in mitigating cognitive decline across multiple domains (working memory, short-term memory, attention, processing speed) during acute NH exposure.

This PhD builds upon the novel and foundational work at IHMC (Coleman et al., 2021), as Study 2 closely replicates that original work. However, a more severe hypoxic exposure of 6096 m (vs. 5029 m) was chosen in this PhD. Similar findings in both studies for mitigation of decline in SpO_2 and in cognitive efficiency during a code substitution task is a central finding to support the utility of R-BD R-βHB KME as a countermeasure to acute NH exposure. Moreover, while conducting my PhD work, other labs have investigated the use of R-BD R-βHB KME to mitigate hypoxic exposure. Prins et al., (2021) used voluntary hypoventilation

(VH) during exercise to induce hypoxia. This protocol induced hypoxia in the circulation (~90%) and at the muscle level (~40%) during VH exercise during KME and PLA. The addition of exercise and the method of inducing hypoxia (i.e. VH) presents challenges when comparing the findings to this PhD. Nonetheless, there are a few interesting findings that are relatable. Prins et al., showed that KME consumption reduced PCO_2 at rest, during VH exercise, and recovery. The reductions of PCO_2 at rest coincided with a reduction in pH and a higher RER than PLA. Interestingly, no changes in VO_2 and VCO_2 were observed during that time point at rest. Moreover, no significant differences in SpO_2 (circulation or in skeletal muscle) or cognitive performance occurred before, during, or after VH exercise. These findings support the ability of acute KME ingestion to alter blood gases at rest (as also found during Study 1 of this thesis) and during exercise. This did not however, translate to mitigation of the decline in SpO_2, potentially due to the greater degree of hypoxia or the method chosen (VH vs. NH in this thesis).

Poffe et al., (2021) evaluated the utility of R-BD R-βHB KME alone or co-administratered with BIC on mitigating declines in SpO_2 and performance during prolonged exercise (>3 h) during NH (~1000-3000 m). Although their research focus was more focused on the physical performance domain and involved no measurements of cognitive performance, it still builds upon the conceptual concepts underpinning of this PhD and supports the use of KME as a countermeasure to hypoxia. This was the first investigation to show a significant elevation in V_E after acute KME ingestion. Acute intake of KME increased V_E by ~4% at the beginning of exercise (IMT180, exposed to ~1000 m) and increased by ~12% by the end of IMT180 compared to CON. In contrast, V_E during KME+BIC was similar to CON at all times. KME significantly lowered PCO_2 during exercise and increased PO_2 compared to CON at the end of the IMT180 (KME: 48±3 mmHg; CON 45±3 mmHg). Ingestion of KME or KME+BIC significantly increased oxygen saturation 60 min following hypoxic exposure (during exercise),

and was 2-3% higher by the end of IMT180. Interestingly, KME+BIC increased pH compared to KME alone and was higher than CON. KME+BIC did not affect V_E compared to CON. It still resulted in a ~3% SpO_2 advantage during prolonged exposure. Altogether, during hypoxic exercise, KME decreased pH and PCO_2 and increased SpO_2 (with and without BIC), V_E, and PO_2. The effect appeared greater when KME was ingested without BIC at the most severe hypoxic exposure (3000 m), coinciding with the largest pH gap between (KME and KME+BIC) (~0.1 unit). These findings highlight that the acid load of KME assists in maintaining a higher SpO_2. The fact that KME+BIC did not induce metabolic acidosis (until after the sprint) or alter blood gases, but still mitigated the decline in oxygen saturation suggests that other mechanisms independent of the acid load and responses in V_E may have also been involved in maintaining a higher SpO_2.

The acute metabolic acidosis in response to hyperketonemia has been reported in a number of previous studies. McCarthy et al., (2023) found that 600 mg/kg of R-BD R-βHB KME ingestion () at rest during a normal breathing produced R-βHB concentration of ~3.5 mM and altered blood gases, pH and RER compared to CON and KME+BIC. KME consumption with or without BIC increased resting RER compared to PLA. Additionally, KME alone significantly increased $PETO_2$ compared to CON (KME: 115±5 mmHg; KME+BIC: 114±3 mmHg; CON: 111±5 mmHg) and significantly lowered $PaCO_2$ compared to both CON and KME+BIC (34±3 mmHg; 36±3 mmHg; 36±3 mmHg; respectively) (McCarthy et al., 2023 - Supplemental Table 1). These findings highlight the effectiveness of KME in altering blood gases at rest, independent of a hypoxic exposure, which should be taken into consideration for future work.

6.3 LIMITATIONS

While this PhD has added to the understanding of EKS as a countermeasure to hypoxia, it was not without some limitations.

Firstly, none of the studies measured cerebral metabolism, CBF, oxygenation, or overall oxidation rate of KB, or βHB specifically. Although inferences can be made from published data, the lack of these measurements limits the ability to support many of the proposed mechanisms for KME as a countermeasure to hypoxia. It is recommended that future studies use positron emission tomography (PET) and near-infrared spectroscopy (NIRS) to measure cerebral metabolism, oxygenation, and ketone body oxidation. Quantifying these measurements will allow for a better understanding of how acute and/or chronic ingestion of EKS modulate peripheral fuel utilization and tissue oxygenation in the brain.

Studies 2, 3, and 4 did not include a measurement of pH to confirm acidosis even though acidosis appears to be a critical mechanism of SpO_2 regulation during hypoxia, and appears to sensitive to acute ingestion of various forms of EKS. Based on the consistent findings in the extant literature, it was assumed that pH decreased but this is not confirmed. Future projects studies should include measurement of pH.

The exact mechanisms responsible for the mitigating effect of KME on the declines in SpO_2 and cognitive performance and whether the latter is casually linked to the former, still needs to be elucidated. Acid balance appears to play an important role, but Poffe et al. (2021) reported an SpO_2 advantage with KME+BIC during hypoxia independent of acid load and V_E, demonstrating other mechanisms are involved.

This thesis evaluated EKS as a countermeasure to severe hypoxic exposure and included in that evaluation was the prevalence of symptoms of AMS. Yet during each study no significant changes in AMS symptoms occurred from baseline in both treatment and PLA

conditions. This lack of change demonstrates that our length of exposure was likely not severe enough to elicit AMS symptoms in the timeline that the questionnaires were administered, and thus conclusions around alleviation around AMS symptoms need to be carefully interpreted.

Multiple subjects were excluded from studies 2 and 3 due to them not completing a full hypoxic exposure and completion of the cognitive testing for analysis on account of their SpO2 values declining below the a priori "safe limit". Exclusion of these 'high responders' to hypoxic exposure may lead to some bias in the data inasmuch as these individuals experienced the greatest hypoxic effect, but it is unknown whether effects on cognitive performance would have paralleled these effects and/or whether EKS would have been more likely or less likely to provide a countermeasure benefit.

HRV was used to evaluate the changes in autonomic function in each of the performed studies, and as such my interpretation of HRV was applied in multiple settings (rest vs exercise) and of varying lengths of time (5 vs 10 vs 15 min), as well as during normoxia and hypoxia conditions. The differences in these parameters in these experiments, and a lack of consensus at present as to whether HRV is a valid and reliable method to evaluate *acutely-stimulated* changes in autonomic function means that these methods are potentially suboptimal and should therefore be interpreted with a requisite amount of caution.

Female participants were only included in one of the four studies. The study was not powered to investigate sex differences. The vast majority of EKS research has focused on male participants (Evans et al. 2022), yet the broader applicability of the results is therefore limited given the influence of endogenous and exogenous sex hormones on metabolism, and that the responses of several genetic, metabolic, and physiological parameters differs between males and females (Ansdell et al., 2020; D'Eon et al., 2002; Fu et al., 2009; Landen et al., 2019; Maher et al., 2010).

6.4 FUTURE DIRECTIONS

6.4.1 EKS

EKS research is a relatively new (<10 years) and emerging area of study that spans multiple scientific domains. The seminal study examining the effect of EKS ingestion on sports performance was a five-part investigation pertaining to the R-BD R-βHB KME as an ergogenic aid (Cox et al., 2016). While this ambitious investigation provided a plethora of new findings to explore further, gaps in foundational physiology remained to be explored. Despite the emergence of multiple new EKS's in recent years, only one rigorous pharmacokinetic investigation has compared R-BD R-βHB KME and KS. (Stubbs et al., 2017), (Evans et al., 2022). There is a need for further pharmacokinetic studies to explore responses to EKS such as non-racemic KS, the AcAc diester, KME+BIC, and new and emerging forms of ketone esters (Crabtree et al., 2023; Falkenhain et al., 2022; Leckey et al., 2017; Lowder et al., 2023; Nieman et al., 2022).

An interesting and novel finding of this PhD was that a non-racemic KS is tolerable and can elicit circulating R-βHB concentrations greater than 2 mM, which is the first investigation to show this response at rest. Prior to this study, the prevailing view, based on studies of racemic KS, indicated that KS are ineffective at elevating R-βHB concentrations to a level where they would be an effective ergogenic aid (Evans et al., 2022). The findings in this PhD thesis need to be replicated in future studies using the same (395 mg/kg) and higher doses of KS in order to make direct comparison with KME, and given their divergent effects on pH provide a unique insight into how the acid load of EKS affects their ability to modulate metabolism. Additional work on how EKS affects gas exchange and ventilation at rest is required before expanding into exercise contexts that provide confounding variables that extensively alter these processes. Much of the work in this field has centred on exercise performance, whereas these foundational metabolic response studies have been neglected to a certain extent. This work could help

standardize EKS dosing and identify possible pH thresholds for upregulating ventilation during exogenous ketosis, ultimately building upon the work of this PhD and other research groups.

Moreover, like most physiology-based research, it would greatly benefit from more studies that include both sexes and exploration of between-sex differences. There likely could be differences in uptake and utilization of these various EKS as they are naturally-derived from FFAs, and females are well-known to have different features of fat metabolism compared to males e.g. females oxidise fat at higher rates and with greater contribution to overall energy provision compared to their male counterparts.

6.4.2 Acute hypoxic exposure

Hypoxic exposure is a more well-established area of research and its effects on cognitive are unequivocal. However, this area would greatly benefit from a standardized cognitive test battery being applied, because a huge variety in the selection of tests, even in the same cognitive domain, makes comparing from study to study challenging (Bliemsrieder et al., 2022). So, standardizing a test battery with multiple options for exposure length (exploring the duration effect) would greatly benefit this field in terms of capabilities for comparison. Additionally, more studies both in EKS and hypoxia would benefit from measurements of cerebral metabolic rate to quantity oxygen utilization and potentially KB utilization with the support of labelled tracer methodology. Finally, a better understanding of the HIF-1 pathway in acute hypoxia versus chronic hypoxia will further the understanding of the acclimatization to hypoxia and allow for the ability to test potential countermeasures utility to accelerate this process.

6.5 CONCLUDING REMARKS

EKS are novel and of increasing interest in a wide range of applications such as: traumatic brain injury (Bernini et al., 2020), glucose control (Walsh et al., 2020a), Alzheimer's disease (Cunnane et al., 2016), heart failure (Nielsen et al., 2019), countermeasure to hypoxia

(Coleman et al., 2021) or low oxygen states such as COPD (Norwitz et al., 2021). There are several forms of EKS and more are being developed, so there is much future work needed understanding the response to those novel forms, and then testing their efficacy in specific use cases. This thesis shows there are important differences between different forms of EKS with respect to magnitude and time course of ketosis, effects on blood gases, and related effects on pH and acid-base balance. Additionally, EKS may provide a novel use as a countermeasure for mitigation of SpO_2 and cognitive performance during acute severe hypoxic exposure. During this thesis, two different altitudes were chosen to test the utility of EKS during severe hypoxic exposure. The higher altitude was the highest altitude that could be safely simulated while allowing time to complete the cognitive performance testing, whereas the lower altitude was to chosen to simulate the highest real-world land exposure that could likely occur in a military setting (i.e. Afghanistan ~4500m). These altitudes and the acute exposure times were deliberately chosen to explore utility as countermeasure to severe hypoxia specifically in the application of pilot populations (commercial/private/military) who are at risk of rapid-onset severe hypoxic exposure due to the loss of oxygen flow or cabin pressure during flight without prior adaptation periods. Further work would be needed to investigate the utility of EKS in other relevant populations e.g. mountaineer rescue populations, who would be acclimatized or have prior exposure that may impact the overall utility of these and other countermeasures. Overall, this thesis shows some promise with the findings EKS at rest modulate blood gases and pH, attenuate declines in SpO_2 and cognitive performance under these conditions, likely stemming from this in pH, but other mechanism likely exist suggesting that future research is warranted to understand both mechanisms and practical use.

REFERENCES

ABDUL KADIR, A., CLARKE, K. & EVANS, R. D. 2020. Cardiac ketone body metabolism. *Biochim Biophys Acta Mol Basis Dis,* 1866**,** 165739.

AINSLIE, P. N., OGOH, S., BURGESS, K., CELI, L., MCGRATTAN, K., PEEBLES, K., MURRELL, C., SUBEDI, P. & BURGESS, K. R. 2008. Differential effects of acute hypoxia and high altitude on cerebral blood flow velocity and dynamic cerebral autoregulation: alterations with hyperoxia. *Journal of Applied Physiology (Bethesda, Md.: 1985),* 104**,** 490-498.

ALLEN, B. W., STAMLER, J. S. & PIANTADOSI, C. A. 2009. Hemoglobin, nitric oxide and molecular mechanisms of hypoxic vasodilation. *Trends in molecular medicine,* 15**,** 452-460.

APRAHAMIAN, I., MARTINELLI, J. E., NERI, A. L. & YASSUDA, M. S. 2009. The Clock Drawing Test: A review of its accuracy in screening for dementia. *Dementia & Neuropsychologia,* 3**,** 74-81.

ASMARO, D., MAYALL, J. & FERGUSON, S. 2013. Cognition at altitude: impairment in executive and memory processes under hypoxic conditions. *Aviat Space Environ Med,* 84**,** 1159-65.

BALASSE, E. O. & FERY, F. 1989. Ketone body production and disposal: effects of fasting, diabetes, and exercise. *Diabetes Metab Rev,* 5**,** 247-70.

BALASSE, E. O., FERY, F. & NEEF, M. A. 1978. Changes induced by exercise in rates of turnover and oxidation of ketone bodies in fasting man. *J Appl Physiol Respir Environ Exerc Physiol,* 44**,** 5-11.

BÄRTSCH, P. & GIBBS, J. S. R. 2007. Effect of Altitude on the Heart and the Lungs. *Circulation,* 116**,** 2191-2202.

BÄRTSCH, P., SWENSON, E. R., PAUL, A., JÜLG, B. & HOHENHAUS, E. 2002. Hypoxic ventilatory response, ventilation, gas exchange, and fluid balance in acute mountain sickness. *High Altitude Medicine & Biology,* 3**,** 361-376.

BÄRTSCH, P., WABER, U., HAEBERLI, A., MAGGIORINI, M., KRIEMLER, S., OELZ, O. & STRAUB, W. P. 1987. Enhanced fibrin formation in high-altitude pulmonary edema. *Journal of Applied Physiology (Bethesda, Md.: 1985),* 63**,** 752-757.

BEER, J. M. A., SHENDER, B. S., CHAUVIN, D., DART, T. S. & FISCHER, J. 2017. Cognitive Deterioration in Moderate and Severe Hypobaric Hypoxia Conditions. *Aerosp Med Hum Perform,* 88**,** 617-626.

BERNINI, A., MASOODI, M., SOLARI, D., MIROZ, J. P., CARTERON, L., CHRISTINAT, N., MORELLI, P., BEAUMONT, M., ABED-MAILLARD, S., HARTWEG, M., FOLTZER, F., ECKERT, P., CUENOUD, B. & ODDO, M. 2020. Modulation of cerebral ketone metabolism following traumatic brain injury in humans. *J Cereb Blood Flow Metab,* 40**,** 177-186.

BIRKHAHN, R. H. 1983. Experience with alternative fuels. *In:* JOHNSTON, I. D. A. (ed.) *Advances in Clinical Nutrition.* USA: Springer/MTP Press.

BIRKHAHN, R. H. & BORDER, J. R. 1978. Intravenous feeding of the rat with short chain fatty acid esters. II. Monoacetoacetin. *Am J Clin Nutr,* 31**,** 436-41.

BIRKHAHN, R. H., MCMENAMY, R. H. & BORDER, J. R. 1977. Intravenous feeding of the rat with short chain fatty acid esters. I. Glycerol monobutyrate. *Am J Clin Nutr,* 30**,** 2078-82.

BIRKHAHN, R. H., MCMENAMY, R. H. & BORDER, J. R. 1979. Monoglyceryl acetoacetate: a ketone body-carbohydrate substrate for parenteral feeding of the rat. *J Nutr,* 109**,** 1168-74.

BJURSTEN, H., EDEROTH, P., SIGURDSSON, E., GOTTFREDSSON, M., SYK, I., EINARSSON, O. & GUDBJARTSSON, T. 2010. S100B profiles and cognitive function at high altitude. *High Altitude Medicine & Biology,* 11**,** 31-38.

BLIEMSRIEDER, K., WEISS, E. M., FISCHER, R., BRUGGER, H., SPERNER-UNTERWEGER, B. & HÜFNER, K. 2022. Cognition and Neuropsychological Changes at Altitude-A Systematic Review of Literature. *Brain Sciences,* 12**,** 1736.

BOGDANOVA, A., PETRUSHANKO, I. Y., HERNANSANZ-AGUSTÍN, P. & MARTÍNEZ-RUIZ, A. 2016. "Oxygen Sensing" by Na,K-ATPase: These Miraculous Thiols. *Frontiers in Physiology,* 7**,** 314.

BOOS, C. J., O'HARA, J. P., MELLOR, A., HODKINSON, P. D., TSAKIRIDES, C., REEVE, N., GALLAGHER, L., GREEN, N. D. C. & WOODS, D. R. 2016. A Four-Way Comparison of Cardiac Function with Normobaric Normoxia, Normobaric Hypoxia, Hypobaric Hypoxia and Genuine High Altitude. *PLoS ONE,* 11**,** e0152868.

BRADWELL, A. R., ASHDOWN, K., RUE, C., DELAMERE, J., THOMAS, O. D., LUCAS, S. J. E., WRIGHT, A. D., HARRIS, S. J. & MYERS, S. D. 2018. Acetazolamide reduces exercise capacity following a 5-day ascent to 4559 m in a randomised study. *BMJ Open Sport — Exercise Medicine,* 4**,** e000302.

BRADWELL, A. R., MYERS, S. D., BEAZLEY, M., ASHDOWN, K., HARRIS, N. G., BRADWELL, S. B., GOODHART, J., IMRAY, C. H., WIMALASENA, Y., EDSELL, M. E., PATTINSON, K. T. S., WRIGHT, A. D., HARRIS, S. J. & BIRMINGHAM MEDICAL RESEARCH EXPEDITIONARY, S. 2014. Exercise limitation of acetazolamide at altitude (3459 m). *Wilderness & Environmental Medicine,* 25**,** 272-277.

BROOKS, E., LAMOTHE, G., NAGPAL, T. S., IMBEAULT, P., ADAMO, K., KARA, J. & DOUCET, É. 2022. Acute Ingestion of Ketone Monoesters and Precursors Do Not Enhance Endurance Exercise Performance: A Systematic Review and Meta-Analysis. *Int J Sport Nutr Exerc Metab,* 32**,** 214-225.

BROWNLOW, M. L., JUNG, S. H., MOORE, R. J., BECHMANN, N. & JANKORD, R. 2017. Nutritional Ketosis Affects Metabolism and Behavior in Sprague-Dawley Rats in Both Control and Chronic Stress Environments. *Front Mol Neurosci,* 10**,** 129.

BRUGNIAUX, J. V., HODGES, A. N. H., HANLY, P. J. & POULIN, M. J. 2007. Cerebrovascular responses to altitude. *Respiratory Physiology & Neurobiology,* 158**,** 212-223.

BRUNENGRABER, H. 1997. Potential of ketone body esters for parenteral and oral nutrition. *Nutrition,* 13**,** 233-5.

BUGA, A., KACKLEY, M. L., CRABTREE, C. D., SAPPER, T. N., MCCABE, L., FELL, B., LAFOUNTAIN, R. A., HYDE, P. N., MARTINI, E. R., BOWMAN, J., PAN, Y., SCANDLING, D., BROWNLOW, M. L., O'CONNOR, A., SIMONETTI, O. P., KRAEMER, W. J. & VOLEK, J. S. 2021. The Effects of a 6-Week Controlled, Hypocaloric Ketogenic Diet, With and Without Exogenous Ketone Salts, on Body Composition Responses. *Front Nutr,* 8**,** 618520.

BURKE, L. M. 2021. Ketogenic low-CHO, high-fat diet: the future of elite endurance sport? *J Physiol,* 599**,** 819-843.

BURKE, L. M. & HAWLEY, J. A. 2018. Swifter, higher, stronger: What's on the menu? *Science,* 362**,** 781-787.

CACIANO, S. L., INMAN, C. L., GOCKEL-BLESSING, E. E. & WEISS, E. P. 2015. Effects of dietary Acid load on exercise metabolism and anaerobic exercise performance. *J Sports Sci Med,* 14**,** 364-71.

CAHILL, G. F., JR. 2006. Fuel metabolism in starvation. *Annu Rev Nutr,* 26**,** 1-22.

CAHILL, G. F., JR. & VEECH, R. L. 2003. Ketoacids? Good medicine? *Trans Am Clin Climatol Assoc,* 114**,** 149-61; discussion 162-3.

CAHILL, G. F., OWEN, O. E. & MORGAN, A. P. 1968. The consumption of fuels during prolonged starvation. *Advances in Enzyme Regulation,* 6**,** 143-150.

CAINE, D. & WATSON, J. D. 2000. Neuropsychological and neuropathological sequelae of cerebral anoxia: a critical review. *Journal of the International Neuropsychological Society: JINS,* 6**,** 86-99.

CAMERON, D., SOTO-MOTA, A., WILLIS, D. R., ELLIS, J., PROCTER, N. E. K., GREENWOOD, R., SAUNDERS, N., SCHULTE, R. F., VASSILIOU, V. S., TYLER, D. J., SCHMID, A. I., RODGERS, C. T., MALCOLM, P. N., CLARKE, K., FRENNEAUX, M. P. & VALKOVIČ, L. 2022. Evaluation of Acute Supplementation With the Ketone Ester (R)-3-Hydroxybutyl-(R)-3-Hydroxybutyrate (deltaG) in Healthy Volunteers by Cardiac and Skeletal Muscle (31)P Magnetic Resonance Spectroscopy. *Front Physiol,* 13**,** 793987.

CARLSTEN, A., HALLGREN, B., JAGENBURG, R., SVANBORG, A. & WERKO, L. 1961. Myocardial metabolism of glucose, lactic acid, amino acids and fatty acids in healthy human individuals at rest and at different work loads. *Scand J Clin Lab Invest,* 13**,** 418-28.

CARR, A. J., HOPKINS, W. G. & GORE, C. J. 2011. Effects of acute alkalosis and acidosis on performance: a meta-analysis. *Sports Med,* 41**,** 801-14.

CAVALERI, F. & BASHAR, E. 2018. Potential Synergies of β-Hydroxybutyrate and Butyrate on the Modulation of Metabolism, Inflammation, Cognition, and General Health. *J Nutr Metab,* 2018**,** 7195760.

CHAILLOU, T. 2018. Skeletal Muscle Fiber Type in Hypoxia: Adaptation to High-Altitude Exposure and Under Conditions of Pathological Hypoxia. *Front Physiol,* 9**,** 1450.

CHANDEL, N. S., MCCLINTOCK, D. S., FELICIANO, C. E., WOOD, T. M., MELENDEZ, J. A., RODRIGUEZ, A. M. & SCHUMACKER, P. T. 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. *The Journal of Biological Chemistry,* 275**,** 25130-25138.

CHANG, Y. K., LABBAN, J. D., GAPIN, J. I. & ETNIER, J. L. 2012. The effects of acute exercise on cognitive performance: A meta-analysis. *Brain Research,* 1453**,** 87-101.

CHAUHAN, P., JETHWA, K., RATHAWA, A., CHAUHAN, G. & MEHRA, S. 2021. The Anatomy of the Hippocampus. *In:* PLUTA, R. (ed.) *Cerebral Ischemia.* Brisbane (AU): Exon Publications.

CHEN, O., BLONQUIST, T. M., MAH, E., SANOSHY, K., BECKMAN, D., NIEMAN, K. M., WINTERS, B. L., ANTHONY, J. C., VERDIN, E., NEWMAN, J. C. & STUBBS, B. J. 2021. Tolerability and Safety of a Novel Ketogenic Ester, Bis-Hexanoyl (R)-1,3-Butanediol: A Randomized Controlled Trial in Healthy Adults. *Nutrients,* 13**,** 2066.

CHEON, S. Y., KIM, J. M., KAM, E. H., HO, C.-C., KIM, E. J., CHUNG, S., JEONG, J.-H., LEE, D. D.-H., LEE, S.-W. & KOO, B.-N. 2017. Cell-penetrating interactomic inhibition of nuclear factor-kappa B in a mouse model of postoperative cognitive dysfunction. *Scientific Reports,* 7**,** 13482.

CHROBOCZEK, M., KOSTRZEWA, M., MICIELSKA, K., GRZYWACZ, T. & LASKOWSKI, R. 2021. Effect of Acute Normobaric Hypoxia Exposure on Executive Functions among Young Physically Active Males. *Journal of Clinical Medicine,* 10**,** 1560.

CLARK, D., MUNTEN, S., HERZIG, K. H. & GAGNON, D. D. 2021. Exogenous Ketone Salt Supplementation and Whole-Body Cooling Do Not Improve Short-Term Physical Performance. *Front Nutr,* 8**,** 663206.

CLARKE, K., TCHABANENKO, K., PAWLOSKY, R., CARTER, E., KING, M. T., MUSA-VELOSO, K., HO, M., ROBERTS, A., ROBERTSON, J., VANITALLIE, T. B. & VEECH, R. L. 2012a. Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory toxicology and pharmacology : RTP,* 63.

CLARKE, K., TCHABANENKO, K., PAWLOSKY, R., CARTER, E., TODD KING, M., MUSA-VELOSO, K., HO, M., ROBERTS, A., ROBERTSON, J., VANITALLIE, T. B. & VEECH, R. L. 2012b. Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regul Toxicol Pharmacol,* 63**,** 401-8.

CLEGG, M. E. 2010. Medium-chain triglycerides are advantageous in promoting weight loss although not beneficial to exercise performance. *Int J Food Sci Nutr,* 61**,** 653-79.

COLEMAN, K., PHILLIPS, J., SCIARINI, M., STUBBS, B., JACKSON, O. & KERNAGIS, D. 2021. A Metabolic Intervention for Improving Human Cognitive Performance During Hypoxia. *Aerosp Med Hum Perform,* 92**,** 556-562.

COMMITTEE ON PSYCHOLOGICAL TESTING, I. V. T., POPULATIONS, B. O. T. H. O. S. & MEDICINE, I. O. 2015. Cognitive Tests and Performance Validity Tests. *Psychological Testing in the Service of Disability Determination.* National Academies Press (US).

COPPEL, J., HENNIS, P., GILBERT-KAWAI, E. & GROCOTT, M. P. 2015. The physiological effects of hypobaric hypoxia versus normobaric hypoxia: a systematic review of crossover trials. *Extrem Physiol Med,* 4**,** 2.

COURCHESNE-LOYER, A., CROTEAU, E., CASTELLANO, C.-A., ST-PIERRE, V., HENNEBELLE, M. & CUNNANE, S. C. 2017. Inverse relationship between brain glucose and ketone metabolism in adults during short-term moderate dietary ketosis: A dual tracer quantitative positron emission tomography study. *Journal of Cerebral Blood Flow & Metabolism,* 37**,** 2485-2493.

COURCHESNE-LOYER, A., FORTIER, M., TREMBLAY-MERCIER, J., CHOUINARD-WATKINS, R., ROY, M., NUGENT, S., CASTELLANO, C.-A. & CUNNANE, S. C. 2013. Stimulation of mild, sustained ketonemia by medium-chain triacylglycerols in healthy humans: estimated potential contribution to brain energy metabolism. *Nutrition (Burbank, Los Angeles County, Calif.),* 29**,** 635-640.

COX, P. J. & CLARKE, K. 2014. Acute nutritional ketosis: implications for exercise performance and metabolism. *Extrem Physiol Med,* 3**,** 17.

COX, P. J., KIRK, T., ASHMORE, T., WILLERTON, K., EVANS, R., SMITH, A., MURRAY, A. J., STUBBS, B., WEST, J., MCLURE, S. W., KING, M. T., DODD, M. S., HOLLOWAY, C., NEUBAUER, S., DRAWER, S., VEECH, R. L., GRIFFIN, J. L. & CLARKE, K. 2016. Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metab,* 24**,** 256-268.

CRABTREE, C. D., BLADE, T., HYDE, P. N., BUGA, A., KACKLEY, M. L., SAPPER, T. N., PANDA, O., ROA-DIAZ, S., ANTHONY, J. C., NEWMAN, J. C., VOLEK, J. S. & STUBBS, B. J. 2022. Bis Hexanoyl (R)-1,3-Butanediol, a Novel Ketogenic Ester, Acutely Increases Circulating r- and s-ß-Hydroxybutyrate Concentrations in Healthy Adults. *J Am Nutr Assoc*.

CRABTREE, C. D., BLADE, T., HYDE, P. N., BUGA, A., KACKLEY, M. L., SAPPER, T. N., PANDA, O., ROA-DIAZ, S., ANTHONY, J. C., NEWMAN, J. C., VOLEK, J. S. & STUBBS, B. J. 2023. Bis Hexanoyl (R)-1,3-Butanediol, a Novel Ketogenic Ester, Acutely Increases Circulating r- and s-ß-Hydroxybutyrate Concentrations in Healthy Adults. *Journal of the American Nutrition Association,* 42**,** 169-177.

CUNNANE, S. C., COURCHESNE-LOYER, A., ST-PIERRE, V., VANDENBERGHE, C., PIEROTTI, T., FORTIER, M., CROTEAU, E. & CASTELLANO, C.-A. 2016a. Can ketones compensate for deteriorating brain glucose uptake during aging? Implications for the risk and treatment of Alzheimer's disease. *Annals of the New York Academy of Sciences,* 1367**,** 12-20.

CUNNANE, S. C., COURCHESNE-LOYER, A., VANDENBERGHE, C., ST-PIERRE, V., FORTIER, M., HENNEBELLE, M., CROTEAU, E., BOCTI, C., FULOP, T. & CASTELLANO, C. A. 2016b. Can Ketones Help Rescue Brain Fuel Supply in Later Life? Implications for Cognitive Health during Aging and the Treatment of Alzheimer's Disease. *Front Mol Neurosci,* 9**,** 53.

CUNNANE, S. C., NUGENT, S., ROY, M., COURCHESNE-LOYER, A., CROTEAU, E., TREMBLAY, S., CASTELLANO, A., PIFFERI, F., BOCTI, C., PAQUET, N., BEGDOURI, H., BENTOURKIA, M., TURCOTTE, E., ALLARD, M., BARBERGER-GATEAU, P., FULOP, T. & RAPOPORT, S. 2011. BRAIN FUEL METABOLISM, AGING AND ALZHEIMER'S DISEASE. *Nutrition (Burbank, Los Angeles County, Calif.),* 27**,** 3-20.

CUNNANE, S. C., TRUSHINA, E., MORLAND, C., PRIGIONE, A., CASADESUS, G., ANDREWS, Z. B., BEAL, M. F., BERGERSEN, L. H., BRINTON, R. D., DE LA MONTE, S., ECKERT, A., HARVEY, J., JEGGO, R., JHAMANDAS, J. H., KANN, O., LA COUR, C. M., MARTIN, W. F., MITHIEUX, G., MOREIRA, P. I., MURPHY, M. P., NAVE, K. A., NURIEL, T., OLIET, S. H. R., SAUDOU, F., MATTSON, M. P., SWERDLOW, R. H. & MILLAN, M. J. 2020. Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. *Nat Rev Drug Discov,* 19**,** 609-633.

D'AGOSTINO, D. P., PILLA, R., HELD, H. E., LANDON, C. S., PUCHOWICZ, M., BRUNENGRABER, H., ARI, C., ARNOLD, P. & DEAN, J. B. 2013. Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. *Am J Physiol Regul Integr Comp Physiol,* 304**,** R829-36.

DAINES, S. A. 2021. The Therapeutic Potential and Limitations of Ketones in Traumatic Brain Injury. *Front Neurol,* 12**,** 723148.

DAUTZENBERG, G., LIJMER, J. & BEEKMAN, A. 2020. Diagnostic accuracy of the Montreal Cognitive Assessment (MoCA) for cognitive screening in old age psychiatry: Determining cutoff scores in

clinical practice. Avoiding spectrum bias caused by healthy controls. *International Journal of Geriatric Psychiatry,* 35**,** 261-269.

DAVIES, D. W. 1959. Acetazolamide therapy with renal complications. *British Medical Journal,* 1**,** 214-215.

DE AQUINO LEMOS, V., ANTUNES, H. K. M., DOS SANTOS, R. V. T., LIRA, F. S., TUFIK, S. & DE MELLO, M. T. 2012. High altitude exposure impairs sleep patterns, mood, and cognitive functions. *Psychophysiology,* 49**,** 1298-1306.

DE OLIVEIRA, L. F., DOLAN, E., SWINTON, P. A., DURKALEC-MICHALSKI, K., ARTIOLI, G. G., MCNAUGHTON, L. R. & SAUNDERS, B. 2022. Extracellular Buffering Supplements to Improve Exercise Capacity and Performance: A Comprehensive Systematic Review and Meta-analysis. *Sports Med,* 52**,** 505-526.

DEARLOVE, D. J., FAULL, O. K., ROLLS, E., CLARKE, K. & COX, P. J. 2019. Nutritional Ketoacidosis During Incremental Exercise in Healthy Athletes. *Front Physiol,* 10**,** 290.

DEARLOVE, D. J., HARRISON, O. K., HODSON, L., JEFFERSON, A., CLARKE, K. & COX, P. J. 2021a. The Effect of Blood Ketone Concentration and Exercise Intensity on Exogenous Ketone Oxidation Rates in Athletes. *Med Sci Sports Exerc,* 53**,** 505-516.

DEARLOVE, D. J., HOLDSWORTH, D., KIRK, T., HODSON, L., CHARIDEMOU, E., KVALHEIM, E., STUBBS, B., BEEVERS, A., GRIFFIN, J. L., EVANS, R., ROBERTSON, J., CLARKE, K. & COX, P. J. 2021b. β-Hydroxybutyrate Oxidation in Exercise Is Impaired by Low-Carbohydrate and High-Fat Availability. *Front Med (Lausanne),* 8**,** 721673.

DEARLOVE, D. J., SOTO MOTA, A., HAUTON, D., PINNICK, K., EVANS, R., MILLER, J., FISCHER, R., MCCULLAGH, J. S. O., HODSON, L., CLARKE, K. & COX, P. J. 2022. The effects of endogenously- and exogenously-induced hyperketonemia on exercise performance and adaptation. *Physiol Rep,* 10**,** e15309.

DESROCHERS, S., DUBREUIL, P., BRUNET, J., JETTÉ, M., DAVID, F., LANDAU, B. R. & BRUNENGRABER, H. 1995. Metabolism of (R,S)-1,3-butanediol acetoacetate esters, potential parenteral and enteral nutrients in conscious pigs. *Am J Physiol,* 268**,** E660-7.

DI PAOLA, M., CALTAGIRONE, C., FADDA, L., SABATINI, U., SERRA, L. & CARLESIMO, G. A. 2008. Hippocampal atrophy is the critical brain change in patients with hypoxic amnesia. *Hippocampus,* 18**,** 719-728.

DIAMOND, A. 2013. Executive Functions. *Annual review of psychology,* 64**,** 135-168.

DINENNO, F. A. 2016. Skeletal muscle vasodilation during systemic hypoxia in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985),* 120**,** 216-225.

DUNHAM-SNARY, K. J., WU, D., SYKES, E. A., THAKRAR, A., PARLOW, L. R. G., MEWBURN, J. D., PARLOW, J. L. & ARCHER, S. L. 2017. Hypoxic Pulmonary Vasoconstriction: From Molecular Mechanisms to Medicine. *Chest,* 151**,** 181-192.

EDWARDS, C., CANFIELD, J., COPES, N., REHAN, M., LIPPS, D. & BRADSHAW, P. C. 2014. D-beta-hydroxybutyrate extends lifespan in C. elegans. *Aging (Albany NY),* 6**,** 621-44.

ELVIDGE, G. P., GLENNY, L., APPELHOFF, R. J., RATCLIFFE, P. J., RAGOUSSIS, J. & GLEADLE, J. M. 2006. Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition: the role of HIF-1alpha, HIF-2alpha, and other pathways. *The Journal of Biological Chemistry,* 281**,** 15215-15226.

EPSTEIN, A. C., GLEADLE, J. M., MCNEILL, L. A., HEWITSON, K. S., O'ROURKE, J., MOLE, D. R., MUKHERJI, M., METZEN, E., WILSON, M. I., DHANDA, A., TIAN, Y. M., MASSON, N., HAMILTON, D. L., JAAKKOLA, P., BARSTEAD, R., HODGKIN, J., MAXWELL, P. H., PUGH, C. W., SCHOFIELD, C. J. & RATCLIFFE, P. J. 2001. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell,* 107**,** 43-54.

EVANS, M., COGAN, K. E. & EGAN, B. 2017. Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *J Physiol,* 595**,** 2857-2871.

EVANS, M. & EGAN, B. 2018. Intermittent Running and Cognitive Performance after Ketone Ester Ingestion. *Med Sci Sports Exerc,* 50**,** 2330-2338.

EVANS, M., MCCLURE, T. S., KOUTNIK, A. P. & EGAN, B. 2022. Exogenous Ketone Supplements in Athletic Contexts: Past, Present, and Future. *Sports Med,* 52**,** 25-67.

EVANS, M., MCSWINEY, F. T., BRADY, A. J. & EGAN, B. 2019. No Benefit of Ingestion of a Ketone Monoester Supplement on 10-km Running Performance. *Med Sci Sports Exerc,* 51**,** 2506-2515.

EVANS, M., PATCHETT, E., NALLY, R., KEARNS, R., LARNEY, M. & EGAN, B. 2018. Effect of acute ingestion of beta-hydroxybutyrate salts on the response to graded exercise in trained cyclists. *Eur J Sport Sci,* 18**,** 376-386.

FAISS, R., PIALOUX, V., SARTORI, C., FAES, C., DÉRIAZ, O. & MILLET, G. P. 2013. Ventilation, oxidative stress, and nitric oxide in hypobaric versus normobaric hypoxia. *Medicine and Science in Sports and Exercise,* 45**,** 253-260.

FALKENHAIN, K., DARAEI, A., FORBES, S. C. & LITTLE, J. P. 2022. Effects of Exogenous Ketone Supplementation on Blood Glucose: A Systematic Review and Meta-Analysis. *Adv Nutr*.

FALLA, M., MICARELLI, A., HÜFNER, K. & STRAPAZZON, G. 2021a. The Effect of Cold Exposure on Cognitive Performance in Healthy Adults: A Systematic Review. *International Journal of Environmental Research and Public Health,* 18**,** 9725.

FALLA, M., PAPAGNO, C., DAL CAPPELLO, T., VÖGELE, A., HÜFNER, K., KIM, J., WEISS, E. M., WEBER, B., PALMA, M., MRAKIC-SPOSTA, S., BRUGGER, H. & STRAPAZZON, G. 2021b. A Prospective Evaluation of the Acute Effects of High Altitude on Cognitive and Physiological Functions in Lowlanders. *Frontiers in Physiology,* 12**,** 670278.

FAULL, O. K., DEARLOVE, D. J., CLARKE, K. & COX, P. J. 2019. Beyond RPE: The Perception of Exercise Under Normal and Ketotic Conditions. *Front Physiol,* 10**,** 229.

FERY, F. & BALASSE, E. O. 1983. Ketone body turnover during and after exercise in overnight-fasted and starved humans. *Am J Physiol,* 245**,** E318-25.

FERY, F. & BALASSE, E. O. 1986. Response of ketone body metabolism to exercise during transition from postabsorptive to fasted state. *Am J Physiol,* 250**,** E495-501.

FERY, F. & BALASSE, E. O. 1988. Effect of exercise on the disposal of infused ketone bodies in humans. *J Clin Endocrinol Metab,* 67**,** 245-50.

FERY, F., FRANKEN, P., NEEF, M. A. & BALASSE, E. O. 1974. Influence of muscular exercise on the rates of uptake and oxidation of infused ketone bodies in normal man. *Arch Int Physiol Biochim,* 82**,** 381-5.

FISCHER, T., OCH, U., KLAWON, I., OCH, T., GRUNEBERG, M., FOBKER, M., BORDEWICK-DELL, U. & MARQUARDT, T. 2018. Effect of a Sodium and Calcium DL-beta-Hydroxybutyrate Salt in Healthy Adults. *J Nutr Metab,* 2018**,** 9812806.

FORD, K. & GLYMOUR, C. 2014. The enhanced warfighter. *Bulletin of the Atomic Scientists,* 70**,** 43-53.

FRAYN, K. N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol,* 55**,** 628-634.

FREUND, G. & WEINSIER, R. L. 1966. Standardized ketosis in man following medium chain triglyceride ingestion. *Metabolism,* 15**,** 980-91.

FROST, S., E ORR, J., OEUNG, B., PUVVULA, N., PHAM, K., BRENA, R., DEYOUNG, P., JAIN, S., SUN, S., MALHOTRA, A. & HEINRICH, E. C. 2021. Improvements in sleep-disordered breathing during acclimatization to 3800 m and the impact on cognitive function. *Physiological Reports,* 9**,** e14827.

FUKUDA, R., ZHANG, H., KIM, J.-W., SHIMODA, L., DANG, C. V. & SEMENZA, G. L. 2007. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell,* 129**,** 111-122.

GARLAND, P. B., RANDLE, P. J. & NEWSHOLME, E. A. 1963. Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes, and starvation. *Nature,* 200**,** 169-70.

GARVER, M. J., SCHEADLER, C. M., SMITH, L. M., TAYLOR, S. J. & HARBACH, C. M. 2018. Simulated Altitude via Re-Breathing Creates Arterial Hypoxemia but Fails to Improve Elements of Running Performance. *International Journal of Exercise Science,* 11**,** 187.

GERTZ, E. W., WISNESKI, J. A., STANLEY, W. C. & NEESE, R. A. 1988. Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J Clin Invest,* 82**,** 2017-25.

GIBSON, G. E. & PETERSON, C. 1982. Decreases in the release of acetylcholine in vitro with low oxygen. *Biochemical Pharmacology,* 31**,** 111-115.

GIBSON, G. E., PULSINELLI, W., BLASS, J. P. & DUFFY, T. E. 1981. Brain dysfunction in mild to moderate hypoxia. *The American Journal of Medicine,* 70**,** 1247-1254.

GNAIGER, E. 2001. Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respiration Physiology,* 128**,** 277-297.

GOM, R. C., BHATT, D., VILLA, B. R., GEORGE, A. G., LOHMAN, A. W., MYCHASIUK, R., RHO, J. M. & TESKEY, G. C. 2021. The ketogenic diet raises brain oxygen levels, attenuates postictal hypoxia, and protects against learning impairments. *Neurobiol Dis,* 154**,** 105335.

GOODALL, S., TWOMEY, R. & AMANN, M. 2014. Acute and chronic hypoxia: implications for cerebral function and exercise tolerance. *Fatigue,* 2**,** 73-92.

GOODWIN, G. W., AHMAD, F., DOENST, T. & TAEGTMEYER, H. 1998a. Energy provision from glycogen, glucose, and fatty acids on adrenergic stimulation of isolated working rat hearts. *Am J Physiol,* 274**,** H1239-47.

GOODWIN, G. W., TAYLOR, C. S. & TAEGTMEYER, H. 1998b. Regulation of energy metabolism of the heart during acute increase in heart work. *J Biol Chem,* 273**,** 29530-9.

GORMSEN, L. C., SVART, M., THOMSEN, H. H., SONDERGAARD, E., VENDELBO, M. H., CHRISTENSEN, N., TOLBOD, L. P., HARMS, H. J., NIELSEN, R., WIGGERS, H., JESSEN, N., HANSEN, J., BOTKER, H. E. & MOLLER, N. 2017. Ketone Body Infusion With 3-Hydroxybutyrate Reduces Myocardial Glucose Uptake and Increases Blood Flow in Humans: A Positron Emission Tomography Study. *J Am Heart Assoc,* 6**,** e005066.

GRAHAM, T. E., BATTRAM, D. S., DELA, F., EL-SOHEMY, A. & THONG, F. S. 2008. Does caffeine alter muscle carbohydrate and fat metabolism during exercise? *Appl. Physiol Nutr. Metab,* 33**,** 1311-1318.

GRISSOM, C. K., ROACH, R. C., SARNQUIST, F. H. & HACKETT, P. H. 1992. Acetazolamide in the treatment of acute mountain sickness: clinical efficacy and effect on gas exchange. *Annals of Internal Medicine,* 116**,** 461-465.

GRIVA, K., STYGALL, J., WILSON, M. H., MARTIN, D., LEVETT, D., MITCHELL, K., MYTHEN, M., MONTGOMERY, H. E., GROCOTT, M. P., AREF-ADIB, G., EDSELL, M., PLANT, T., IMRAY, C., COOKE, D., HARRINGTON, J., KHOSRAVI, M. & NEWMAN, S. P. 2017. Caudwell Xtreme Everest: A prospective study of the effects of environmental hypoxia on cognitive functioning. *PLoS ONE,* 12**,** e0174277.

GUZY, R. D., HOYOS, B., ROBIN, E., CHEN, H., LIU, L., MANSFIELD, K. D., SIMON, M. C., HAMMERLING, U. & SCHUMACKER, P. T. 2005. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metabolism,* 1**,** 401-408.

HACES, M. L., HERNÁNDEZ-FONSECA, K., MEDINA-CAMPOS, O. N., MONTIEL, T., PEDRAZA-CHAVERRI, J. & MASSIEU, L. 2008. Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. *Experimental Neurology,* 211**,** 85-96.

HAGENFELDT, L. & WAHREN, J. 1968. Human forearm muscle metabolism during exercise. 3. Uptake, release and oxidation of beta-hydroxybutyrate and observations on the beta-hydroxybutyrate/acetoacetate ratio. *Scand J Clin Lab Invest,* 21**,** 314-20.

HAGENFELDT, L. & WAHREN, J. 1971. Human forearm muscle metabolism during exercise. VI. Substrate utilization in prolonged fasting. *Scand J Clin Lab Invest,* 27**,** 299-306.

HARRIS, G. A., CLELAND, J., COLLIE, A. & MCCRORY, P. 2009. Cognitive assessment of a trekking expedition to 5100 m: a comparison of computerized and written testing methods. *Wilderness & Environmental Medicine,* 20**,** 261-268.

HARVEY, P. D. 2019. Domains of cognition and their assessment. *Dialogues in Clinical Neuroscience,* 21**,** 227-237.

HASBROUCQ, T., TANDONNET, C., MICALLEF-ROLL, J., BLIN, O. & POSSAMAÏ, C.-A. 2003. An electromyographic analysis of the effect of levodopa on the response time of healthy subjects. *Psychopharmacology,* 165**,** 313-316.

HASHIM, S. A., ROHOLT, H. B. & VAN ITALLIE, T. B. 1962. Pancreatogenous steatorrhea treated with medium chain triglycerides. *Clin. Res,* 10**,** 394.

HASHIM, S. A. & VANITALLIE, T. B. 2014. Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. *J Lipid Res,* 55**,** 1818-26.

HASSELBALCH, S. G., KNUDSEN, G. M., JAKOBSEN, J., HAGEMAN, L. P., HOLM, S. & PAULSON, O. B. 1995. Blood-brain barrier permeability of glucose and ketone bodies during short-term starvation in humans. *The American Journal of Physiology,* 268**,** E1161-1166.

HASSELBALCH, S. G., MADSEN, P. L., HAGEMAN, L. P., OLSEN, K. S., JUSTESEN, N., HOLM, S. & PAULSON, O. B. 1996. Changes in cerebral blood flow and carbohydrate metabolism during acute hyperketonemia. *Am J Physiol,* 270**,** E746-51.

HEMMINGSSON, T. & LINNARSSON, D. 2009. Lower exhaled nitric oxide in hypobaric than in normobaric acute hypoxia. *Respiratory Physiology & Neurobiology,* 169**,** 74-77.

HIGENBOTTAM, T., OGG, C. S. & SAXTON, H. M. 1978. Acute renal failure from the use of acetazolamide (Diamox). *Postgraduate Medical Journal,* 54**,** 127-128.

HO, K. L., KARWI, Q. G., WAGG, C., ZHANG, L., VO, K., ALTAMIMI, T., UDDIN, G. M., USSHER, J. R. & LOPASCHUK, G. D. 2021. Ketones can become the major fuel source for the heart but do not increase cardiac efficiency. *Cardiovasc Res,* 117**,** 1178-1187.

HOCHACHKA, P. W., BUCK, L. T., DOLL, C. J. & LAND, S. C. 1996. Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proceedings of the National Academy of Sciences of the United States of America,* 93**,** 9493-9498.

HOHENAUER, E., FREITAG, L., COSTELLO, J. T., WILLIAMS, T. B., KÜNG, T., TAUBE, W., HERTEN, M. & CLIJSEN, R. 2022. The effects of normobaric and hypobaric hypoxia on cognitive performance and physiological responses: A crossover study. *PLOS ONE,* 17**,** e0277364.

HOILAND, R. L., BAIN, A. R., RIEGER, M. G., BAILEY, D. M. & AINSLIE, P. N. 2016. Hypoxemia, oxygen content, and the regulation of cerebral blood flow. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 310**,** R398-R413.

HOLDSWORTH, D. A., COX, P. J., KIRK, T., STRADLING, H., IMPEY, S. G. & CLARKE, K. 2017. A Ketone Ester Drink Increases Postexercise Muscle Glycogen Synthesis in Humans. *Med Sci Sports Exerc,* 49**,** 1789-1795.

HONIGMAN, B., THEIS, M. K., KOZIOL-MCLAIN, J., ROACH, R., YIP, R., HOUSTON, C., MOORE, L. G. & PEARCE, P. 1993. Acute mountain sickness in a general tourist population at moderate altitudes. *Annals of Internal Medicine,* 118**,** 587-592.

HUANG, R., HUESTIS, M., GAN, E. S., OOI, E. E. & OHH, M. 2021. Hypoxia and viral infectious diseases. *JCI Insight,* 6.

IMRAY, C., WRIGHT, A., SUBUDHI, A. & ROACH, R. 2010. Acute Mountain Sickness: Pathophysiology, Prevention, and Treatment. *Progress in Cardiovascular Diseases,* 52**,** 467-484.

ISSA, A. N., HERMAN, N. M., WENTZ, R. J., TAYLOR, B. J., SUMMERFIELD, D. C. & JOHNSON, B. D. 2016. Association of Cognitive Performance with Time at Altitude, Sleep Quality, and Acute Mountain Sickness Symptoms. *Wilderness & Environmental Medicine,* 27**,** 371-378.

JAMES, S. & KJERULF GREER, B. 2019. Influence of Exogenous β-Hydroxybutyrate on Walking Economy and Rating of Perceived Exertion. *J Diet Suppl,* 16**,** 463-469.

JELLEMA, R. K., LIMA PASSOS, V., ZWANENBURG, A., OPHELDERS, D. R. M. G., DE MUNTER, S., VANDERLOCHT, J., GERMERAAD, W. T. V., KUYPERS, E., COLLINS, J. J. P., CLEUTJENS, J. P. M., JENNEKENS, W., GAVILANES, A. W. D., SEEHASE, M., VLES, H. J., STEINBUSCH, H., ANDRIESSEN, P., WOLFS, T. G. A. M. & KRAMER, B. W. 2013. Cerebral inflammation and mobilization of the peripheral immune system following global hypoxia-ischemia in preterm sheep. *Journal of Neuroinflammation,* 10**,** 13.

JENSEN, J. D. & VINCENT, A. L. 2023. High Altitude Pulmonary Edema. *StatPearls.* Treasure Island (FL): StatPearls Publishing.

JEUKENDRUP, A. E., SARIS, W. H., BROUNS, F., HALLIDAY, D. & WAGENMAKERS, J. M. 1996. Effects of carbohydrate (CHO) and fat supplementation on CHO metabolism during prolonged exercise. *Metabolism,* 45**,** 915-21.

JEUKENDRUP, A. E., THIELEN, J. J., WAGENMAKERS, A. J., BROUNS, F. & SARIS, W. H. 1998. Effect of medium-chain triacylglycerol and carbohydrate ingestion during exercise on substrate utilization and subsequent cycling performance. *Am J Clin Nutr,* 67**,** 397-404.

JEUKENDRUP, A. E. & WALLIS, G. A. 2005. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int. J. Sports Med,* 26**,** S28-S37.

JO, E., SILVA, S. C., T., A. A., ARREGLADO, J. P., ELAM, N. L., OSMOND, A. D., STEINBERG, R. & WONG, M. W. H. 2022. The Effects of 10-Day Exogenous Ketone Consumption on Repeated Time Trial Running Performances: A Randomized-Control Trial. *J Diet Suppl,* 19**,** 19-34.

JONES, W. P., LOE, S. A., KRACH, S. K., RAGER, R. Y. & JONES, H. M. 2008. Automated neuropsychological assessment metrics (ANAM) and Woodcock-Johnson III Tests of Cognitive Ability: a concurrent validity study. *The Clinical Neuropsychologist,* 22**,** 305-320.

KACKLEY, M. L., SHORT, J. A., HYDE, P. N., LAFOUNTAIN, R. A., BUGA, A., MILLER, V. J., DICKERSON, R. M., SAPPER, T. N., BARNHART, E. C., KRISHNAN, D., MCELROY, C. A., MARESH, C. M., KRAEMER, W. J. & VOLEK, J. S. 2020. A Pre-Workout Supplement of Ketone Salts, Caffeine, and Amino Acids Improves High-Intensity Exercise Performance in Keto-Naïve and Keto-Adapted Individuals. *J Am Coll Nutr,* 39**,** 290-300.

KALYANARAMAN, B. 2013. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. *Redox Biology,* 1**,** 244-257.

KARAMANLI, H., ILIK, F., KAYHAN, F. & PAZARLI, A. C. 2015. Assessment of cognitive impairment in long-term oxygen therapy-dependent COPD patients. *Int J Chron Obstruct Pulmon Dis,* 10**,** 2087-94.

KARINEN, H., PELTONEN, J. & TIKKANEN, H. 2008. Prevalence of acute mountain sickness among Finnish trekkers on Mount Kilimanjaro, Tanzania: an observational study. *High Altitude Medicine & Biology,* 9**,** 301-306.

KARWI, Q. G. & LOPASCHUK, G. D. 2021. CrossTalk proposal: Ketone bodies are an important metabolic fuel for the heart. *J Physiol,* 600**,** 1001-1004.

KASHIWAYA, Y., KING, M. T. & VEECH, R. L. 1997. Substrate signaling by insulin: a ketone bodies ratio mimics insulin action in heart. *Am J Cardiol,* 80**,** 50a-64a.

KELLER, U., LUSTENBERGER, M., MULLER-BRAND, J., GERBER, P. P. & STAUFFACHER, W. 1989. Human ketone body production and utilization studied using tracer techniques: regulation by free fatty acids, insulin, catecholamines, and thyroid hormones. *Diabetes Metab Rev,* 5**,** 285-98.

KEMPPAINEN, J., FUJIMOTO, T., KALLIOKOSKI, K. K., VILJANEN, T., NUUTILA, P. & KNUUTI, J. 2002. Myocardial and skeletal muscle glucose uptake during exercise in humans. *J Physiol,* 542**,** 403-12.

KESL, S. L., POFF, A. M., WARD, N. P., FIORELLI, T. N., ARI, C., VAN PUTTEN, A. J., SHERWOOD, J. W., ARNOLD, P. & D'AGOSTINO, D. P. 2016. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague-Dawley rats. *Nutr Metab (Lond),* 13**,** 9.

KIES, C., TOBIN, R. B., FOX, H. M. & MEHLMAN, M. A. 1973. Utilization of 1,3-butanediol and nonspecific nitrogen in human adults. *J Nutr,* 103**,** 1155-63.

KILLGORE, W. D. S. 2010. Effects of sleep deprivation on cognition. *Progress in Brain Research,* 185**,** 105-129.

KIM, D. K., HEINEMAN, F. W. & BALABAN, R. S. 1991. Effects of beta-hydroxybutyrate on oxidative metabolism and phosphorylation potential in canine heart in vivo. *Am J Physiol,* 260**,** H1767-73.

KIM, J.-W., TCHERNYSHYOV, I., SEMENZA, G. L. & DANG, C. V. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. *Cell Metabolism,* 3**,** 177-185.

KIMURA, I., INOUE, D., MAEDA, T., HARA, T., ICHIMURA, A., MIYAUCHI, S., KOBAYASHI, M., HIRASAWA, A. & TSUJIMOTO, G. 2011. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A,* 108**,** 8030-5.

KOLWICZ, S. C., JR. 2018. An "Exercise" in Cardiac Metabolism. *Front Cardiovasc Med,* 5**,** 66.

KOUTNIK, A. P., D'AGOSTINO, D. P. & EGAN, B. 2019. Anticatabolic Effects of Ketone Bodies in Skeletal Muscle. *Trends Endocrinol Metab,* 30**,** 227-229.

KUO, C. C., FATTOR, J. A., HENDERSON, G. C. & BROOKS, G. A. 2005. Lipid oxidation in fit young adults during postexercise recovery. *J Appl. Physiol,* 99**,** 349-356.

KUO, P. T. & HUANG, N. N. 1965. The effect of medium chain triglyceride upon fat absorption and plasma lipid and depot fat of children with cystic fibrosis of the pancreas. *J Clin Invest,* 44**,** 1924-33.

KUSCHINSKY, W., SUDA, S. & SOKOLOFF, L. 1981. Local cerebral glucose utilization and blood flow during metabolic acidosis. *The American Journal of Physiology,* 241**,** H772-777.

LAFFEL, L. 1999. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev,* 15**,** 412-26.

LANCHA JUNIOR, A. H., PAINELLI VDE, S., SAUNDERS, B. & ARTIOLI, G. G. 2015. Nutritional Strategies to Modulate Intracellular and Extracellular Buffering Capacity During High-Intensity Exercise. *Sports Med,* 45 Suppl 1**,** S71-81.

LARSON, E. B., ROACH, R. C., SCHOENE, R. B. & HORNBEIN, T. F. 1982. Acute mountain sickness and acetazolamide. Clinical efficacy and effect on ventilation. *JAMA,* 248**,** 328-332.

LASSEN, N. A. & HARPER, A. M. 1975. Letter: High-altitude cerebral oedema. *Lancet (London, England),* 2**,** 1154.

LATSHANG, T. D., LO CASCIO, C. M., STÖWHAS, A.-C., GRIMM, M., STADELMANN, K., TESLER, N., ACHERMANN, P., HUBER, R., KOHLER, M. & BLOCH, K. E. 2013. Are Nocturnal Breathing, Sleep, and Cognitive Performance Impaired at Moderate Altitude (1,630-2,590 m)? *Sleep,* 36**,** 1969-1976.

LAUGHLIN, M. R., TAYLOR, J., CHESNICK, A. S. & BALABAN, R. S. 1994. Nonglucose substrates increase glycogen synthesis in vivo in dog heart. *Am J Physiol,* 267**,** H219-23.

LEAF, D. E. & GOLDFARB, D. S. 2007. Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness. *J Appl Physiol (1985),* 102**,** 1313-22.

LECKEY, J. J., ROSS, M. L., QUOD, M., HAWLEY, J. A. & BURKE, L. M. 2017. Ketone Diester Ingestion Impairs Time-Trial Performance in Professional Cyclists. *Front Physiol,* 8**,** 806.

LEFFERTS, W. K., DEBLOIS, J. P., SORIANO, J. E., MANN, L., RAMPURI, Z., HERRINGTON, B., THRALL, S., BIRD, J., HARMAN, T. S., DAY, T. A., HEFFERNAN, K. S. & BRUTSAERT, T. D. 2020. Preservation of Neurovascular Coupling to Cognitive Activity in Anterior Cerebrovasculature During Incremental Ascent to High Altitude. *High Altitude Medicine & Biology,* 21**,** 20-27.

LEGG, S. J., GILBEY, A., HILL, S., RAMAN, A., DUBRAY, A., IREMONGER, G. & MÜNDEL, T. 2016. Effects of mild hypoxia in aviation on mood and complex cognition. *Appl Ergon,* 53 Pt B**,** 357-63.

LEZAK, M. D., HOWIESON, D. B., BIGLER, E. D. & TRANEL, D. 2012. *Neuropsychological assessment, 5th ed,* New York, NY, US, Oxford University Press.

LIM, D. C. & PACK, A. I. 2014. Obstructive sleep apnea and cognitive impairment: addressing the blood-brain barrier. *Sleep Medicine Reviews,* 18**,** 35-48.

LIM, S. Y. & KWAK, Y.-S. 2019. Effect of nutrients and exhaustive exercise on brain function. *Journal of Exercise Rehabilitation,* 15**,** 341-345.

LINCOLN, B. C., DES ROSIERS, C. & BRUNENGRABER, H. 1987. Metabolism of S-3-hydroxybutyrate in the perfused rat liver. *Arch Biochem Biophys,* 259**,** 149-56.

LITTLE, J. R., GOTO, M. & SPITZER, J. J. 1970. Effect of ketones on metabolism of FFA by dog myocardium and skeletal muscle in vivo. *Am J Physiol,* 219**,** 1458-63.

LIU, Z., YANG, Z., GU, Y., LIU, H. & WANG, P. 2021. The effectiveness of eye tracking in the diagnosis of cognitive disorders: A systematic review and meta-analysis. *PLoS ONE,* 16**,** e0254059.

LOCKWOOD, A. H., PEEK, K. E., IZUMIYAMA, M., YAP, E. W. & LABOVE, J. 1989. Effects of moderate hypoxemia and unilateral carotid ligation on cerebral glucose metabolism and acid-base balance in the rat. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism,* 9**,** 342-349.

LOEPPKY, J. A., ICENOGLE, M., SCOTTO, P., ROBERGS, R., HINGHOFER-SZALKAY, H. & ROACH, R. C. 1997. Ventilation during simulated altitude, normobaric hypoxia and normoxic hypobaria. *Respiration Physiology,* 107**,** 231-239.

LØKKEN, N., STORGAARD, J. H., REVSBECH, K. L., VOERMANS, N. C., VAN HALL, G., VISSING, J. & ØRNGREEN, M. C. 2022. No effect of oral ketone ester supplementation on exercise capacity in patients with McArdle disease and healthy controls: a randomized placebo-controlled cross-over study. *J Inherit Metab Dis,* 45**,** 502-516.

LOWDER, J., FALLAH, S., VENDITTI, C., MUSA-VELOSO, K. & KOTLOV, V. 2023. An open-label, acute clinical trial in adults to assess ketone levels, gastrointestinal tolerability, and sleepiness following consumption of (R)-1,3-butanediol (Avela™). *Front Physiol,* 14**,** 1195702.

LUMB, A. B. & SLINGER, P. 2015. Hypoxic Pulmonary Vasoconstriction. *Anesthesiology,* 122**,** 932-946.

MAALOUF, M., SULLIVAN, P. G., DAVIS, L., KIM, D. Y. & RHO, J. M. 2007. KETONES INHIBIT MITOCHONDRIAL PRODUCTION OF REACTIVE OXYGEN SPECIES PRODUCTION FOLLOWING GLUTAMATE EXCITOTOXICITY BY INCREASING NADH OXIDATION. *Neuroscience,* 145**,** 256-264.

MADISON, L. L., MEBANE, D., UNGER, R. H. & LOCHNER, A. 1964. The Hypoglycemic Action of Ketones. II. Evidence for a Stimulatory Feedback of Ketones on the Pancreatic Beta Cells *. *Journal of Clinical Investigation,* 43**,** 408-415.

MAGGIORINI, M., BÜHLER, B., WALTER, M. & OELZ, O. 1990. Prevalence of acute mountain sickness in the Swiss Alps. *BMJ (Clinical research ed.),* 301**,** 853-855.

MAHAJAN, V. R., NADEL, J. A., KING, M. T., PAWLOSKY, R. J., DAVIS, M. I., VEECH, R. L., LOVINGER, D. M. & SALINAS, A. G. 2022. Ketone ester-enriched diet ameliorates motor and dopamine release deficits in MitoPark mice. bioRxiv.

MAIZELS, E. Z., RUDERMAN, N. B., GOODMAN, M. N. & LAU, D. 1977. Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat. *Biochem J,* 162**,** 557-68.

MALLE, C., GINON, B. & BOURRILHON, C. 2016. Brief Working Memory and Physiological Monitoring During a High-Altitude Expedition. *High Altitude Medicine & Biology,* 17**,** 359-364.

MALLE, C., QUINETTE, P., LAISNEY, M., BOURRILHON, C., BOISSIN, J., DESGRANGES, B., EUSTACHE, F. & PIÉRARD, C. 2013. Working memory impairment in pilots exposed to acute hypobaric hypoxia. *Aviat Space Environ Med,* 84**,** 773-9.

MANK, M. M., REED, L. F., WALTON, C. J., BARUP, M. L. T., ATHER, J. L. & POYNTER, M. E. 2022. Therapeutic ketosis decreases methacholine hyperresponsiveness in mouse models of inherent obese asthma. *Am J Physiol Lung Cell Mol Physiol,* 322**,** L243-l257.

MANSOR, L. S. & WOO, G. H. 2020. Ketones for Post-exercise Recovery: Potential Applications and Mechanisms. *Front Physiol,* 11**,** 613648.

MARDIMAE, A., BALABAN, D. Y., MACHINA, M. A., HAN, J. S., KATZNELSON, R., MINKOVICH, L. L., FEDORKO, L., MURPHY, P. M., WASOWICZ, M., NAUGHTON, F., MEINERI, M., FISHER, J. A. & DUFFIN, J. 2012. The interaction of carbon dioxide and hypoxia in the control of cerebral blood flow. *Pflügers Archiv - European Journal of Physiology,* 464**,** 345-351.

MARGOLIS, L. M. & O'FALLON, K. S. 2020. Utility of Ketone Supplementation to Enhance Physical Performance: A Systematic Review. *Adv Nutr,* 11**,** 412-419.

MARTEN, B., PFEUFFER, M. & SCHREZENMEIR, J. 2006. Medium-chain triglycerides. *International Dairy Journal,* 16**,** 1374-1382.

MARTIN-ARROWSMITH, P. W., LOV, J., DAI, J., MORAIS, J. A. & CHURCHWARD-VENNE, T. A. 2020. Ketone Monoester Supplementation Does Not Expedite the Recovery of Indices of Muscle Damage After Eccentric Exercise. *Front Nutr,* 7**,** 607299.

MCCARTHY, C. G., WAIGI, E. W., SINGH, G., CASTANEDA, T. R., MELL, B., CHAKRABORTY, S., WENCESLAU, C. F. & JOE, B. 2021a. Physiologic, Metabolic, and Toxicologic Profile of 1,3-Butanediol. *J Pharmacol Exp Ther,* 379**,** 245-252.

MCCARTHY, D. G., BOSTAD, W., BONE, J., POWLEY, F. J., RICHARDS, D. L. & GIBALA, M. J. 2023. Effect of Acute Ketone Monoester Ingestion on Cardiorespiratory Responses to Exercise and the Influence of Blood Acidosis. *Medicine and Science in Sports and Exercise,* 55**,** 1286-1295.

MCCARTHY, D. G., BOSTAD, W., POWLEY, F. J., LITTLE, J. P., RICHARDS, D. L. & GIBALA, M. J. 2021b. Increased cardiorespiratory stress during submaximal cycling after ketone monoester ingestion in endurance-trained adults. *Appl Physiol Nutr Metab,* 46**,** 986-993.

MCMORRIS, T., HALE, B. J., BARWOOD, M., COSTELLO, J. & CORBETT, J. 2017. Effect of acute hypoxia on cognition: A systematic review and meta-regression analysis. *Neurosci Biobehav Rev,* 74**,** 225-232.

MCMORRIS, T., TURNER, A., HALE, B. J. & SPROULE, J. 2016. Beyond the Catecholamines Hypothesis for an Acute Exercise–Cognition Interaction. *Exercise-Cognition Interaction.* Elsevier.

MICHIELS, C. 2004. Physiological and pathological responses to hypoxia. *Am J Pathol,* 164**,** 1875-82.

MIKKELSEN, K. H., SEIFERT, T., SECHER, N. H., GRONDAL, T. & VAN HALL, G. 2015. Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-beta-hydroxybutyratemia in post-absorptive healthy males. *J Clin Endocrinol Metab,* 100**,** 636-43.

MILLAR, L. J., SHI, L., HOERDER-SUABEDISSEN, A. & MOLNÁR, Z. 2017. Neonatal Hypoxia Ischaemia: Mechanisms, Models, and Therapeutic Challenges. *Frontiers in Cellular Neuroscience,* 11**,** 78.

MILLER, V. J., LAFOUNTAIN, R. A., BARNHART, E., SAPPER, T. S., SHORT, J., ARNOLD, W. D., HYDE, P. N., CRABTREE, C. D., KACKLEY, M. L., KRAEMER, W. J., VILLAMENA, F. A. & VOLEK, J. S. 2020. A ketogenic diet combined with exercise alters mitochondrial function in human skeletal muscle while improving metabolic health. *Am J Physiol Endocrinol Metab,* 319**,** E995-e1007.

MILLIGAN, L. P. & MCBRIDE, B. W. 1985. Energy costs of ion pumping by animal tissues. *The Journal of Nutrition,* 115**,** 1374-1382.

MUJICA-PARODI, L. R., AMGALAN, A., SULTAN, S. F., ANTAL, B., SUN, X., SKIENA, S., LITHEN, A., ADRA, N., RATAI, E. M., WEISTUCH, C., GOVINDARAJAN, S. T., STREY, H. H., DILL, K. A., STUFFLEBEAM, S. M., VEECH, R. L. & CLARKE, K. 2020. Diet modulates brain network stability, a biomarker for brain aging, in young adults. *Proc Natl Acad Sci U S A,* 117**,** 6170-6177.

MURMAN, D. L. 2015. The Impact of Age on Cognition. *Seminars in Hearing,* 36**,** 111-121.

MURRAY, A. J., KNIGHT, N. S., COLE, M. A., COCHLIN, L. E., CARTER, E., TCHABANENKO, K., PICHULIK, T., GULSTON, M. K., ATHERTON, H. J., SCHROEDER, M. A., DEACON, R. M., KASHIWAYA, Y., KING, M. T., PAWLOSKY, R., RAWLINS, J. N., TYLER, D. J., GRIFFIN, J. L., ROBERTSON, J., VEECH, R. L. & CLARKE, K. 2016. Novel ketone diet enhances physical and cognitive performance. *Faseb j,* 30**,** 4021-4032.

MYETTE-COTE, E., CALDWELL, H. G., AINSLIE, P. N., CLARKE, K. & LITTLE, J. P. 2019. A ketone monoester drink reduces the glycemic response to an oral glucose challenge in individuals with obesity: a randomized trial. *Am J Clin Nutr,* 110**,** 1491-1501.

MYETTE-CÔTÉ, É., NEUDORF, H., RAFIEI, H., CLARKE, K. & LITTLE, J. P. 2018. Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. *J Physiol,* 596**,** 1385-1395.

MYETTE-CÔTÉ, É., SOTO-MOTA, A. & CUNNANE, S. C. 2021. Ketones: potential to achieve brain energy rescue and sustain cognitive health during ageing. *Br J Nutr***,** 1-17.

NAKAGATA, T., TAMURA, Y., KAGA, H., SATO, M., YAMASAKI, N., SOMEYA, Y., KADOWAKI, S., SUGIMOTO, D., SATOH, H., KAWAMORI, R. & WATADA, H. 2021. Ingestion of an exogenous ketone monoester improves the glycemic response during oral glucose tolerance test in individuals with impaired glucose tolerance: A cross-over randomized trial. *Journal of Diabetes Investigation,* 12**,** 756-762.

NAUGHTON, M. T., ROCHFORD, P. D., PRETTO, J. J., PIERCE, R. J., CAIN, N. F. & IRVING, L. B. 1995. Is normobaric simulation of hypobaric hypoxia accurate in chronic airflow limitation? *American Journal of Respiratory and Critical Care Medicine,* 152**,** 1956-1960.

NEWMAN, J. C. & VERDIN, E. 2017. β-Hydroxybutyrate: A Signaling Metabolite. *Annu Rev Nutr,* 37**,** 51-76.

NEWSHOLME, E. A., RANDLE, P. J. & MANCHESTER, K. L. 1962. Inhibition of the phosphofructokinase reaction in perfused rat heart by respiration of ketone bodies, fatty acids and pyruvate. *Nature,* 193**,** 270-1.

NEYRA, J. A., ALVAREZ-MAZA, J. C. & NOVAK, J. E. 2014. Anuric Acute Kidney Injury Induced by Acute Mountain Sickness Prophylaxis With Acetazolamide. *Journal of Investigative Medicine High Impact Case Reports,* 2**,** 2324709614530559.

NIELSEN, R., MØLLER, N., GORMSEN, L. C., TOLBOD, L. P., HANSSON, N. H., SORENSEN, J., HARMS, H. J., FRØKIÆR, J., EISKJAER, H., JESPERSEN, N. R., MELLEMKJAER, S., LASSEN, T. R., PRYDS, K., BØTKER, H. E. & WIGGERS, H. 2019. Cardiovascular Effects of Treatment With the Ketone Body 3-Hydroxybutyrate in Chronic Heart Failure Patients. *Circulation,* 139**,** 2129-2141.

NORWITZ, N. G., DEARLOVE, D. J., LU, M., CLARKE, K., DAWES, H. & HU, M. T. 2020. A Ketone Ester Drink Enhances Endurance Exercise Performance in Parkinson's Disease. *Front Neurosci,* 14**,** 584130.

NORWITZ, N. G., WINWOOD, R., STUBBS, B. J., D'AGOSTINO, D. P. & BARNES, P. J. 2021. Case Report: Ketogenic Diet Is Associated With Improvements in Chronic Obstructive Pulmonary Disease. *Front Med (Lausanne),* 8**,** 699427.

O'MALLEY, T., MYETTE-COTE, E., DURRER, C. & LITTLE, J. P. 2017. Nutritional ketone salts increase fat oxidation but impair high-intensity exercise performance in healthy adult males. *Appl Physiol Nutr Metab,* 42**,** 1031-1035.

OCHI, G., KANAZAWA, Y., HYODO, K., SUWABE, K., SHIMIZU, T., FUKUIE, T., BYUN, K. & SOYA, H. 2018. Hypoxia-induced lowered executive function depends on arterial oxygen desaturation. *J Physiol Sci,* 68**,** 847-853.

OECHMICHEN, M. & MEISSNER, C. 2006. Cerebral hypoxia and ischemia: the forensic point of view: a review. *Journal of Forensic Sciences,* 51**,** 880-887.

OGOH, S., NAKAHARA, H., UEDA, S., OKAZAKI, K., SHIBASAKI, M., SUBUDHI, A. W. & MIYAMOTO, T. 2014. Effects of acute hypoxia on cerebrovascular responses to carbon dioxide. *Experimental Physiology,* 99**,** 849-858.

OGOH, S., SATO, K., NAKAHARA, H., OKAZAKI, K., SUBUDHI, A. W. & MIYAMOTO, T. 2013. Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. *Experimental Physiology,* 98**,** 692-698.

OMORI, N. E., WOO, G. H. & MANSOR, L. S. 2022. Exogenous Ketones and Lactate as a Potential Therapeutic Intervention for Brain Injury and Neurodegenerative Conditions. *Frontiers in Human Neuroscience,* 16**,** 846183.

PAN, J. W., TELANG, F. W., LEE, J. H., DE GRAAF, R. A., ROTHMAN, D. L., STEIN, D. T. & HETHERINGTON, H. P. 2001. Measurement of beta-hydroxybutyrate in acute hyperketonemia in human brain. *Journal of Neurochemistry,* 79**,** 539-544.

PASIAKOS, S. M., KARL, J. P. & MARGOLIS, L. M. 2021. Challenging traditional carbohydrate intake recommendations for optimizing performance at high altitude. *Curr Opin Clin Nutr Metab Care,* 24**,** 483-489.

PAULSON, O. B., STRANDGAARD, S. & EDVINSSON, L. 1990. Cerebral autoregulation. *Cerebrovascular and Brain Metabolism Reviews,* 2**,** 161-192.

PAVLOV, N., HAYNES, A. G., STUCKI, A., JÜNI, P. & OTT, S. R. 2018. Long-term oxygen therapy in COPD patients: population-based cohort study on mortality. *Int J Chron Obstruct Pulmon Dis,* 13**,** 979-988.

PEACOCK, O. J., GONZALEZ, J. T., ROBERTS, S. P., SMITH, A., DRAWER, S. & STOKES, K. A. 2022. Ketone Monoester Ingestion Alters Metabolism and Simulated Rugby Performance in Professional Players. *Int J Sport Nutr Exerc Metab,* 32**,** 334-341.

PETIET, C. A., TOWNES, B. D., BROOKS, R. J. & KRAMER, J. H. 1988. Neurobehavioral and psychosocial functioning of women exposed to high altitude in mountaineering. *Perceptual and Motor Skills,* 67**,** 443-452.

PETRICK, H. L., BRUNETTA, H. S., PIGNANELLI, C., NUNES, E. A., VAN LOON, L. J. C., BURR, J. F. & HOLLOWAY, G. P. 2020. In vitro ketone-supported mitochondrial respiration is minimal when other substrates are readily available in cardiac and skeletal muscle. *J Physiol,* 598**,** 4869-4885.

PIERRE, K. & PELLERIN, L. 2005. Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *Journal of Neurochemistry,* 94**,** 1-14.

POFF, A. M., KOUTNIK, A. P. & EGAN, B. 2020. Nutritional Ketosis with Ketogenic Diets or Exogenous Ketones: Features, Convergence, and Divergence. *Curr Sports Med Rep,* 19**,** 251-259.

POFF, A. M., MOSS, S., SOLIVEN, M. & D'AGOSTINO, D. P. 2021. Ketone Supplementation: Meeting the Needs of the Brain in an Energy Crisis. *Front Nutr,* 8**,** 783659.

POFFÉ, C. & HESPEL, P. 2020. Ketone bodies: beyond their role as a potential energy substrate in exercise. *J Physiol,* 598**,** 4749-4750.

POFFÉ, C., RAMAEKERS, M., BOGAERTS, S. & HESPEL, P. 2020. Exogenous ketosis impacts neither performance nor muscle glycogen breakdown in prolonged endurance exercise. *J Appl Physiol (1985),* 128**,** 1643-1653.

POFFÉ, C., RAMAEKERS, M., BOGAERTS, S. & HESPEL, P. 2021a. Bicarbonate Unlocks the Ergogenic Action of Ketone Monoester Intake in Endurance Exercise. *Med Sci Sports Exerc,* 53**,** 431-441.

POFFÉ, C., RAMAEKERS, M., VAN THIENEN, R. & HESPEL, P. 2019. Ketone ester supplementation blunts overreaching symptoms during endurance training overload. *J Physiol,* 597**,** 3009-3027.

POFFÉ, C., ROBBERECHTS, R., PODLOGAR, T., KUSTERS, M., DEBEVEC, T. & HESPEL, P. 2021b. Exogenous ketosis increases blood and muscle oxygenation but not performance during exercise in hypoxia. *Am J Physiol Regul Integr Comp Physiol,* 321**,** R844-r857.

POFFÉ, C., ROBBERECHTS, R., STALMANS, M., VANDERROOST, J., BOGAERTS, S. & HESPEL, P. 2023. Exogenous ketosis increases circulating dopamine concentration and maintains mental alertness in ultra-endurance exercise. *J Appl Physiol (1985),* 134**,** 1456-1469.

POFFÉ, C., WYNS, F., RAMAEKERS, M. & HESPEL, P. 2021c. Exogenous Ketosis Impairs 30-min Time-Trial Performance Independent of Bicarbonate Supplementation. *Med Sci Sports Exerc,* 53**,** 1068-1078.

POSCH, A. M., DANDORF, S. & HILE, D. C. 2018. The Effects of Acetazolamide on Exercise Performance at Sea Level and in Hypoxic Environments: A Review. *Wilderness Environ Med,* 29**,** 541-545.

PRADHAN, G. N., OTTESTAD, W., MELAND, A., KÅSIN, J. I., HØISETH, L. Ø., CEVETTE, M. J. & STEPANEK, J. 2021. Oculometric Feature Changes During Acute Hypoxia in a Simulated High-Altitude Airdrop Scenario. *Aerospace Medicine and Human Performance,* 92**,** 928-936.

PREGNOLATO, S., CHAKKARAPANI, E., ISLES, A. & LUYT, K. 2019. Glutamate Transport and Preterm Brain Injury. *Frontiers in physiology,* 10.

PRINS, P. J., BUXTON, J. D., MCCLURE, T. S., D'AGOSTINO, D. P., AULT, D. L., WELTON, G. L., JONES, D. W., ATWELL, A. D., SLACK, M. A., SLACK, M. L., WILLIAMS, C. E., BLANCHFLOWER, M. E., KANNEL, K. K., FAULKNER, M. N., SZMACIASZ, H. L., CROLL, S. M., STANFORTH, L. M., HARRIS, T. D., GWALTNEY, H. C. & KOUTNIK, A. P. 2021. Ketone Bodies Impact on Hypoxic CO2 Retention Protocol During Exercise. *Front Physiol,* 12**,** 780755.

PRINS, P. J., D'AGOSTINO, D. P., ROGERS, C. Q., AULT, D. L., WELTON, G. L., JONES, D. W., HENSON, S. R., ROTHFUSS, T. J., AIKEN, K. G., HOSE, J. L., ENGLAND, E. L., ATWELL, A. D., BUXTON, J. D. & KOUTNIK, A. P. 2020a. Dose response of a novel exogenous ketone supplement on physiological, perceptual and performance parameters. *Nutr Metab (Lond),* 17**,** 81.

PRINS, P. J., KOUTNIK, A. P., D'AGOSTINO, D. P., ROGERS, C. Q., SEIBERT, J. F., BRECKENRIDGE, J. A., JACKSON, D. S., RYAN, E. J., BUXTON, J. D. & AULT, D. L. 2020b. Effects of an Exogenous Ketone Supplement on Five-Kilometer Running Performance. *J Hum Kinet,* 72**,** 115-127.

PUCHALSKA, P. & CRAWFORD, P. A. 2021. Metabolic and Signaling Roles of Ketone Bodies in Health and Disease. *Annu Rev Nutr,* 41**,** 49-77.

PUN, M., HARTMANN, S. E., FURIAN, M., DYCK, A. M., MURALT, L., LICHTBLAU, M., BADER, P. R., RAWLING, J. M., ULRICH, S., BLOCH, K. E. & POULIN, M. J. 2018. Effect of Acute, Subacute, and Repeated Exposure to High Altitude (5050 m) on Psychomotor Vigilance. *Frontiers in Physiology,* 9**,** 677.

QAZI, A. S., MOORE, A. R. & HOLLAND-WINKLER, A. M. 2022. Anaerobic, aerobic, and cognitive outcomes are similar after supplementing with exogenous ketone salts and a sports drink. *Int J Health Nutr Exerc Sci,* 1**,** 101-122.

QUINONES, M. D. & LEMON, P. W. R. 2022a. Acute Ketone Salts-Caffeine-Taurine-Leucine Supplementation but not Ketone Salts-Taurine-Leucine, Improves Endurance Cycling Performance. *Int J Sport Nutr Exerc Metab,* 32**,** 238-245.

QUINONES, M. D. & LEMON, P. W. R. 2022b. Ketone Ester Supplementation Improves Some Aspects of Cognitive Function during a Simulated Soccer Match after Induced Mental Fatigue. *Nutrients,* 14**,** 4376.

RANDLE, P. J., NEWSHOLME, E. A. & GARLAND, P. B. 1964. Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. *Biochem J,* 93**,** 652-65.

REAVEN, G. 2005. All obese individuals are not created equal: insulin resistance is the major determinant of cardiovascular disease in overweight/obese individuals. *Diabetes & Vascular Disease Research,* 2**,** 105-112.

RESEARCHANDMARKETS.COM. 2021. *Ketones - Global Market Trajectory & Analytics* [Online]. Available: https://www.researchandmarkets.com/r/pzjjgx [Accessed 02/04/2022 2022].

RIHET, P., POSSAMAÏ, C.-A., MICALLEF-ROLL, J., BLIN, O. & HASBROUCQ, T. 2002. Dopamine and human information processing: a reaction-time analysis of the effect of levodopa in healthy subjects. *Psychopharmacology,* 163**,** 62-67.

RITCHIE, N. D., BAGGOTT, A. V. & ANDREW TODD, W. T. 2012. Acetazolamide for the Prevention of Acute Mountain Sickness—A Systematic Review and Meta-analysis. *Journal of Travel Medicine,* 19**,** 298-307.

RITTIG, N., SVART, M., THOMSEN, H. H., VESTERGAARD, E. T., REHFELD, J. F., HARTMANN, B., HOLST, J. J., JOHANNSEN, M., MØLLER, N. & JESSEN, N. 2020. Oral D/L-3-Hydroxybutyrate Stimulates Cholecystokinin and Insulin Secretion and Slows Gastric Emptying in Healthy Males. *J Clin Endocrinol Metab,* 105**,** dgaa483.

ROACH, R. C., LOEPPKY, J. A. & ICENOGLE, M. V. 1996. Acute mountain sickness: increased severity during simulated altitude compared with normobaric hypoxia. *Journal of Applied Physiology (Bethesda, Md.: 1985),* 81**,** 1908-1910.

ROBACH, P., CALBET, J. A., THOMSEN, J. J., BOUSHEL, R., MOLLARD, P., RASMUSSEN, P. & LUNDBY, C. 2008. The ergogenic effect of recombinant human erythropoietin on VO2max depends on the severity of arterial hypoxemia. *PLoS One,* 3**,** e2996.

ROBINSON, A. M. & WILLIAMSON, D. H. 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev,* 60**,** 143-87.

RODGER, S., PLEWS, D., LAURSEN, P. & DRILLER, M. W. 2017. Oral β-hydroxybutyrate salt fails to improve 4-minute cycling performance following submaximal exercise. *J Sci Cycling,* 6**,** 26-31.

ROLFE, D. F. & BROWN, G. C. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev,* 77**,** 731-758.

ROSA, E. D., HANNIGAN, C., BRENNAN, S., REILLY, R., RAPČAN, V. & ROBERTSON, I. H. 2014. Reliability and validity of the Automatic Cognitive Assessment Delivery (ACAD). *Frontiers in Aging Neuroscience,* 6**,** 34.

ROSENBERG, G. A. 2017. Extracellular matrix inflammation in vascular cognitive impairment and dementia. *Clinical Science (London, England: 1979),* 131**,** 425-437.

RUSSELL, R. R., 3RD, CLINE, G. W., GUTHRIE, P. H., GOODWIN, G. W., SHULMAN, G. I. & TAEGTMEYER, H. 1997. Regulation of exogenous and endogenous glucose metabolism by insulin and acetoacetate in the isolated working rat heart. A three tracer study of glycolysis, glycogen metabolism, and glucose oxidation. *J Clin Invest,* 100**,** 2892-9.

RYU, S., SHCHUKINA, I., YOUM, Y. H., QING, H., HILLIARD, B., DLUGOS, T., ZHANG, X., YASUMOTO, Y., BOOTH, C. J., FERNÁNDEZ-HERNANDO, C., SUÁREZ, Y., KHANNA, K., HORVATH, T. L., DIETRICH, M. O., ARTYOMOV, M., WANG, A. & DIXIT, V. D. 2021. Ketogenic diet restrains aging-induced exacerbation of coronavirus infection in mice. *Elife,* 10.

SARIS, C. G. J. & TIMMERS, S. 2022. Ketogenic diets and Ketone suplementation: A strategy for therapeutic intervention. *Frontiers in Nutrition,* 9**,** 947567.

SARKAR, M., NIRANJAN, N. & BANYAL, P. K. 2017. Mechanisms of hypoxemia. *Lung India,* 34**,** 47-60.

SAVOUREY, G., LAUNAY, J.-C., BESNARD, Y., GUINET, A. & TRAVERS, S. 2003. Normo- and hypobaric hypoxia: are there any physiological differences? *European Journal of Applied Physiology,* 89**,** 122-126.

SAVOUREY, G., LAUNAY, J.-C., BESNARD, Y., GUINET-LEBRETON, A., ALONSO, A., SAUVET, F. & BOURRILHON, C. 2007. Normo or hypobaric hypoxic tests: propositions for the determination of the individual susceptibility to altitude illnesses. *European Journal of Applied Physiology,* 100**,** 193-205.

SAXENA, K. & JOLLY, M. K. 2019. Acute vs. Chronic vs. Cyclic Hypoxia: Their Differential Dynamics, Molecular Mechanisms, and Effects on Tumor Progression. *Biomolecules,* 9**,** 339.

SCHMIT, C., HAUSSWIRTH, C., LE MEUR, Y. & DUFFIELD, R. 2017. Cognitive Functioning and Heat Strain: Performance Responses and Protective Strategies. *Sports Medicine (Auckland, N.Z.),* 47**,** 1289-1302.

SCHNEIDER, M., BERNASCH, D., WEYMANN, J., HOLLE, R. & BARTSCH, P. 2002. Acute mountain sickness: influence of susceptibility, preexposure, and ascent rate. *Medicine and Science in Sports and Exercise,* 34**,** 1886-1891.

SCHÖNFELD, P. & WOJTCZAK, L. 2016. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. *J Lipid Res,* 57**,** 943-54.

SCOFIELD, R. F., BRADY, P. S., SCHUMANN, W. C., KUMARAN, K., OHGAKU, S., MARGOLIS, J. M. & LANDAU, B. R. 1982. On the lack of formation of L-(+)-3-hydroxybutyrate by liver. *Arch Biochem Biophys,* 214**,** 268-72.

SCOTT, B. E., LAURSEN, P. B., JAMES, L. J., BOXER, B., CHANDLER, Z., LAM, E., GASCOYNE, T., MESSENGER, J. & MEARS, S. A. 2019. The effect of 1,3-butanediol and carbohydrate supplementation on running performance. *J Sci Med Sport,* 22**,** 702-706.

SELF, D. A., MANDELLA, J. G., PRINZO, O. V., FORSTER, E. M. & SHAFFSTALL, R. M. 2011. Physiological equivalence of normobaric and hypobaric exposures of humans to 25,000 feet (7620 m). *Aviation, Space, and Environmental Medicine,* 82**,** 97-103.

SEMENZA, G. L. 2007. Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. *The Biochemical Journal,* 405**,** 1-9.

SEMENZA, G. L., JIANG, B. H., LEUNG, S. W., PASSANTINO, R., CONCORDET, J. P., MAIRE, P. & GIALLONGO, A. 1996. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem,* 271**,** 32529-37.

SEMENZA, G. L., ROTH, P. H., FANG, H. M. & WANG, G. L. 1994. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *The Journal of Biological Chemistry,* 269**,** 23757-23763.

SENIOR, B. & LORIDAN, L. 1968. Direct regulatory effect of ketones on lipolysis and on glucose concentrations in man. *Nature,* 219**,** 83-84.

SEO, Y., BURNS, K., FENNELL, C., KIM, J.-H., GUNSTAD, J., GLICKMAN, E. & MCDANIEL, J. 2015. The Influence of Exercise on Cognitive Performance in Normobaric Hypoxia. *High Altitude Medicine & Biology,* 16**,** 298-305.

SHAH, P., LIU, X., HODGETTS, T. & SII, F. 2018. Acetazolamide can cause acute hypercrystalluria. *BMJ***,** k3400.

SHARP, F. R. & BERNAUDIN, M. 2004. HIF1 and oxygen sensing in the brain. *Nature Reviews. Neuroscience,* 5**,** 437-448.

SHAW, D. M., CABRE, G. & GANT, N. 2021. Hypoxic Hypoxia and Brain Function in Military Aviation: Basic Physiology and Applied Perspectives. *Front Physiol,* 12**,** 665821.

SHAW, D. M., MERIEN, F., BRAAKHUIS, A., MAUNDER, E. & DULSON, D. K. 2020. Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States. *Sports Med,* 50**,** 641-656.

SHAW, D. M., MERIEN, F., BRAAKHUIS, A., PLEWS, D., LAURSEN, P. & DULSON, D. K. 2019. The Effect of 1,3-Butanediol on Cycling Time-Trial Performance. *Int J Sport Nutr Exerc Metab,* 29**,** 466-473.

SHERWIN, R. S., HENDLER, R. G. & FELIG, P. 1976. Effect of diabetes mellitus and insulin on the turnover and metabolic response to ketones in man. *Diabetes,* 25**,** 776-84.

SHI, Q. H., GE, D., ZHAO, W., MA, X., HU, K. Y., LU, Y., LIU, Z. X., RAN, J. H., LI, X. L., ZHOU, Y. & FU, J. F. 2016. A Computerized Evaluation of Sensory Memory and Short-term Memory Impairment After Rapid Ascent to 4280 m. *Biomedical and environmental sciences: BES,* 29**,** 457-460.

SHIVVA, V., COX, P. J., CLARKE, K., VEECH, R. L., TUCKER, I. G. & DUFFULL, S. B. 2016. The Population Pharmacokinetics of D-beta-hydroxybutyrate Following Administration of (R)-3-Hydroxybutyl (R)-3-Hydroxybutyrate. *Aaps j,* 18**,** 678-88.

SHUKRALLA, A. A., DOLAN, E. & DELANTY, N. 2022. Acetazolamide: Old drug, new evidence? *Epilepsia Open,* 7**,** 378-392.

SOETERS, M. R., SERLIE, M. J., SAUERWEIN, H. P., DURAN, M., RUITER, J. P., KULIK, W., ACKERMANS, M. T., MINKLER, P. E., HOPPEL, C. L., WANDERS, R. J. & HOUTEN, S. M.

2012. Characterization of D-3-hydroxybutyrylcarnitine (ketocarnitine): an identified ketosis-induced metabolite. *Metabolism,* 61**,** 966-73.

SOTO-MOTA, A., NORWITZ, N. G., EVANS, R., CLARKE, K. & BARBER, T. M. 2021. Exogenous ketosis in patients with type 2 diabetes: Safety, tolerability and effect on glycaemic control. *Endocrinol Diabetes Metab,* 4**,** e00264.

SOTO-MOTA, A., NORWITZ, N. G., EVANS, R. D. & CLARKE, K. 2022. Exogenous d-β-hydroxybutyrate lowers blood glucose in part by decreasing the availability of L-alanine for gluconeogenesis. *Endocrinology, Diabetes & Metabolism,* 5**,** e00300.

SOTO-MOTA, A., VANSANT, H., EVANS, R. D. & CLARKE, K. 2019. Safety and tolerability of sustained exogenous ketosis using ketone monoester drinks for 28 days in healthy adults. *Regul Toxicol Pharmacol,* 109**,** 104506.

STALEY, K. J., SOLDO, B. L. & PROCTOR, W. R. 1995. Ionic Mechanisms of Neuronal Excitation by Inhibitory GABAA Receptors. *Science,* 269**,** 977-981.

STEINBACK, C. D. & POULIN, M. J. 2007. Ventilatory responses to isocapnic and poikilocapnic hypoxia in humans. *Respiratory Physiology & Neurobiology,* 155**,** 104-113.

STELLINGWERFF, T., SPRIET, L. L., WATT, M. J., KIMBER, N. E., HARGREAVES, M., HAWLEY, J. A. & BURKE, L. M. 2006. Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *Am. J. Physiol Endocrinol. Metab,* 290**,** E380-E388.

STEPANEK, J., PRADHAN, G. N., COCCO, D., SMITH, B. E., BARTLETT, J., STUDER, M., KUHN, F. & CEVETTE, M. J. 2014. Acute hypoxic hypoxia and isocapnic hypoxia effects on oculometric features. *Aviat Space Environ Med,* 85**,** 700-7.

STUBBS, B. J., BLADE, T., MILLS, S., THOMAS, J., YUFEI, X., NELSON, F. R., HIGLEY, N., NIKIFOROV, A. I., RHINER, M. O., VERDIN, E. & NEWMAN, J. C. 2021a. In vitro stability and in vivo pharmacokinetics of the novel ketogenic ester, bis hexanoyl (R)-1,3-butanediol. *Food Chem Toxicol,* 147**,** 111859.

STUBBS, B. J., COX, P. J., EVANS, R. D., CYRANKA, M., CLARKE, K. & DE WET, H. 2018. A Ketone Ester Drink Lowers Human Ghrelin and Appetite. *Obesity (Silver Spring),* 26**,** 269-273.

STUBBS, B. J., COX, P. J., EVANS, R. D., SANTER, P., MILLER, J. J., FAULL, O. K., MAGOR-ELLIOTT, S., HIYAMA, S., STIRLING, M. & CLARKE, K. 2017. On the metabolism of exogenous ketones in humans. *Front Physiol,* 8**,** 848.

STUBBS, B. J., COX, P. J., KIRK, T., EVANS, R. D. & CLARKE, K. 2019. Gastrointestinal Effects of Exogenous Ketone Drinks are Infrequent, Mild, and Vary According to Ketone Compound and Dose. *Int J Sport Nutr Exerc Metab,* 29**,** 596-603.

STUBBS, B. J., KOUTNIK, A. P., GOLDBERG, E. L., UPADHYAY, V., TURNBAUGH, P. J., VERDIN, E. & NEWMAN, J. C. 2020. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. *Med (N Y),* 1**,** 43-65.

STUBBS, B. J., NIKIFOROV, A. I., RIHNER, M. O., WESTON, S., HIGLEY, N., ROY, S., DAKOULAS, E., VERDIN, E. & NEWMAN, J. C. 2021b. Genetic Toxicity Studies of the Ketogenic Ester Bis Hexanoyl (R)-1,3-Butanediol. *Int J Toxicol,* 40**,** 242-249.

STUBBS, B. J., NIKIFOROV, A. I., RIHNER, M. O., WESTON, S. L., HIGLEY, N., STUMP, D. G., KRANE, G. A., GADUPUDI, G., VERDIN, E. & NEWMAN, J. C. 2021c. Toxicological evaluation of the ketogenic ester bis hexanoyl (R)-1,3-butanediol: Subchronic toxicity in Sprague Dawley rats. *Food Chem Toxicol,* 150**,** 112084.

SUBUDHI, A. W., BOURDILLON, N., BUCHER, J., DAVIS, C., ELLIOTT, J. E., EUTERMOSTER, M., EVERO, O., FAN, J.-L., HOUTEN, S. J.-V., JULIAN, C. G., KARK, J., KARK, S., KAYSER, B., KERN, J. P., KIM, S. E., LATHAN, C., LAURIE, S. S., LOVERING, A. T., PATERSON, R., POLANER, D. M., RYAN, B. J., SPIRA, J. L., TSAO, J. W., WACHSMUTH, N. B. & ROACH, R. C. 2014. AltitudeOmics: The Integrative Physiology of Human Acclimatization to Hypobaric Hypoxia and Its Retention upon Reascent. *PLOS ONE,* 9**,** e92191.

SUBUDHI, A. W., DIMMEN, A. C. & ROACH, R. C. 2007. Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. *J Appl Physiol (1985),* 103**,** 177-83.

SUBUDHI, A. W., PANERAI, R. B. & ROACH, R. C. 2009. Acute hypoxia impairs dynamic cerebral autoregulation: results from two independent techniques. *Journal of Applied Physiology,* 107**,** 1165-1171.

SUBUDHI, A. W., PANERAI, R. B. & ROACH, R. C. 2010. Effects of hypobaric hypoxia on cerebral autoregulation. *Stroke,* 41**,** 641-646.

SUN, M. K. & ALKON, D. L. 2002. Carbonic anhydrase gating of attention: memory therapy and enhancement. *Trends Pharmacol Sci,* 23**,** 83-9.

SUN, M. K., ZHAO, W. Q., NELSON, T. J. & ALKON, D. L. 2001. Theta rhythm of hippocampal CA1 neuron activity: gating by GABAergic synaptic depolarization. *Journal of Neurophysiology,* 85**,** 269-279.

SVART, M., GORMSEN, L. C., HANSEN, J., ZEIDLER, D., GEJL, M., VANG, K., AANERUD, J. & MOELLER, N. 2018. Regional cerebral effects of ketone body infusion with 3-hydroxybutyrate in humans: Reduced glucose uptake, unchanged oxygen consumption and increased blood flow by positron emission tomography. A randomized, controlled trial. *PLoS One,* 13**,** e0190556.

SVART, M., RITTIG, N., PEDERSEN, S. B., JESSEN, N. & MØLLER, N. 2021. Oral 3-hydroxybutyrate ingestion decreases endogenous glucose production, lipolysis, and hormone-sensitive lipase phosphorylation in adipose tissue in men: a human randomized, controlled, crossover trial. *Diabet Med,* 38**,** e14385.

TAGGART, A. K., KERO, J., GAN, X., CAI, T. Q., CHENG, K., IPPOLITO, M., REN, N., KAPLAN, R., WU, K., WU, T. J., JIN, L., LIAW, C., CHEN, R., RICHMAN, J., CONNOLLY, D., OFFERMANNS, S., WRIGHT, S. D. & WATERS, M. G. 2005. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *J Biol Chem,* 280**,** 26649-52.

TAYLOR, A. T. 2011. High-Altitude Illnesses: Physiology, Risk Factors, Prevention, and Treatment. *Rambam Maimonides Medical Journal,* 2**,** e0022.

THOMAS, L. K., ITTMANN, M. & COOPER, C. 1982. The role of leucine in ketogenesis in starved rats. *Biochem J,* 204**,** 399-403.

TIEU, K., PERIER, C., CASPERSEN, C., TEISMANN, P., WU, D. C., YAN, S. D., NAINI, A., VILA, M., JACKSON-LEWIS, V., RAMASAMY, R. & PRZEDBORSKI, S. 2003. D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J Clin Invest,* 112**,** 892-901.

TORP, K. D., MODI, P., POLLARD, E. J. & SIMON, L. V. 2023. Pulse Oximetry. *StatPearls.* Treasure Island (FL): StatPearls Publishing.

TOUSSAINT, C. M., KENEFICK, R. W., PETRASSI, F. A., MUZA, S. R. & CHARKOUDIAN, N. 2021. Altitude, Acute Mountain Sickness, and Acetazolamide: Recommendations for Rapid Ascent. *High Alt Med Biol,* 22**,** 5-13.

TSAI, Y. C., CHOU, Y. C., WU, A. B., HU, C. M., CHEN, C. Y., CHEN, F. A. & LEE, J. A. 2006. Stereoselective effects of 3-hydroxybutyrate on glucose utilization of rat cardiomyocytes. *Life Sci,* 78**,** 1385-91.

TUCKER, A., REEVES, J. T., ROBERTSHAW, D. & GROVER, R. F. 1983. Cardiopulmonary response to acute altitude exposure: water loading and denitrogenation. *Respiration Physiology,* 54**,** 363-380.

TURNER, C. E., BARKER-COLLO, S. L., CONNELL, C. J. & GANT, N. 2015. Acute hypoxic gas breathing severely impairs cognition and task learning in humans. *Physiol Behav,* 142**,** 104-10.

TUTTOLOMONDO, A., DI RAIMONDO, D., DI SCIACCA, R., PINTO, A. & LICATA, G. 2008. Inflammatory cytokines in acute ischemic stroke. *Current Pharmaceutical Design,* 14**,** 3574-3589.

ULLAH, M. S., DAVIES, A. J. & HALESTRAP, A. P. 2006. The Plasma Membrane Lactate Transporter MCT4, but Not MCT1, Is Up-regulated by Hypoxia through a HIF-1α-dependent Mechanism*. *Journal of Biological Chemistry,* 281**,** 9030-9037.

VALENZUELA, P. L., CASTILLO-GARCÍA, A., MORALES, J. S. & LUCIA, A. 2021. Perspective: Ketone Supplementation in Sports-Does It Work? *Adv Nutr,* 12**,** 305-315.

VAN RIJT, W. J., VAN HOVE, J. L. K., VAZ, F. M., HAVINGA, R., ALLERSMA, D. P., ZIJP, T. R., BEDOYAN, J. K., HEINER-FOKKEMA, M. R., REIJNGOUD, D. J., GERAGHTY, M. T., WANDERS, R. J. A., OOSTERVEER, M. H. & DERKS, T. G. J. 2021. Enantiomer-specific pharmacokinetics of D,L-3-hydroxybutyrate: Implications for the treatment of multiple acyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis,* 44**,** 926-938.

VAN THIENEN, R., MASSCHELEIN, E., D'HULST, G., THOMIS, M. & HESPEL, P. 2017. Twin Resemblance in Muscle HIF-1α Responses to Hypoxia and Exercise. *Frontiers in Physiology,* 7**,** 676.

VAN ZYL, C. G., LAMBERT, E. V., HAWLEY, J. A., NOAKES, T. D. & DENNIS, S. C. 1996. Effects of medium-chain triglyceride ingestion on fuel metabolism and cycling performance. *J Appl Physiol (1985),* 80**,** 2217-25.

VANDOORNE, T., DE SMET, S., RAMAEKERS, M., VAN THIENEN, R., DE BOCK, K., CLARKE, K. & HESPEL, P. 2017. Intake of a Ketone Ester Drink during Recovery from Exercise Promotes mTORC1 Signaling but Not Glycogen Resynthesis in Human Muscle. *Front Physiol,* 8**,** 310.

VEECH, R. L. 2004. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids,* 70**,** 309-19.

VESTBERG, T., REINEBO, G., MAUREX, L., INGVAR, M. & PETROVIC, P. 2017. Core executive functions are associated with success in young elite soccer players. *PLoS ONE,* 12**,** e0170845.

VESTERGAARD, E. T., ZUBANOVIC, N. B., RITTIG, N., MØLLER, N., KUHRE, R. E., HOLST, J. J., REHFELD, J. F. & THOMSEN, H. H. 2021. Acute ketosis inhibits appetite and decreases plasma concentrations of acyl ghrelin in healthy young men. *Diabetes Obes Metab,* 23**,** 1834-1842.

VESTERGAARD, M. B., LINDBERG, U., AACHMANN-ANDERSEN, N. J., LISBJERG, K., CHRISTENSEN, S. J., LAW, I., RASMUSSEN, P., OLSEN, N. V. & LARSSON, H. B. W. 2016. Acute hypoxia increases the cerebral metabolic rate – a magnetic resonance imaging study. *Journal of Cerebral Blood Flow & Metabolism,* 36**,** 1046-1058.

VOLEK, J. S., NOAKES, T. & PHINNEY, S. D. 2015. Rethinking fat as a fuel for endurance exercise. *Eur J Sport Sci,* 15**,** 13-20.

VOSS, M. W., KRAMER, A. F., BASAK, C., PRAKASH, R. S. & ROBERTS, B. 2010. Are expert athletes 'expert' in the cognitive laboratory? A meta-analytic review of cognition and sport expertise. *Applied Cognitive Psychology,* 24**,** 812-826.

WAHLQVIST, M. L., KAIJSER, L., LASSERS, B. W., LÖW, H. & CARLSON, L. A. 1973. The role of fatty acid and of hormones in the determination of myocardial carbohydrate metabolism in healthy fasting men. *Eur J Clin Invest,* 3**,** 57-65.

WAHREN, J., SATO, Y., OSTMAN, J., HAGENFELDT, L. & FELIG, P. 1984. Turnover and splanchnic metabolism of free fatty acids and ketones in insulin-dependent diabetics at rest and in response to exercise. *J Clin Invest,* 73**,** 1367-76.

WALDMAN, H. S., BASHAM, S. A., PRICE, F. G., SMITH, J. W., CHANDER, H., KNIGHT, A. C., KRINGS, B. M. & MCALLISTER, M. J. 2018. Exogenous ketone salts do not improve cognitive responses after a high-intensity exercise protocol in healthy college-aged males. *Appl Physiol Nutr Metab,* 43**,** 711-717.

WALDMAN, H. S., BRYANT, A. R., SHEPHERD, B. D., EGAN, B. & MCALLISTER, M. J. 2022. No Effect of a Ketone Monoester on Markers of Stress and Performance in a Live-Burn Search and Rescue in Firefighters. *J Strength. Cond. Res,* 36**,** 763-711.

WALDMAN, H. S., SHEPHERD, B. D., EGAN, B. & MCALLISTER, M. J. 2020. Exogenous Ketone Salts Do Not Improve Cognitive Performance During a Dual-Stress Challenge. *Int J Sport Nutr Exerc Metab,* 30**,** 120–127.

WALSH, J. J., CALDWELL, H. G., NEUDORF, H., AINSLIE, P. N. & LITTLE, J. P. 2021a. Short-term ketone monoester supplementation improves cerebral blood flow and cognition in obesity: A randomized cross-over trial. *J Physiol,* 599**,** 4763-4778.

WALSH, J. J., MYETTE-CÔTÉ, É. & LITTLE, J. P. 2020a. The Effect of Exogenous Ketone Monoester Ingestion on Plasma BDNF During an Oral Glucose Tolerance Test. *Front Physiol,* 11**,** 1094.

WALSH, J. J., MYETTE-CÔTÉ, É., NEUDORF, H. & LITTLE, J. P. 2020b. Potential Therapeutic Effects of Exogenous Ketone Supplementation for Type 2 Diabetes: A Review. *Curr Pharm Des,* 26**,** 958-969.

WALSH, J. J., NEUDORF, H. & LITTLE, J. P. 2021b. 14-Day Ketone Supplementation Lowers Glucose and Improves Vascular Function in Obesity: A Randomized Crossover Trial. *J Clin Endocrinol Metab,* 106**,** e1738-e1754.

WANG, J., KE, T., ZHANG, X., CHEN, Y., LIU, M., CHEN, J. & LUO, W. 2013a. Effects of acetazolamide on cognitive performance during high-altitude exposure. *Neurotoxicology and Teratology,* 35**,** 28-33.

WANG, J., KE, T., ZHANG, X., CHEN, Y., LIU, M., CHEN, J. & LUO, W. 2013b. Effects of acetazolamide on cognitive performance during high-altitude exposure. *Neurotoxicol Teratol,* 35**,** 28-33.

WANG, K., SMITH, Z. M., BUXTON, R. B., SWENSON, E. R. & DUBOWITZ, D. J. 2015. Acetazolamide during acute hypoxia improves tissue oxygenation in the human brain. *J Appl Physiol (1985),* 119**,** 1494-500.

WANG, X., CUI, L. & JI, X. 2022. Cognitive impairment caused by hypoxia: from clinical evidences to molecular mechanisms. *Metabolic Brain Disease,* 37**,** 1-16.

WEBBER, R. J. & EDMOND, J. 1977. Utilization of L(+)-3-hydroxybutyrate, D(-)-3-hydroxybutyrate, acetoacetate, and glucose for respiration and lipid synthesis in the 18-day-old rat. *J Biol Chem,* 252**,** 5222-6.

WEIGLE, D. S., BUBEN, A., BURKE, C. C., CARROLL, N. D., COOK, B. M., DAVIS, B. S., DUBOWITZ, G., FISHER, R. E., FREEMAN, T. C., GIBBONS, S. M., HANSEN, H. A., HEYS, K. A., HOPKINS, B., JORDAN, B. L., MCELWAIN, K. L., POWELL, F. L., REINHART, K. E., ROBBINS, C. D., SUMMERS, C. C., WALKER, J. D., WEBER, S. S. & WEINHEIMER, C. J. 2007. Adaptation to altitude as a vehicle for experiential learning of physiology by university undergraduates. *Advances in Physiology Education,* 31**,** 270-278.

WEST, J. B. 2004. The Physiologic Basis of High-Altitude Diseases. *Annals of Internal Medicine,* 141**,** 789.

WHEATON, W. W. & CHANDEL, N. S. 2011. Hypoxia. 2. Hypoxia regulates cellular metabolism. *American Journal of Physiology - Cell Physiology,* 300**,** C385-C393.

WHITE, H., HEFFERNAN, A. J., WORRALL, S., GRUNSFELD, A. & THOMAS, M. 2021. A Systematic Review of Intravenous β-Hydroxybutyrate Use in Humans - A Promising Future Therapy? *Front Med (Lausanne),* 8**,** 740374.

WHITFIELD, J., BURKE, L. M., MCKAY, A. K. A., HEIKURA, I. A., HALL, R., FENSHAM, N. & SHARMA, A. P. 2021. Acute Ketogenic Diet and Ketone Ester Supplementation Impairs Race Walk Performance. *Med Sci Sports Exerc,* 53**,** 776-784.

WILLIAMS, T. B., CORBETT, J., MCMORRIS, T., YOUNG, J. S., DICKS, M., ANDO, S., THELWELL, R. C., TIPTON, M. J. & COSTELLO, J. T. 2019. Cognitive performance is associated with cerebral oxygenation and peripheral oxygen saturation, but not plasma catecholamines, during graded normobaric hypoxia. *Exp Physiol,* 104**,** 1384-1397.

WILLIAMSON, J. R. & KREBS, H. A. 1961. Acetoacetate as fuel of respiration in the perfused rat heart. *Biochem J,* 80**,** 540-7.

WILSON, M. H., NEWMAN, S. & IMRAY, C. H. 2009. The cerebral effects of ascent to high altitudes. *The Lancet. Neurology,* 8**,** 175-191.

WOODHOUSE, J., HEYANKA, D. J., SCOTT, J., VINCENT, A., ROEBUCK-SPENCER, T., DOMBOSKI-DAVIDSON, K., O'MAHAR, K. & ADAMS, R. 2013. Efficacy of the ANAM General Neuropsychological Screening Battery (ANAM GNS) for detecting neurocognitive impairment in a mixed clinical sample. *The Clinical Neuropsychologist,* 27**,** 376-385.

YAMADA, T., ZHANG, S. J., WESTERBLAD, H. & KATZ, A. 2010. {beta}-Hydroxybutyrate inhibits insulin-mediated glucose transport in mouse oxidative muscle. *Am J Physiol Endocrinol Metab,* 299**,** E364-73.

YOUM, Y. H., NGUYEN, K. Y., GRANT, R. W., GOLDBERG, E. L., BODOGAI, M., KIM, D., D'AGOSTINO, D., PLANAVSKY, N., LUPFER, C., KANNEGANTI, T. D., KANG, S., HORVATH, T. L., FAHMY, T. M., CRAWFORD, P. A., BIRAGYN, A., ALNEMRI, E. & DIXIT, V. D. 2015. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med,* 21**,** 263-9.

YURISTA, S. R., NGUYEN, C. T., ROSENZWEIG, A., DE BOER, R. A. & WESTENBRINK, B. D. 2021. Ketone bodies for the failing heart: fuels that can fix the engine? *Trends Endocrinol Metab,* 32**,** 814-826.

ZANDER, R. & VAUPEL, P. 1985. Proposal for using a standardized terminology on oxygen transport to tissue. *Advances in Experimental Medicine and Biology,* 191**,** 965-970.

ZHANG, G., ZHOU, S.-M., YUAN, C., TIAN, H.-J., LI, P. & GAO, Y.-Q. 2013. The effects of short-term and long-term exposure to a high altitude hypoxic environment on neurobehavioral function. *High Altitude Medicine & Biology,* 14**,** 338-341.

ZHOU, G., DADA, L. A., CHANDEL, N. S., IWAI, K., LECUONA, E., CIECHANOVER, A. & SZNAJDER, J. I. 2008. Hypoxia-mediated Na-K-ATPase degradation requires von Hippel Lindau protein. *The FASEB Journal,* 22**,** 1335-1342.