Impact of keton ester supplementation on indices of cognitive function in young healthy subjects: A randomized cross-over pilot study

Impacto da suplementação com ésteres de cetona nos indicadores da função cognitiva em sujeitos jovens e saudáveis: Um estudo piloto cruzado e randomizado

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Abstract

Cognitive function undergoes numerous modulations throughout lifetime, with exogenous components capable of increasing biomarkers that influence cognitive enhancement. The main objective of this study was to assess whether cognitive indices were enhanced by the ingestion of ketone esters and if they could elevate the brain-derived neurotrophic factor (BDNF) and one of its isoforms and precursor (proBDNF), neurotrophins associated with improved cognitive functions. Six subjects (age 22-40) were recruited. Initially, they underwent anthropometric evaluations and a maximal oxygen consumption (VO_2max) test. Each subject had a continuous ketone monitoring system (CKM) mounted and ingested a ketone ester drink on ketone exercise (KE) and ketone rest (KR). They then did the N-back test to assess improvements in memory and learning skills. Ten blood samples were collected during each session to measure ß-hydroxybutyrate (BOH). A discomfort rating questionnaire was applied throughout the trials to monitor gastrointestinal side effects. The supplement was well accepted and significantly increased blood ketones to 18-fold and BOH to 9-fold above baseline. There was a strong correlation (r=0,8991) between ketones measured with the CKM and the BOH analysis, with the CKM showing higher absolute values compared to BOH. However, the supplementation did not affect BDNF and proBDNF nor the N-back scores, and consequently, did not impact cognitive function. This way, ketone esters effectively raise blood ketones, but they do not influence cognitive function in short-term.

Key-words: Ketone bodies; hydroxybutyrates; cognitive function; brain-derived neurotrophic factor; performance-enhancing substances

Resumo

A função cognitiva sofre numerosas modulações ao longo da vida e há compostos exógenos capazes de aumentar biomarcadores que potenciam a função cognitiva. O principal objetivo deste estudo foi avaliar se os indicadores cognitivos melhoravam com a ingestão de ésteres de cetona e se estes podiam elevar o fator neurotrófico derivado do cérebro (BDNF) e uma das suas isoformas e precursor (proBDNF), neurotrofinas estas associadas à melhoria da função cognitiva. Seis indivíduos (idade 22-40) foram recrutados. Inicialmente, fizeram-se avaliações antropométricas e testes do consumo máximo de oxigénio (VO_2max). Foi implementado um sistema de monitorização contínua de cetonas (SMCC) e ingerida uma bebida de ésteres de cetona nas sessões que conjugaram o suplemento com exercício e com repouso. Realizou-se o teste "N-back" para avaliar melhorias nas capacidades de memorização e aprendizagem. Dez amostras de sangue foram recolhidas em cada sessão para medir β-hidroxibutirato (BOH). Um questionário de avaliação de desconforto serviu para monitorizar efeitos colaterais gastrointestinais. O suplemento foi bem aceite e aumentou significativamente as cetonas sanguíneas, 18 vezes, e o BOH, 9 vezes, comparativamente à *baseline*. Verificou-se uma forte correlação (r=0,8991) entre as cetonas medidas pelo SMCC e a análise de BOH, tendo o SMCC demonstrado valores absolutos mais altos. A suplementação não afetou o BDNF, o proBDNF, nem os resultados do "N-back" e não impactou a função cognitiva. Assim, os ésteres de cetona aumentam as cetonas sanguíneas, não influenciando a função cognitiva a curto prazo.

Palavras-chave: Corpos cetónicos; hidroxibutiratos; função cognitiva; fator neurotrófico derivado do cérebro; substâncias que melhoram o desempenho

List of abbreviations and acronyms

BDNF – Brain-derived neurotrophic factor

BOH - ß-hydroxybutyrate

CKM - Continuous Ketone Monitoring System

KE – Ketone Exercise

KR – Ketone Rest

mBDNF - Mature brain-derived neurotrophic factor

PL – Placebo

proBDNF - Brain-derived neurotrophic factor precursor

TrkB – Brain-derived neurotrophic factor receptor

VO_2max - Maximal oxygen consumption

Summary

Introduction

1. Cognition and brain health

It is widely acknowledged that the human brain is a highy complex organ, with much remaining to be elucidated regarding its mechanisms and signaling pathways. In recent years, considerable interest among researchers has been directed towards gaining a deeper comprehension of the brain's synaptic plasticity capacity and identifying significant neurogenesis biomarkers crucial for brain health and cognitive performance enhancement.

According to the World Health Organization, brain health is a comprehensive and complex field involving cognitive, sensory, social-emotional, behavioral, and motor functions, allowing individuals to reach their full potential, even with disorders. It is influenced by factors like physical health, healthy environments, safety, lifelong learning, social connections, and access to quality services. Throughout life, various brain and nervous system conditions can arise, leading to growth disruptions, structural damage, or impaired functioning. [1]

Recent scientific interest has identified certain substances both endogenous and exogenous to the body, extreme nutritional interventions, and physical exercise adaptations that can modulate learning and memory. Some of these factors can cross the blood-brain barrier and stimulate the synthesis or release of brain-derived neurotrophic factor (BDNF).[2-9]

2. Brain-derived neurotrophic factor

In order to further comprehend the mechanisms through which memory and learning skills may be enhanced, BDNF has been extensively investigated for its several beneficial effects on human cognition. BDNF assumes a crucial role within

the central nervous system, contributing to homeostasis and acting as stimulator of synaptogenesis by promoting axonal and dendritic growth as well as branching, enhancing spatial learning and memory retention, among other functions.[2, 6, 7, 10-12]

BDNF can be released in two distinct forms: brain-derived neurotrophic factor precursor (proBDNF) and mature brain-derived neurotrophic factor (mBDNF), each with distinct functions. Specifically, mBDNF promotes neuron survival, proliferation, and enhances memory consolidation, whereas proBDNF induces apoptosis.[2] Serum levels of proBDNF are significantly associated with changes in ß-hydroxybutyrate (BOH) concentration and demonstrate superior repeatability compared to mBDNF in serum measurements, meaning that proBDNF levels are more consistently reproducible over repeated tests, indicating a more stable biomarker.[2] Exercise-induced increases in BOH may enhance the conversion of proBDNF to mBDNF, contributing to overall higher levels of BDNF in the brain.[7] BDNF can be found circulating in plasma or bound to platelets, with blood serum containing a greater concentration of this neurotrophin compared to plasma.[13]

3. BDNF biomarkers and pathways

BDNF biomarkers include its mRNA levels in the brain and peripheral tissues like blood, and BDNF protein levels in the brain, cerebrospinal fluid, plasma or serum. The expression levels of BDNF's primary receptor, TrkB, and low-affinity nerve growth factor receptors are also key biomarkers. BDNF gene transcription is regulated by molecular elements, including the CREB factor. BDNF and TrkB are expressed in neurons of the central nervous system and other tissues such as cardiac, hepatic, skeletal muscle, and adipose tissue. BDNF exerts its effects through pathways involving TrkB, where BDNF binds to its receptor and initiates

signaling cascades. Factors like BOH and exercise promote BDNF expression, with BOH acting via the cAMP/PKA/CREB signaling pathway to upregulate BDNF transcription. BDNF and its pathways are crucial for neuronal survival, synaptic plasticity, neurogenesis, and cognitive function, playing significant roles in brain health. Ketosis can influence BDNF levels, suggesting that lifestyle interventions can modulate BDNF expression and benefit the brain.[4, 7, 11, 13]

4. Mechanisms of ketone bodies

Ketone bodies, produced endogenously from acetyl-CoA during fasting, can also be obtained exogenously through ketone supplements, extremely popular among athletes.[14, 15] BOH is an example of a ketone body, four-carbon short-chain organic acid derived from free fatty acids, providing energy to tissues outside the liver.[16]

The cognitive benefits of ketosis arise from ketones serving as an efficient brain energy source during glucose deprivation, reducing oxidative stress and inflammation contributing to neuroprotection, and increasing BDNF levels to support neuronal growth and synaptic plasticity.[17]

Ketone supplements significantly impact plasma ketone levels, peaking within 1-2 hours.[14] However, in another research study, a ketone monoester drink elevated plasma BOH concentrations 30 minutes post-ingestion, with levels remaining high until 210 minutes of follow-up.[18]

Human studies show that BOH administration significantly elevates BDNF serum levels, correlating with increased hippocampal proBDNF and mBDNF levels.[5]

Ketone ester supplementation combined with physical exercise, promote brain health and cognitive function.

5. Role of exercise

During exercise, ketone bodies are readily oxidized by skeletal muscle, similar to glucose, and aid post-exercise recovery by replenishing muscle glycogen.[14]

Exercise enhances cognition by stimulating BDNF production. This occurs through different potential mechanisms such as the release of FNDC5 protein, which activates BDNF expression via PGC-1alpha pathway in the hippocampus.[13] Others inlcude GLDP1, a byproduct of glycogen metabolism in the liver, that has been identified as a factor capable of influencing BDNF levels through unknown mechanisms. Also lactate, a byproduct of anaerobic metabolism in muscles, is released into the bloodstream during exercise and crosses the blood-brain barrier serving as an energy substrate for neurons to promote BDNF synthesis. Lactate levels in the blood and hippocampus will induce synaptic plasticity genes like BDNF, improving spatial learning and memory retention.[12] BOH can enter the brain and enhance energy availability in neurons, promoting a conducive environment for BDNF synthesis. Exercise-induced shear stress also boosts blood flow to the brain and muscles, increasing the release of platelet-bound BDNF.[13] Cathepsin B, released during exercise, crosses the blood-brain barrier and promotes BDNF expression in the hippocampus, facilitating neurogenesis and elevating neuroprotective proteins.[3]

It has been proved that practicing aerobic exercise for three months can increase hippocampal volume by 12% in healthy individuals, and older adults with mild cognitive impairment show elevated peripheral BDNF levels after 16 weeks of exercise.[3] Moreover, adults who practiced aerobic exercise three times per week for six months demonstrated significant development in brain areas such as the frontal and parietal cortices, improving attention and resolution capacity.[19]

Prolonged exercise also increases arterial and jugular venous BDNF levels, indicating substantial BDNF being released into the bloodstream and also crossing the blood-brain barrier.[13] After four hours of exercise below the lactate threshold, BDNF levels in jugular venous plasma were 2.5 times higher than at rest, with brain BDNF levels increasing by up to 84%.[13]

The findings of another research, indicate that BDNF responses to low-intensity exercise are primarily influenced by elevated circulating platelet levels. Increasing both the duration and, to a greater extent, the intensity of exercise is necessary to release free, unbound BDNF into circulation. The increase in BDNF during exercise may be related to the rise in circulating platelets, which store large amounts of this neurotrophin.[8]

6. Knowledge gaps

There is a notable deficiency in research addressing the variability in individual responses to ketone supplementation. It is crucial to understand the relative efficacy of ketone supplements particularly through comparative studies with other cognitive-enhancing interventions, such as exercise, which are currently limited. Furthermore, additional research is required to establish effective and safe dosages of ketone supplements for cognitive enhancement, along with a comprehensive evaluation of potential side effects. While some studies indicate improvements in specific cognitive functions, the consistency and width of these effects across various cognitive domains remain unclear.

Objectives

The aim of the present pilot study was to investigate the cognitive indices triggered by the ingestion of exogenous keton esters in a fasted state and to

determine whether these compounds can elevate BDNF and proBDNF levels in circulating blood. Given that this neurotrophin is capable of modulating memory skills, the study places particular emphasis on assessing the enhancement of subject's mental recall, comprehension and retention of knowledge throughout the trials. Additionally, the study aimed to understand the role of light to moderate-intensity exercise in increasing BDNF and proBDNF levels and the potential enhancement of cognitive function.

Methodology

1. Study design and subjects

The population of this study includes six healthy adults that were recruited via email. A balanced male-to-female ratio was selected to accurately reflect the general population, rather than to specifically study sex differences. Exclusion criteria consisted of chronic illnesses and the use of daily medication that could interfere with the ketone ester absorption. Participants were considered eligible for inclusion if they had 20-40 years, healthy status and practiced exercise regularly. The data collection was completed between march and april 2024 at the Swedish School of Sports and Health Science and a study protocol was elaborated (Appendix A).

Table 1. Descriptive statistics about the participants

	Age (Y)	Weight (Kg)	Height (cm)	Fat Free Mass (Kg)	Body Fat (%)	VO_2max (ml/min)	Watt at 65%	HR Ex (bpm)	RPE
Male (n=3)	29±7	85,9±7,0	184±1,8	71,4±2,7	17±7,7	4374±771	215±56	143±5,2	13,7±0,6
Female (n=3)	29±9,6	68,3±7,6	174±6,0	49,6±6,8	25±3,2	3254±522	135±20	142±15	13,7±0,6
Average (n=6)	29±7,5	77,1±11,6	179±6,4	60,5±12,8	21±7,1	3814±850	175±57,9	143±10	13,7±0,5

Data are described as mean ± standard deviation. Y = years; Kg = kilograms; Cm = centimeters, g = grams; % = percentage; ml/min = milliliters of oxygen consumed in one minute; Watt at 65% = load to exercise at 65% VO_2; bpm = beats per minute during the exercise trial; RPE = Rate of perceived exertion during the exercise trial

2. Initial testing

On a separate day prior to experimental trials, all subjects did a pre-intervention visit where anthropometric measurements such as weight and height were assessed. A VO_2max evaluation was performed in a COSMED ergometer following a protocol consisting of 3 different loads, incremented every 3 minutes and ramps with higher loads until subjects reached volitional exhaustion or were unable to maintain a cadence >60 rpm. Individual's VO_2 peak at 65% capacity, power and maximal heart rate were calculated using Excel. All subjects were asked to fill a health questionnaire to check if they had any serious health conditions.

Subjects were instructed to fast overnight before each session and were only allowed to drink water and/or coffee without any sugar. A continuous ketone monitoring system (CKM) was installed on the *triceps* muscle in order to keep track of blood ketone levels. It was also performed a whole-body examination on all participants using the DEXA (Dual-energy X-ray absorptiometry) method.

3. Experimental trials

A catheter was inserted into an antecubital vein in a supine position for sampling. During each session, about 5 mL of blood was drawn at 10 different time points into heparinized plasma containers, and at 5 of these time points, 5 mL of blood was also collected into sterile serum tubes. In order to monitor the blood levels of lactate and glucose, 20 µL of blood were pipetted to microtubes with an anti-haemolysing solution before being put on the Biosen analyzer to do the measurements with a high degree of accuracy.

It was elaborated and applied a comfort rating questionnaire (Appendix B) on the following moments: baseline, pre-intervention, middle, post-intervention, post

60min and post 120min to check on how individuals were feeling due to any gastrointestinal discomfort caused by the supplement. Additionally, it was made a protocol for manual measurements (Appendix C) with the levels of the keton bodies captured by the CKM, blood concentrations of glucose and lactate, heart rate and rate of perceived exertion[20], these last two were specifically recorded by the test leader for the KE session.

Participants completed the sessions (Placebo, Ketone and Rest, Ketone and Exercise) in a randomized way. In each session individuals did a cognitive test at baseline level and post 60 minutes of the intervention itself (exercise or rest for 30 minutes). It consisted in a platform with the PsychoPy algorithm and the Python programming language where subjects tested their memory skills by completing three different tests: on the first one, the N-back, they had to remember one number backwards, on the 2-back, they had to recall the second number counting backwards and on the 3-back, they had to remember the third number counting backwards and this repetead for a few rounds. Afterwards, some metrics were collected and used for statistical analysis such as: the mean of the reaction time (in milliseconds) it took participants to answer right or wrong and the total score.

On the KR and KE sessions, and 20 minutes before cycling, subjects ingested a 25 mL bottle of the deltaG Ketone Performance drink® with the nutritional composition of 140kcal per serving, 0g fat, 0g protein, 0g sodium, 4g total carbohydrates and 3g total sugars. On the other hand, on the PL session participants ingested the placebo drink consisting of 30g of glucose mixed with 200mL of water in order for it to have the same calories as the supplement.

In order to monitor subjects' heart rate while they were cycling on the KE session, they were given a chest-strap with a heart rate monitor that was connected to

the Wattbike. By the end of the exercising bout, it was possible to evaluate the general performance, specially, the cadence, power, speed and total of energy spent because the main goal was to be bellow the lactate threshold (around 2mmol/L).

After the sessions all samples were centrifugated at 2000 rpm to isolate plasma and 3000 rpm for separating serum at 4ºC for 10 min and both were stored in microtubes and then frozen at -80ºC until analysed.

The following step was to analyse and calculate the BOH concentration on the plasma samples based on the BOH Spectrophotometry assay. The assay was performed by enzymatic conversion of BOH by BOH-dehydrogenase coupled to conversion of NAD to NADH followed by its measurement at 340 nm, with dilutions optimized for our samples based on preliminary testing. Its reproducibility was tested by experimenting multiple concentrations of the samples and of the standards. Moreover, a comparison was done between the BOH concentration obtained from the assay and the blood ketones concentration captured by the CKM. The concentration of proBDNF was also analysed using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Regarding the statisctical analysis, descriptive statistics was performed to calculate the mean of the age, sex, fat free mass, body fat, weight, height, VO_2max, heart rate, watts at 65% and RPE. For the main outcomes it was evaluated plasma BDNF - time x condition, plasma proBDNF - time x condition both using the ANOVA test and plasma BOH vs. CKM ketones to assess the accuracy of the monitor using the Spearman Correlation on the GraphPad Prism 10 software. For the secondary outcomes the results of N-back and its changes from pre-intervention

to post-intervention, regarding the condition were analysed using a mixed model ANOVA due to missing values, blood glucose – time x condition, blood lactate – time x condition and discomfort rating – time X condition using ANOVA. Statistical significance was set at $P< 0.05$.

Results

1. **Ketones levels**

   After supplement intake, both the ketone levels measured by the CKM and the BOH increased significantly after 20 min and remained elevated throughout the duration of KE and KR trials. Regarding the CKM, the values increased to approximately 18-fold above baseline and the peak concentration was 5,3 mmol/L at 35 min on the KR session ($p<0,05$ for time, Fig 1A). BOH was responsive to both KE and KR interventions due to the its increase to approximately 9-fold above baseline. At 35 min, BOH levels reached a peak concentration of 3,9 mmol/L on the KR group ($p<0,05$ for time, Fig 1B). There is a strong ($r=0,8991$) and significant ($p=0,0001$) correlation between the concentrations of CKM ketones and BOH. However, CKM indicates higher ketone levels than the BOH analyses (Fig 1C).

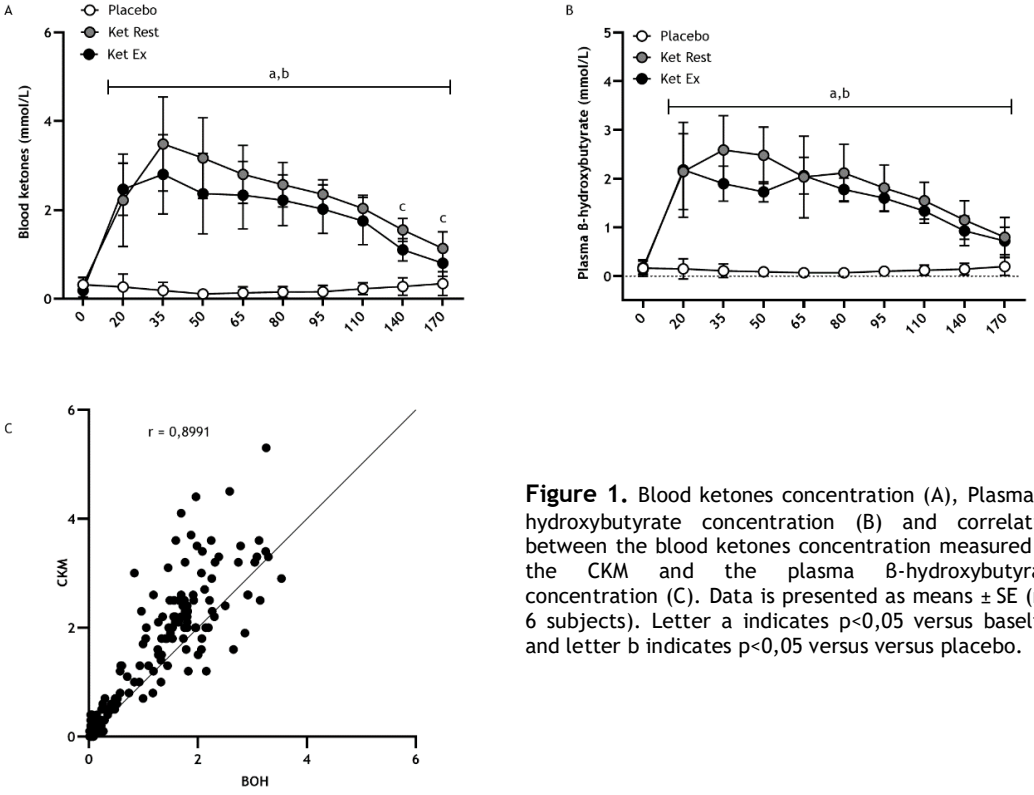

Figure 1. Blood ketones concentration (A), Plasma ß-hydroxybutyrate concentration (B) and correlation between the blood ketones concentration measured by the CKM and the plasma ß-hydroxybutyrate concentration (C). Data is presented as means ± SE (n = 6 subjects). Letter a indicates p<0,05 versus baseline and letter b indicates p<0,05 versus versus placebo.

2. Glucose and lactate levels

Glucose concentrations began to decrease during pre-intervention and remained lower during KE and KR trials but increased after the intervention and returned to values close to baseline (p<0,05 for time, Fig 2A). However, no significant differences were found between KE and KR trials. Blood glucose increased to approximately 2-fold above baseline in the PL group. In this group, it reached a peak concentration of 6,59mmol/L and returned to baseline after 90 minutes of follow-up. As expected, lactate was responsive to the KE intervention and increased approximately 4-fold above baseline reaching a peak of 2,6mmol/L. Lactate levels returned to baseline 15 min after the exercise bout (Fig 2B).

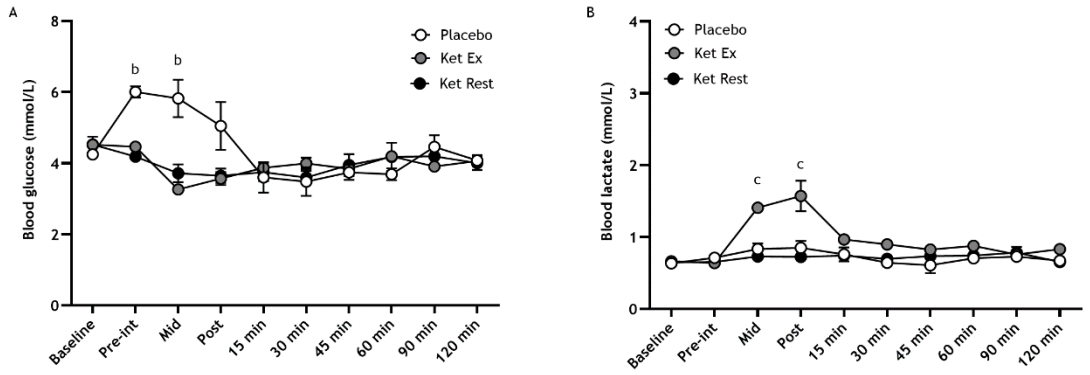

Figure 2. Blood glucose concentration (A) and blood lactate concentration (B). Data is presented as means ± SE (n = 6 subjects). Letter b indicates p<0,05 versus placebo and letter c indicates p<0,05 between KR and KE.

3. BDNF and proBDNF levels

For plasma levels of BDNF there was a main effect of time ($p<0.05$), without any significant differences between trials, indicating a general increase due to the intervention but not the supplementation (Fig 3A). Plasma proBDNF levels remained stable across the interventions, with a small non-significant increase at 50 min time point, with a peak of 168 pg × ml-1 in the KE trial and its levels returned to baseline after 65 min (Fig 3B).

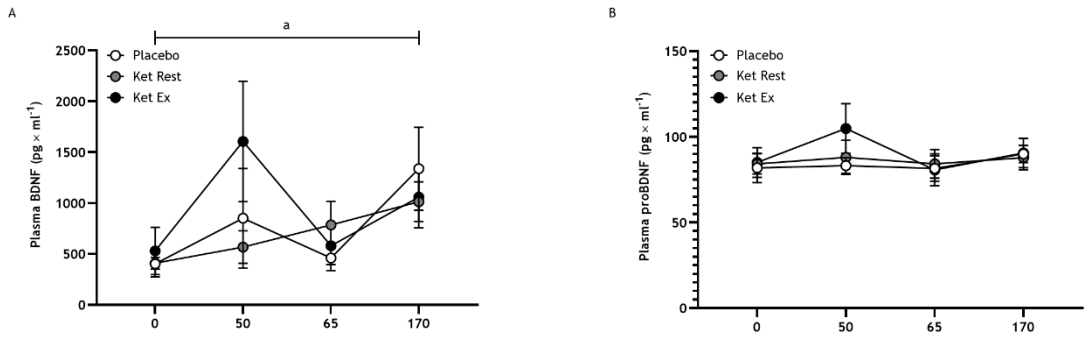

Figure 3. Plasma BDNF concentration (A) and plasma proBDNF concentration (B). Data is presented as means ± SE (n = 6 subjects).

4. N-back test and discomfort rating scores

The N-back score was not statistically significant between trials (p=0,9558) and time points (p=0,5386). On the PL and KE trials, the highest score was

304 on the post-intervention test (Fig. 4A). Due to interpersonal variability, there are significant differences between subjects on the discomfort rating score but not between trials and time points (Fig. 4B).

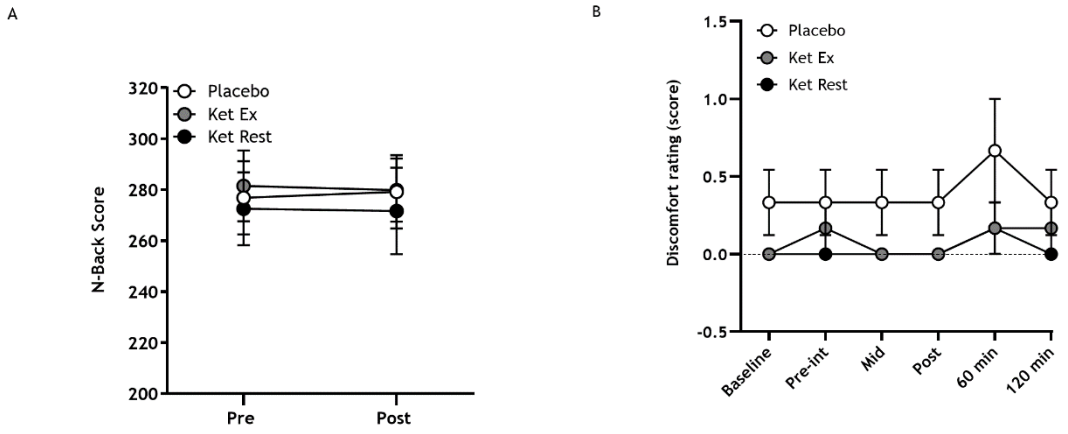

Figure 4. N-back score (A) and discomfort rating score (B). Data is presented as means ± SE (n = 6 subjects).

Discussion

The aim of this study was to investigate whether ketone supplement intake could increase BDNF levels and enhance memory and learning skills, as well as to explore the potential role of exercise in enhancing these effects.

The results showed that the supplement had a robust effect on blood ketones (Fig.1A,1B), and was well tolerated (Fig. 4B). Even though it was verified a strong correlation between the ketones captured by the CKM and the BOH analysis, CKM measured higher absolute values in comparison with BOH levels (Fig.1C). The supplement induced a very rapid increase of blood ketone levels within the same range as described in literature. [18, 21-27] As mentioned, it was also well tolerated and caused no gastrointestinal discomfort on subjects because ketone esters, such as those administered in our study, are preferable to ketone salts in this regard. [15, 28] Additionally, our participants either rested or practiced cycling at a light to

moderate-intensity, which is more beneficial for the gastrointestinal system compared to running or engaging in high-intensity exercise.[14, 16] It is interesting to highlight that the CKM demonstrated to be effective in measuring blood ketones, despite the absence of published validations for the use of this monitor. It should be acknowledged as a limitation that the higher absolute values captured with CKM are likely attributed to the monitor's capability to measure all blood ketone bodies (including acetoacetate and acetones) which are not measured in the BOH assay.

BDNF and proBDNF levels increased after 50 minutes, however, this change was not statistically significant, indicating that the supplementation had no effect on elevating the concentration of these neurotrophins (Fig.3). These results contrast with findings in animal studies, where BDNF levels were measured directly in the brain and ketone supplementation was conducted over an extended period of time, in comparison to our study.[2, 29-32] In human studies, acute changes in blood BDNF levels do not necessarily reflect acute changes in the concentration of this neurotrophin in the human brain because *de novo* synthesis of BDNF is not observed that acutely in blood.[14, 15, 22, 24, 33, 34]

The N-back score did not differ significantly from pre to post intervention neither between trials, suggesting that the supplement had no effect on the cognitive functions assessed with this test (Fig. 4A), which is not observed in studies with longer interventions.[9] Ketones did not influence cognitive function because they did not elevate BDNF levels, which are known to enhance memory and learning abilities.

Conclusion

This study did not find evidence that ketone supplementation affects BDNF and proBDNF levels or influences cognitive function in a short-term intervention. Furthermore, our study supports that the CKM is a highly efficient device for monitoring blood ketone levels, but it needs to be applied in other human studies to validate its potential for investigating changes in blood ketones. Additionally, it was found that the supplement was well tolerated during light to moderate exercise, as its physical impact was minimal and did not affect negatively the gastrointestinal system. There is still much to unravel regarding the therapeutic potential and enhancement of sports performance with ketone ester supplements, for example, because of their benefits for muscle recovery and mental fatigue in athletes, and improvement of cognitive dysfunction in psychological diseases without the need for extreme diets such as the ketogenic diet. To further investigate the potential enhancement of cognitive function by ketones, longer-term studies are needed to allow for *de novo* synthesis of BDNF.

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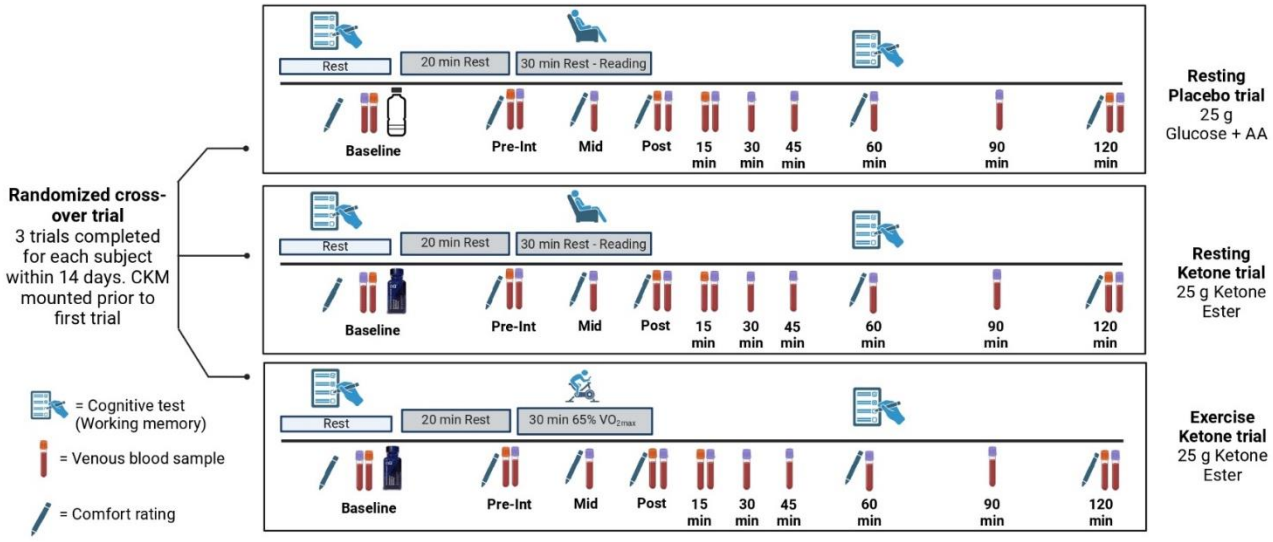
Appendices

Index of appendices:

Appendix A - Study Protocol



Appendix B – Comfort Rating questionnaire

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Comfort Rating - Protocol (Appendix 1):

Info							
		Baseline	Pre-Int	Mid	Post	After 60 min	After 120 min
Symptoms	Abdominal cramps						
	Dry retching (vomit-like feelings)						
	Reflux						
	Nausea						
	Headaches						
	Dizziness						
	Fatigue						

Rating criteria:

No symptoms (0)

Minor symptoms: Fatigue (1)

Moderate sympoms: Nausea, headaches and dizziness (2)

Major symptoms: abdominal cramps, dry retching and reflux (3)

Appendix C – Manual measurements protocol

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Manual measurements - Protocol:

Participant information

ID:_____**Date/time:**_____

Trial:_____

Info	Time	CKM	Glucose	Lactate	Heart Rate	Rate of perceived exertion
Baseline						
Pre-Int						
Mid						
Post						
After 15 min						
After 30 min						
After 45 min						
After 60 min						
After 90 min						
After 120 min						

Comments: