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Circulating endocannabinoids are associated with mental alertness during ultra-endurance exercise.

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Abstract

Introduction: Ultra-endurance exercise events result in central fatigue, impacting on mental alertness and decision making. Endocannabinoids are typically elevated during endurance exercise and have been implicated in central processes such as learning and memory, but their role in central fatigue has never been studied.

Materials & Methods: Twenty-four recreational male ultrarunners participated in a 100-km trail run, and eighteen of them completed at least 60 km and were included in the analyses. A cognitive test battery to assess median reaction time (MRT) and median movement time (MMT) during a reaction time task, and median response latency (MRL) during a rapid visual information processing task was completed prior to and immediately after the trail. Blood serum samples pre and post exercise were analyzed for endocannabinoids and related lipids (anadamide: AEA; 2-arachidonoylglycerol: 2-AG; palmitoylethanolamide: PEA; oleoylethanolamide: OEA; stearoylethanolamine: SEA) via liquid chromatography-mass spectrometry.

Results: Ultra-endurance exercise worsened all cognitive parameters and increased abundance of AEA, PEA, OEA and SEA but not 2-AG. Interestingly, the exercise-induced change in MRT showed moderate, positive correlations with the change in different endocannabinoids, i.e. AEA ($r=0.5164$, $p=0.0338$), PEA ($r=0.5466$, $p=0.0251$) and OEA ($r=0.5442$, $p=0.0239$).

Conclusion: These results indicate a potential role of endocannabinoids on mental alertness following ultra-endurance exercise.

Key words: endocannabinoid system; central fatigue; ultra-endurance exercise; trail running ; ketones

Introduction

Ultra-endurance events are increasingly popular, and pose a significant mental and physical demand to the human body. Central fatigue gradually develops during ultra-endurance events, which has a profound impact on mental alertness, decision making and ultimately performance[1,2]. Different causes of central fatigue following exercise include an increase in brain noradrenaline activity and a suppression of dopamine activity, but it remains unclear whether the endocannabinoid system is also involved in the regulation of mental alertness following ultra-endurance events.

The endocannabinoid system is a widespread signaling system that consists of lipid mediators called endocannabinoids, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), that are present in the blood and in many tissues such as the brain[3]. eCBs can bind to cannabinoid receptors that are highly expressed in neural and metabolic tissues. Other lipid mediators, such as palmitoylethanolamide (PEA), oleoylethanolamide (OEA) or stearoylethanolamine (SEA), share the metabolic pathways of AEA, and are thus part of the endocannabinoid system although they do not bind to the classic cannabinoid receptors. For convenience, the endocannabinoids AEA and 2-AG and related lipid mediators PEA, OEA and SEA will referred to as eCBs.

The central endocannabinoid system, including circulating eCBs[4,5] and central cannabinoid receptors, is activated following endurance exercise, for instance in the regulation of the 'runner's high'[6] that is typically observed following endurance-like exercise events. The 'runner's high' refers to a transient state of euphoria with lowered anxiety and a higher pain threshold. The endocannabinoid system is also involved in other cognitive processes, such as learning and memory, but it has never been studied whether endocannabinoids are related with mental alertness following ultra-endurance exercise. Recently, ketone ester supplementation during an ultra-endurance event was shown to improve mental alertness following the event[7]. Therefore, the present study investigated 1) whether circulating eCBs are affected by ultra-endurance events, 2) whether circulating eCBs associate with the changes in mental alertness induced by ultra-endurance events, and 3) whether ketone ester supplementation affects the levels of circulating eCBs during an ultra-endurance event.

Materials & Methods

Ethical Approval and Participants

Twenty-four recreational male ultrarunners participated in this study, which was approved by the Ethics Committee Research UZ/KU Leuven (B3222021000483) and conforms to the Declaration of Helsinki (registered at www.clinicaltrials.gov: NCT05407753). All subjects were fully informed of the content and potential risks of all experimental procedures before giving their written informed consent.

Participants were requested to run a 100-km trail run as fast as possible. Eighteen out of 24 initial recruits completed 60 km (n=4), 80 km (n=6) or 100 km (full trail; n=8) and were included in the analyses (Suppl. Table 1). Six participants prematurely terminated the trail due to perceived hindrance from a pre-exercise skeletal muscle biopsy, and were excluded from analyses (Fig. 1).

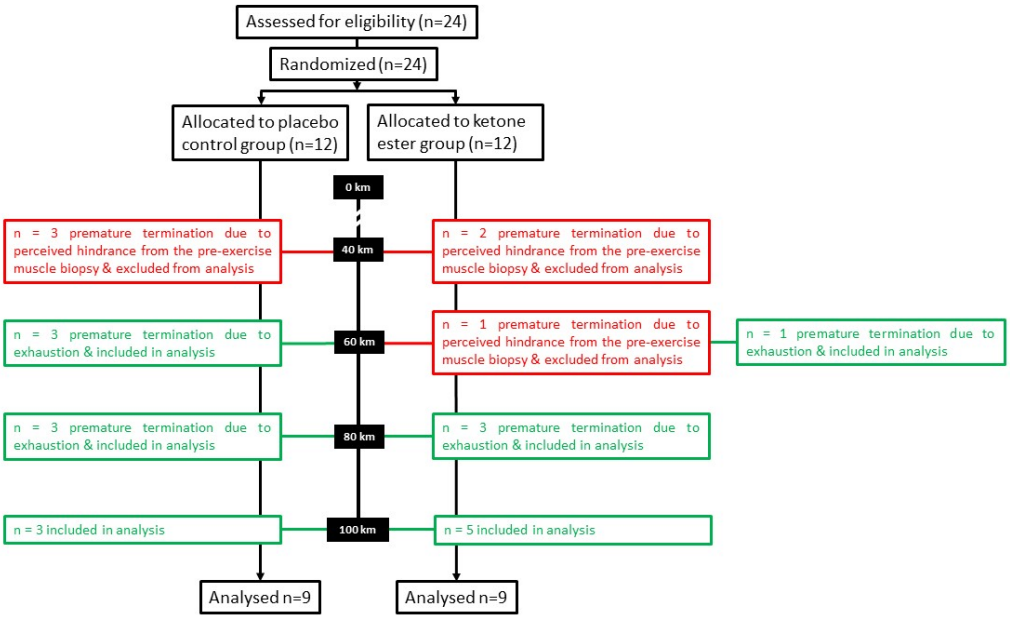


Fig. 1. CONSORT Flow Diagram. Flow-chart showing eligibility assessment, randomization, inclusion and exclusion throughout the study.

Trail run

Participants reported to a makeshift laboratory at the start of the trail and received a standardized meal (620 kcal; 72% carbohydrate, 15% fat, 13% protein). Two hours following the meal, subjects started the 100-km off-road trail on a 20-km loop (95% off-road) and accumulated 250 m vertical altitude per loop. Environmental conditions were permanent rain at 8°C–13°C and 70%–80% relative humidity. Nutritional intake during the trail was chosen by each participant and was registered at each loop.

Power calculation & Randomization

To obtain a moderate effect size (η^2_p=0.06) for an interaction effect (time x condition) for AEA, a sample size of 16 is required, when 1-β=0.8, α=0.05, two time points and correlations among repeated measures=0.08 (G*Power v.3.1).

Participants were randomized to receive either a ketone ester drink (KE, n=12) or a corresponding control drink (CON, n=12) during the trail. Randomization was based on previous ultramarathon performance and experience, and the aerobic threshold assessed during a submaximal incremental running test (8 km/h + 1.5 km/h each 8 minutes until the lactate threshold was reached), and was performed by an investigator who was otherwise not involved in the study.

Nutritional Intervention

Participants in KE received pure (R)-ßHB (R)1,3-butanediol ketone ester (KetoneAid Inc., Falls Church, VA) at regular intervals. Participants received 25 g 30 min before the trail, and additional boluses of 12.5 g were provided at the start and every 30 min thereafter. An additional bolus of 25 g was provided immediately postexercise. Participants in CON received a taste-matched noncaloric placebo dissolved in water.

Experimental Sessions

Before (PRE; 60min following meal) and after the trail (POST; 10min after completion of the run), cognitive function was assessed and blood was sampled.

Cognitive Test Battery

The cognitive test battery (~15min; Cantab, Cambridge Cognition, Cambridge, UK) consisted of two validated cognitive tests, i.e., 1) a reaction time task, and 2) a rapid visual information processing task. These tests are sensitive and specific to evaluate alterations in the context of exercise[8]. Cognitive tasks were administered on a tablet in a quiet, comfortable and standardized setting, and were performed with the dominant index finger. Detailed explanation of the task procedures is available at www.cambridgecognition.com/cantab/cognitive-tests/. Outcome parameters included in the current study are: 1) reaction time task: median reaction time (MRT), median movement time (MMT); 2) rapid visual information processing task: median response latency (MRL).

Blood Sample Analyses

Venous blood samples were obtained from an antecubital vein (Venoject, Terumo, Tokyo, Japan) and collected into vacuum tubes containing Silica Clot Activator [Becton Dickinson (BD) Vacutainer]. Blood was centrifuged (1,500 rpm for 15 min at 4°C) and the supernatant was stored at -80°C until analysis. Serum eCBs (AEA, 2-AG, PEA, OEA, SEA) were analyzed by liquid chromatography-mass spectrometry (UHPLC-MS/MS). Briefly, lipids were extracted by a liquid/liquid extraction (CH_2Cl_2-MeOH-H_2O) in the presence of the internal standards (d4-AEA, d4-PEA, d4-OEA and d5-2-AG), and purified by solid phase extraction. Then, the endocannabinoid-containing fraction was analyzed with a Xevo-TQS mass spectrometer (Waters, Milford, MA). The UHPLC-MS/MS method used is described elsewhere[4]. Hereafter the

Statistics

Differences between KE and CON over time were evaluated by a two-way repeated-measures analysis of variance (group x time). Correlations between the change in blood eCBs (Δ; post-trial minus pre-trail) and the change in cognitive measure (Δ; post-trial minus pre-trail) were analyzed via Pearson correlations as they were normally distributed. All statistical analyses were performed in GraphPad Prism version 8.4.3 (GraphPad Software, La Jolla, CA).

Results

Ultra-endurance running increases circulating eCBs

As previously reported, MRT, MMT and MRL all increased in the placebo group, but not in the KE group (Fig. 2a-c). The blood serum levels of four out of five lipid mediators, i.e. AEA (+55%; $p<0.0001$), PEA (+58%; $p<0.0001$), OEA (+70%; $p<0.0001$) and SEA (+43%; $p=0.0002$), were higher after the ultra-endurance running (Fig. 2d-h). Only for PEA, there was an interaction effect, indicating a smaller increase upon running in KE (+39%) *vs.* CON (+77%; $p=0.0404$; Fig. 2e).

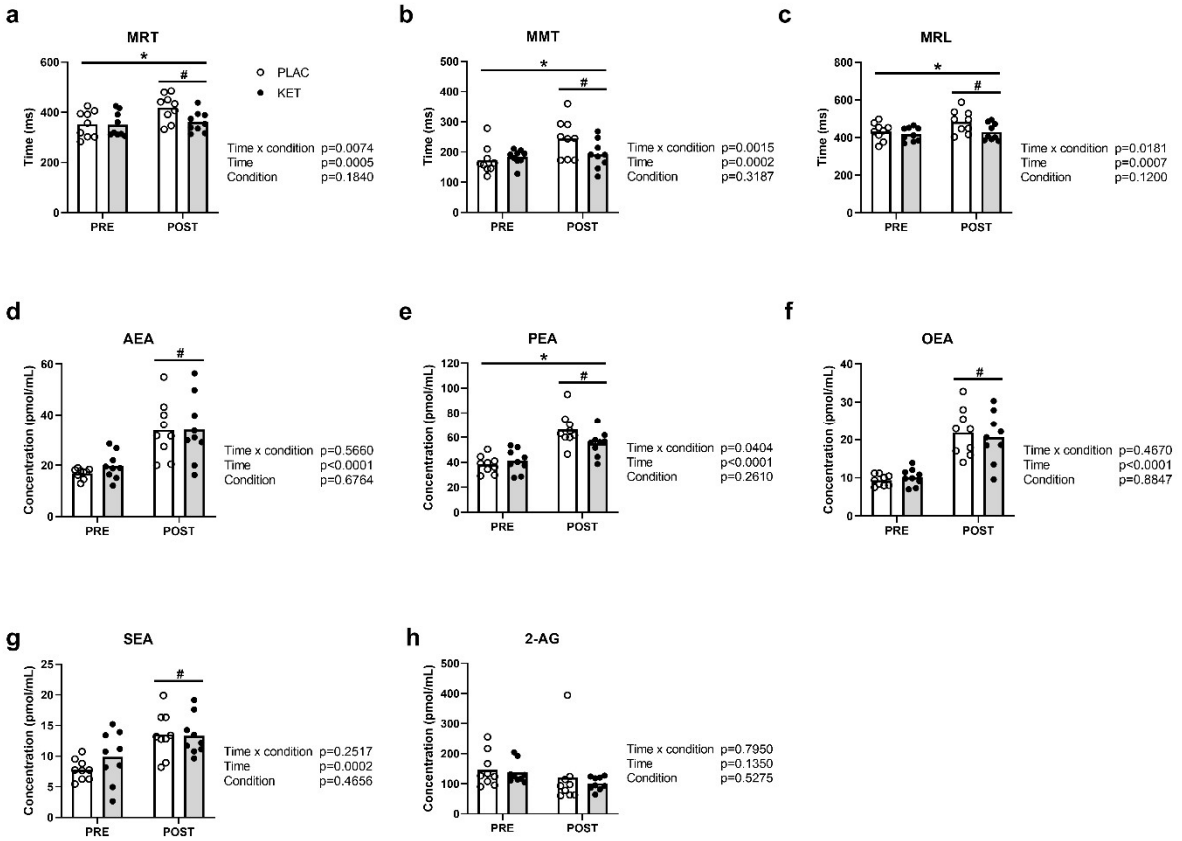
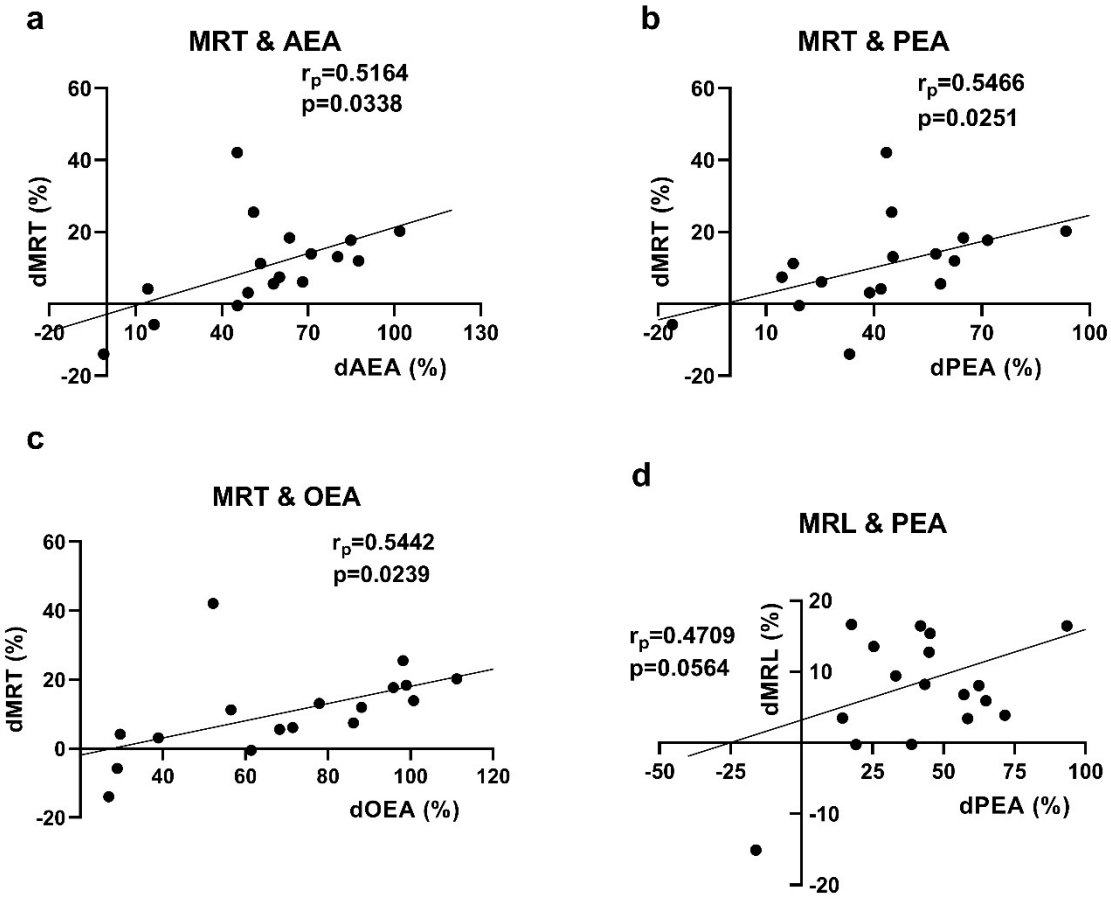


Fig. 2. The effect of ketone ester supplementation during ultra-endurance running on cognitive performance and serum eCB levels. Data are shown as mean (bar graph) and individual data points, and represent a: median reaction time (MRT), b: median movement time (MMT), c: median reaction latency (MRL), d: anandamide (AEA), e: palmitoylethanolamide (PEA), f: oleoylethanolamide (OEA), g: stearoylethanolamine (SEA), h: 2-arachidonoylglycerol (2-AG). During running, participants received either ketone ester supplements (KE, n = 9) or a placebo (CON, n = 9). Main and interaction effects were achieved via two-way repeated-measures ANOVA. *$p < 0.05$ for a significant interaction effect (Time * Condition); #$p < 0.05$ for a significant time effect (Pre vs. Post).

eCB levels correlate with mental alertness upon ultra-endurance running

Moderate, positive correlations between ΔMRT and ΔeCBs, i.e. ΔAEA ($r_p=0.5164$, $p=0.0338$; Fig. 3a), ΔPEA ($r_p=0.5466$, $p=0.0251$; Fig. 3b) and ΔOEA ($r_p=0.5442$, $p=0.0239$; Fig. 3c), were observed. ΔMRL tended to positively correlate with ΔPEA ($r_p=0.4709$, $p=0.0564$; Fig. 3d). None of the other combinations between ΔeCBs and Δcognitive measures reached significance.



148

149 Fig. 3. The correlation between the change in mental alertness (post-trail – pre-trail) and the change in
150 serum eCB levels (post-trail – pre-trail) in the CON (n=9) and KE (n=9) group combined. Presented
151 measures of mental alertness include median reaction time (MRT) and median reaction latency (MRL).
152 Blood eCBs include anandamide (AEA), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA).
153 Data were normally distributed and analyzed via Pearson correlations. Pearson correlations coefficients
154 (rp) and significances are presented. d refers to delta or Δ.

Discussion

It has been shown that eCBs are involved in the regulation of central processes such as the 'runner's high' during exercise in mice[6,9], but there is little evidence on whether eCBs are also involved in central processes upon exercise in humans. The present study showed that only systemic *N*-acylethanolamines sharing common metabolic pathways (i.e; AEA, PEA, OEA), but not 2-AG, are elevated upon ultra-endurance exercise in humans, and that the change in levels associates with the change in mental alertness.

Mechanistically, it has been shown that eCB production by the gut microbiome elevates brain dopamine levels during exercise[9]. eCBs control dopamine neurotransmission at midbrain and forebrain loci, partly via cannabinoid receptor 1[10]. Indeed, previous studies showed that elevated eCB levels associate with improved exercise-induced cognitive functions such as motor sequence memory[11]. In the present study, dopamine levels increased more following ultra-endurance exercise in KE *vs.* CON (Suppl. Fig. 1a). However, the change in dopamine levels did not correlate with the change in markers of mental alertness (Suppl. Fig. 1b-d) or with the change in eCB levels (Suppl. Fig. 1e-h), whereas higher eCB levels were associated with impaired mental alertness. It should be highlighted that, in contrast to eCBs, dopamine does not cross the blood-brain barrier, and that central and circulating dopamine concentrations do not correlate[12]. Therefore, eCBs are superior biomarkers of mental alertness following ultra-endurance exercise compared to (circulating) dopamine levels. The eCB – brain dopamine axis requires further investigation, especially in humans and in the context of exercise.

Alternatively, the energetic stress due to ultra-endurance exercise, which is not always present during common endurance exercise, might have increased eCB levels such as AEA in the brain[13], which is a protective response to stimulate food intake via CB1 activation[14]. Therefore, it can be hypothesized that participants with higher energetic stress, and thus higher eCB levels, had lower energy availability to fuel the brain. Participants in the KE group performed better on the cognitive test battery[7] and exhibited lower circulating levels of the eCB PEA (Fig. 1) than the CON group. However, it is unclear whether they exhibited lower energetic stress, as the brain energy substrate glucose was lower and the ketone body D-β-Hydroxybutyrate was higher in KE compared to CON[7]. More work is needed to explore the metabolic stress - eCB axis in the context of cognitive performance following (ultra-)endurance exercise.

It is surprising that increased eCB levels upon exercise are related with improved memory[11] and, at the same time, with decreased mental alertness in the present study. This discrepancy might be attributed to the fact that ultra-endurance running (~85 km; ~10.2 hours) in the present study induces central fatigue whereas mental alertness might have increased upon shorter exercise bouts (15-30 minutes) in other studies. It can also be hypothesized that acute eCB-induced CB1 activation in the brain, during common exercise (15-90 minutes), improves the cognitive function[15,16], whereas longer

CB1 activation, during ultra-endurance exercise (~600 minutes), might have desensitized and downregulated CB1, and thereby cognitive function. However, it is not clear whether ~10h activation is sufficient to induce CB1 desensitization and downregulation in the human brain.

Ketone ester supplementation improved mental alertness following ultra-endurance exercise[7]. Interestingly, ketone esters also attenuated the ultra-endurance-induced increase in PEA levels, which might explain some of the effects on mental alertness (Fig. 1e). Indeed, increased PEA levels significantly correlated with a decrease in mental alertness (i.e. increased MRT; Fig. 2b). In contrast, preclinical evidence showed that PEA treatment improved learning and memory in a murine Alzheimer's disease model[17] and recent work found that 6-week PEA supplementation increased circulating BDNF levels and improved memory in healthy adults[18]. Together, these data suggest that PEA plays a role in mental processes, be it in a contradictory way. Further work is definitely required to uncover the dual role of PEA in mental processes and to test the therapeutic potential of PEA supplementation in situations where cognition and/or mental alertness are challenged, such as ultra-endurance exercise and disease.

Together, these data suggest that eCBs play a dual role in central processes following endurance exercise, potentially depending on modalities such as exercise duration. The present study included only 18 white, male, trained participants, which limits the external validity of the findings. Future studies with more (diverse) participants and different cognitive challenges are needed to further increase our understanding of the source(s) of increased eCB levels during exercise, and of the mechanism(s) that link eCBs with central processes such as mental alertness. The cognitive test batteries applied in this study (i.e. reaction time and rapid visual processing task) shed light on a limited aspect of mental alertness. The inclusion of other cognitive tests that assess more cognitive processes, e.g. attention and memory, might provide broader insights into how eCBs modulate mental alertness upon ultra-endurance exercise.

Authorship contribution statement

Conceptualization: SD, CP, KK; Methodology: CP; Formal analysis: SD, CP, WL, RR, MS, RM, GM; Writing original draft: SD; Reviewing & editing: SD, CP, WL, RR, MS, GM, KK; Supervision: CP, KK; Funding acquisition: SD, CP, KK. All authors have read and agreed to the published version of the manuscript.

Author Disclosure Statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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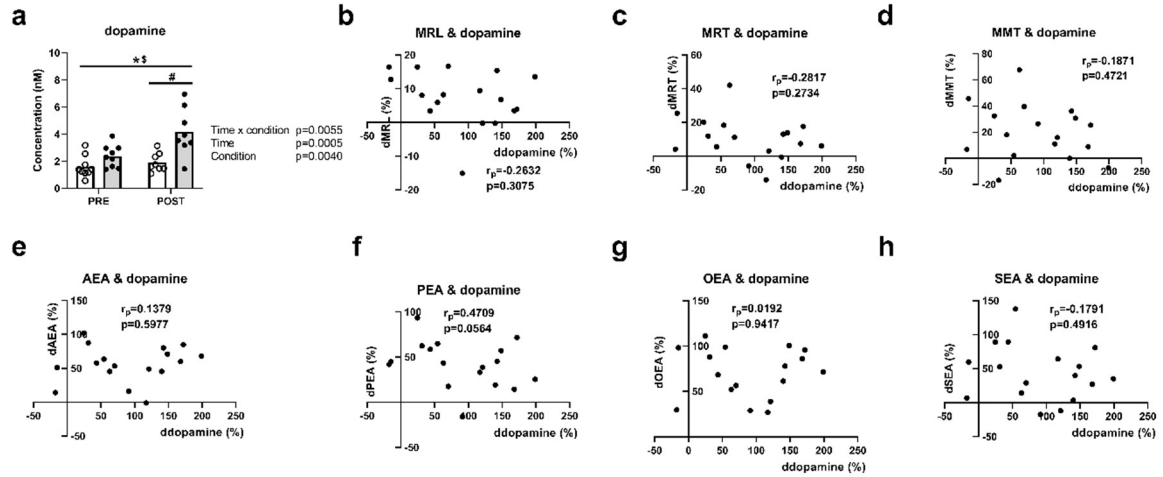
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Supplementary table 1. Participant characteristics

	All	CON	KE	P value
Sample size	18	9	9	
Age (year)	35.6 ± 7.9	34.1 ± 8.7	37.1 ± 7.2	0.44
Weight (kg)	73.4 ± 7.0	73.8 ± 8.2	73.1 ± 6.0	0.85
Height (m)	1.82 ± 0.07	1.83 ± 0.08	1.80 ± 0.05	0.39
BMI (kg/m²)	22.3 ± 1.9	22.0 ± 2.1	22.5 ± 1.8	0.60
VO_2max (ml/kg/min)	53.5 ± 3.5	53.4 ± 3.8	53.6 ± 3.4	0.95
AT (km/h)	13.3 ± 0.6	13.4 ± 0.7	13.3 ± 0.5	0.82
LT (km/h)	15.3 ± 1.1	15.4 ± 1.2	15.2 ± 1.0	0.71
Training volume (km/wk)	74 ± 22	72 ± 24	75 ± 21	0.80
No. of ultraruns ≥100 km completed	4 ± 4	4 ± 4	4 ± 4	0.95

Data are means ± SD and represent baseline characteristics of the subjects receiving either placebo (CON; n = 9) or ketone ester (KE; n = 9). Except for a single subject in both KE (maximum distance: 80 km) and CON (maximum distance: 85 km), all participants completed at least one ultrarun of ≥100 km. BMI, body mass index; AT, aerobic threshold; LT, lactate threshold.



Suppl. Fig. 1. *The effect of ketone ester supplementation during ultra-endurance running on circulating dopamine levels (a) and the correlation between the change in dopamine levels (post-trail – pre-trail), on one hand, and the change in mental alertness (post-trail – pre-trail; b-d) and the change in serum eCB levels (post-trail – pre-trail; e-h), on the other hand, in the CON (n=9) and KE (n=9) group combined. Data are shown as mean (bar graph) and individual data points (a). During running, participants received either ketone ester supplements (KE, n = 9) or a placebo (CON, n = 9). Main and interaction effects were achieved via two-way repeated-measures ANOVA (a). *p < 0.05 for a significant interaction effect (Time * Condition); #p < 0.05 for a significant time effect (Pre vs. Post); $p < 0.05 for a significant condition effect (KE vs. CON) (a). Presented measures of mental alertness include median reaction time (MRT), median reaction latency (MRL) and median movement time (MMT). Blood eCBs include anandamide (AEA), palmitoylethanolamide (PEA), oleoylethanolamide (OEA) and stearoylethanolamine (SEA). Data were normally distributed and analyzed via Pearson correlations. Pearson correlation coefficients (r_p) and significances are presented. d refers to delta or Δ (b-h).*