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Short-term ketone monoester supplementation improves cerebral blood flow and cognition in obesity: A randomized cross-over trial

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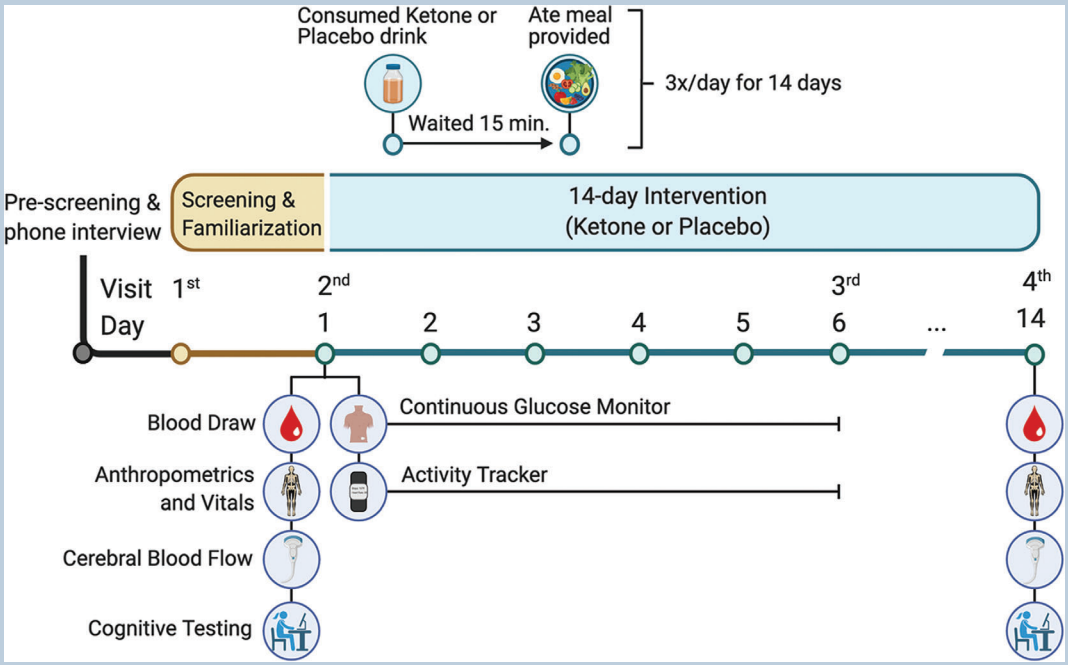

Abstract Adults with obesity are at increased risk of neurocognitive impairments, partly as a result of reduced cerebral blood flow and brain-derived neurotrophic factor (BDNF). Ketone

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supplements containing β-hydroxybutyrate (β-OHB) are a purported therapeutic strategy for improving brain health in at-risk populations. We tested the hypothesis that short-term β-OHB supplementation will elevate cerebral blood flow and BDNF, as well as improve cognition in adults with obesity. In a placebo-controlled double-blind, cross-over design, 14 adults with obesity (10 females; aged 56 $\pm$ 12 years; body mass index = 33.8 $\pm$ 6.9 kg m^{-2}) consumed 30 mL (12 g) of β-OHB or placebo thrice-daily for 14 days. Blood flow (Q) and cerebrovascular conductance (CVC) were measured in the common carotid (CCA), internal carotid (ICA) and vertebral (VA) arteries by duplex ultrasound. BDNF was measured by an enzyme-linked immunosorbent assay. Cognition was assessed by the digit-symbol substitution (DSST), Stroop and task-switching tests. Following 14 days of ketone supplementation, we observed significant improvements in cerebrovascular outcomes including Q_{CCA} (+12%), Q_{VA} (+11%), VA_{CVC} (+12%) and VA shear rate (+10%). DSST performance significantly improved following ketone supplementation (+2.7 correct responses) and improved DSST performance was positively associated improvements in cerebrovascular outcomes including Q_{CCA}, CCA_{CVC}, Q_{VA} and VA_{CVC}. By contrast to one hypothesis, β-OHB did not impact fasting serum and plasma BDNF. β-OHB supplementation improved cognition in adults with obesity, which may be partly facilitated by improvements in cerebral blood flow. β-OHB supplementation was well-tolerated and appears to be safe for cerebrovascular health, suggesting potential therapeutic benefits of β-OHB in a population at risk of neurocognitive impairment.

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Abstract figure legend Schematic of our placebo-controlled crossover trial that investigated the effect of 14-days ketone supplementation on metabolic, vascular, and cognitive function in adults with obesity

Key points

- People with obesity are at increased risk of neurocognitive dysfunction, partly as a result of -induced reductions in cerebral blood flow (CBF) and brain-derived neurotrophic factor (BDNF).
- Ketone supplements containing β-hydroxybutyrate (β-OHB) reduce postprandial hyperglycaemia, which may increase CBF and BDNF, thereby protecting against obesity-related cognitive dysfunction.
- We show for the first time that 14 days of thrice-daily β-OHB supplementation improves aspects of cognition and increases cerebrovascular flow, conductance and shear rate in the extracranial arteries of adults with obesity.
- Our preliminary data indicate a significant positive relationship between elevated CBF and improved cognition following β-OHB supplementation.
- This trial provides a foundation for the potential non-pharmacological therapeutic application of β-OHB supplementation in patient groups at risk of hyperglycaemic cerebrovascular disease and cognitive dysfunction.

Introduction

Adults with obesity are at increased risk for numerous cardiometabolic pathologies, including hyperglycaemia, insulin resistance, cerebrovascular disease and cognitive dysfunction (Alosco & Gunstad, 2014; Prickett *et al.* 2018). Both acute (Duckrow, 1995) and chronic (Duckrow *et al.* 1987) hyperglycaemia reduce cerebral blood flow (CBF) in animals, and reduced glucose tolerance is associated with poor cognitive performance and hippocampal atrophy in cognitively normal older adults (Convit *et al.* 2003). Impaired glucose tolerance and obesity are independently associated with the reduced levels of circulating brain-derived neurotrophic factor (BDNF) (Krabbe *et al.* 2007). It is now established that BDNF is a key orchestrator of activity-dependent brain plasticity, as well as the maintenance of neuronal integrity, and is critical for cognitive functions (Griffin *et al.* 2009; Marosi & Mattson, 2014). Accordingly, interventions that can mitigate metabolic dysfunction and protect against

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hyperglycaemia may have important implications for brain health in adults with obesity.

Supplementation with a ketone monoester containing the ketone body β-hydroxybutyrate (β-OHB) represents a therapeutic strategy that may protect and improve brain health in adults with obesity. β-OHB is an endogenous, alternative fuel source for the brain, heart and skeletal muscle during periods of starvation, and exerts pleiotropic effects as a signalling molecule (Newman & Verdin, 2014). Until recently, elevating circulating β-OHB levels was only achievable via prolonged fasting or severe carbohydrate restriction. The recent advent of exogenous oral ketone supplements capable of inducing rapid, nutritional ketosis and attenuating hyperglycaemia (Clarke *et al.* 2012*b*; Myette-Côté *et al.* 2018, 2019; Soto-Mota *et al.* 2019) represents a potential therapeutic strategy for protecting and improving brain health, especially in adults with obesity.

The human brain can readily uptake and oxidize β-OHB in a linear fashion with increasing blood-borne concentrations within a high, physiological range (Mikkelsen *et al.* 2015). This shift in cerebral metabolism is accompanied by immediate functional effects because the acute ingestion of exogenous β-OHB improves brain network stability in normoglycaemic adults (Mujica-Parodi *et al.* 2020) and acute infusion of β-OHB transiently improves working memory in adults with type 2 diabetes (T2D) (Jensen *et al.* 2020). Improvements in cognitive function may be partly facilitated by increases in CBF and/or increased expression of BDNF. For example, infusion of β-OHB increases CBF by 30–40% in young (Hasselbalch *et al.* 1996) and middle-to-older aged humans (Svart *et al.* 2018). Although the mechanisms remain unclear and independent of changes in arterial P_{CO_2} or pH, subtle alternations in neuronal redox potential (Vlassenko *et al.* 2006; Xin *et al.* 2018), reductions in oxidative stress(Shimazu *et al.* 2013) and/or direct effects vascular function (Kimura *et al.* 2011; Han *et al.* 2018; Wu *et al.* 2020) may all contribute. Currently, however, these effects are described solely in terms acute β-OHB exposure (i.e. hours) and the impact of regular short-term (i.e. days) ketone supplementation on CBF is unknown.

Improvements in cognitive function with β-OHB may be facilitated by BDNF (Arentoft *et al.* 2009; Erickson *et al.* 2012; Sun *et al.* 2018). Animal and *in vitro* models show that β-OHB upregulates BDNF in neurons (Sleiman *et al.* 2016; Marosi *et al.* 2016; Hu *et al.* 2018) and we have shown that β-OHB ingestion (0.45 mL kg^{-1} body weight; 3.4 mM peak) prior to an oral-glucose tolerance test (OGTT) increases plasma BDNF in adults with obesity (Walsh *et al.* 2020). Accordingly, exogenous β-OHB supplementation may be a viable strategy for increasing BDNF and potentially proffering some neuro-protective effects, especially in vulnerable individuals.

The effect of a practical short-term β-OHB supplementation intervention on cognitive function, CBF and BDNF in adults with obesity is currently unknown. Therefore, the present placebo-controlled, double-blind, cross-over trial aimed to investigate the effect of 14 days of thrice-daily ketone supplementation on CBF, cognitive function and BDNF in adults with obesity. We hypothesized that ketone supplementation would lead to elevations in CBF and circulating BDNF and this would be reflected by improved cognitive function.

Methods

The present study reports secondary outcomes from a trial registered as 'Ketone Supplementation, Glucose Control, and Cardiovascular Function' (ClinicalTrials.gov Identifier NCT03817749). The primary outcome of this study postprandial glucose and the main findings have been reported elsewhere (Walsh *et al.* 2021). This trial was approved by the University of British Columbia Clinical Research Ethics Board (ID H18-02930). The trial conformed with the standards set by the *Declaration of Helsinki* and subsequent revisions, and all participants provided their written informed consent prior to commencement of the trial.

Participants

Participants between the ages of 30–69 years were recruited from the greater Kelowna, BC, area between March 2019 and January 2020. Inclusion criteria were the presence of at least one of the following: (i) elevated waist circumference of >102 cm for males and >88 cm for females; (ii) body mass index >30 kg m^{-2}; or (iii) diagnoses of prediabetes based on A1C (5.7–6.4%) and/or fasting plasma glucose (5.6–6.9 mmol L^{-1}) (American Diabetes Association, 2020). Exclusion criteria were actively attempting to lose weight, history of previous cardiovascular events, presence of T2D, presence of a chronic inflammatory disease, currently following ketogenic diet or taking ketone supplements, current tobacco or drug use, or history of hypoglycaemia. A telephone screening interview was performed to assess participant eligibility. If deemed eligible for the study, participants were invited for an in-person familiarization visit. Female participants completed a menstrual cycle questionnaire and all female participants reported being postmenopausal and were not taking hormone supplements.

Recruitment was closed once 15 participants were enrolled. Following the familiarization visit, participants were randomly assigned to either the ketone or placebo condition for 14 days and, after at least 14 days of washout, completed the alternate condition. The randomization

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schedule was prepared using an online generator using block sizes of 2 and 4 (https://www.sealedenvelope.com/simple-randomiser/v1/lists). Sealed opaque envelopes were used to reveal randomization sequence, which was denoted as A–B or B–A with identity of the ketone and placebo supplement blinded to both researchers and participants. The final allocation ratio for Trial 1 was 7:8 (Condition A:Condition B). All experimental visits were conducted in the Exercise Metabolism and Inflammation Laboratory at the University of British Columbia, Okanagan Campus, BC, Canada.

Intervention design

The trial comprised a randomized, placebo-controlled, double-blind, cross-over trial following the 2010 CONSORT guidelines (*The Lancet*, 2010). Each participant attended the laboratory three times per condition (pre-intervention, continuous glucose monitor removal and post-intervention). The present study presents data from the the pre- and post-intervention visits.

Participants consumed either a ketone or placebo supplement 15 min prior to breakfast, lunch and dinner for 14 days. Supplements were clear, taste-matched drinks and were provided in single serving opaque bottles (42 bottles per condition). Participants were provided with all meals for each 14 day intervention period. Breakfast, lunch and dinner meals were provided by a local meal preparation service and comprised ∼50% energy from carbohydrate, 30% from fat and 20% from protein.

Daily meals were supplemented with participant-selected, pre-packaged snacks prior to their first intervention period, aiming to meet individual estimated daily energy requirements based on the Harris–Benedict equation with an activity factor of 1.4 (Harris & Benedict, 1918). An example menu plan is provided elsewhere (Walsh *et al*. 2021). Meals and snacks were delivered to participants prior to their first visit for both conditions to ensure the exact same foods were eaten prior to pre-intervention measures. Following the pre-intervention visit, participants received meals, snacks and supplements equivalent to a 5 day supply. Subsequent food and supplement exchanges were co-ordinated between researchers and participants on an individual basis. Participants recorded all food and drink consumed during their first trial condition in a food log and any deviations from the meal plan were matched during the second trial condition. Compliance with the supplement regimen was assessed by self-report log and returning of empty supplement bottles. Participants were asked to report their feelings of hunger and fullness following their meals, as well as gastrointestinal symptoms following supplement ingestion, during the first 4 days of each condition using visual analogue scales (Walsh *et al*. 2021).

Supplement details

The ketone supplement used in this study was a (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate ketone monoester (ΔG; HVMN Inc., San Francisco, CA< USA), which contained 0.4 g mL^{-1} β-OHB, natural flavouring and <2% stevia leaf extract. The taste-matched placebo drink contained the same natural flavouring as the ketone supplement, stevia leaf extract, 5 mL of Bittrex stock (0.005 g of denatonium benzoate powder in 40 mL of water) and 1.5 mL of arrowroot stock (2 g of arrowroot powder in 50 mL of water) to match the bitter flavour and viscosity of the ketone monoester. Supplements were dispensed into opaque 30 mL bottles (12 g $dose^{-1}$ β-OHB; 36 g day^{-1} β-OHB) and labelled either A or B by a third-party researcher. Blinding was maintained until completion of data collection and analysis, in its entirety. The intention of our dosing regimen was to maintain elevated circulating β-OHB levels during the waking hours of the day. Clarke *et al*. (2012*a*) reported that ingestion of a relatively low β-OHB dose (0.14 g kg^{-1} body weight) significantly elevates β-OHB for up to 4 h in healthy adults, and we confirmed that our dosing regimen significantly elevated β-OHB within 15 min of ingestion (Walsh *et al*. 2021).

Trial outcomes

For the pre- and post-intervention visits, participants arrived at the laboratory between 08.00 h and 09.00 h (local time) following an overnight fast (≥10 h), having abstained from caffeine and alcohol for at least 12 h, as well as any exercise beyond their regular activities for at least 24 h. During these visits, a battery of measures was performed and these have been reported in detail elsewhere (Walsh *et al*. 2021). Measures included anthropometry, body composition, peripheral vascular function (brachial artery flow-mediated dilatation) and fasting blood samples to determine plasma cytokines and immune cell function. The present study presents CBF, cognitive function and BDNF measures taken during these visits. All measures were performed in the same sequence and by the same researchers for the entire study.

Haemodynamic and respiratory measurements

All haemodynamic and cerebrovascular outcomes were assessed before and after each intervention period, following 20 min of quiet, supine rest with participants in a fasting, non-supplemented state. At least three automated brachial blood pressure measurements (model BP769CAN; Omron Healthcare, Kyoto, Japan), including heart rate were performed in the supine position immediately following the cerebrovascular assessment. Mean arterial pressure (MAP) was calculated as: 1/3

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systolic blood pressure + 2/3 diastolic blood pressure. The partial pressure of end-tidal carbon dioxide (P_{ETCO_2}) and respiratory rate were measured using a capnograph (EMMA, Masimo, Irvine, CA, USA) immediately prior to the cerebrovascular assessment.

Cerebral blood flow assessment

Blood velocity and vessel diameter of the internal carotid artery (ICA), common carotid artery (CCA), and vertebral artery (VA) were measured using a 10 MHz multifrequency linear array Duplex ultrasound (Terason uSmart 3300; Teratech, Burlington, MA, USA) using the previously described location and standardization techniques (Thomas *et al.* 2015). Pulse-wave mode was used to measure peak blood velocity and arterial diameter in the sagittal axis was measured concurrently using B-mode imaging. The ICA and CCA blood velocity and vessel diameter were measured $\geq$1.5 cm from the carotid bifurcation to avoid any turbulent or retrograde flow patterns. The VA blood velocity and diameter were measured between C4 and C5 or C5 and C6. The vessel location was decided on an individual basis to allow for reliable image acquisition, with the same location repeated within participants and between trials. Additionally, the insonation angle (60°) was unchanged throughout each test and, following acquisition of the first ultrasound image, there was no alteration of B-mode gain or dynamic range to avoid changes in arterial wall brightness/thickness. The between-day coefficients of variation in the current study for ICA, VA and CCA diameter were 6.1%, 2.2% and 1.7%, respectively. There were difficulties in obtaining reliable images and blood velocity recordings for some participants as a result of anatomical constraints (e.g. very deep vessel structures resulting from excess adiposity and/or carotid stenosis/plaque with turbulent blood flow); therefore, the following sample sizes were included: ICA, $n = 8$, and VA, $n = 8$. The CCA measurements were conducted in $n = 7$ as a surrogate for 'bulk CBF' in the remaining participants where Q_{ICA} could not be obtained.

Data processing

The Q_{ICA}, Q_{CCA} and Q_{VA} recordings were at least 1 min for each measurement including at least 12 cardiac cycles for analyses (Thomas *et al.* 2015). Duplex ultrasound recordings were screen captured and saved for offline analysis using custom edge-detection and wall tracking software (BloodFlow Analysis, version 5.1). This analysis method utilizes integration of diameter and velocity traces to calculate mean beat-to-beat flow at 30 Hz independent of observer bias (Woodman *et al.* 2001).

Calculations

Blood flow was calculated as:

$$Q = \text{peak envelope blood velocity}/2 \times \left(\pi\left(0.5 \times \text{diameter}\right)^2\right) \times 60.$$

Mean shear rate was calculated as:

$$\text{Shear rate} = 4 \times \text{peak envelope blood velocity / arterial diameter}$$

Cerebrovascular conductance (CVC) was calculated as:

$$Q_{ICA},\ Q_{VA} \text{ or } Q_{CCA}/\text{MAP}$$

Cognitive function assessment

Cognitive function was assessed using a battery of three psychometrically valid tests on a tablet (BrainBaseline; Digital Artefacts, Iowa City, IA, USA). The test battery consisted of the digit symbol substitution task (DSST), the Stroop task and task-switching task (TST). The DSST requires participants to match symbols with digits based on nine corresponding digit-symbol pairings. The primary end-point of the DSST is the number of correct items processed in 90 s. For the Stroop task, participants were asked to indicate the colour of the ink that a word was written in via button press. Three types of stimuli were used for the Stroop task: (i) congruent-when ink colour and words are the same (e.g. RED written in red ink); (ii) incongruent-when ink colour and words are different (e.g. RED written in green ink); and (iii) neutral-when the presented word is not related to a colour (e.g. HOUSE written in red ink). The primary end-point for the Stroop task is Stroop cost (calculated as: Incongruent minus congruent response time (ms)). The TST requires participants to switch between two separate tasks depending on the colour of stimuli presented. If a blue box is presented, participants must determine whether the number in the box is higher or lower than five; if the box is pink, participants must determine whether the number is odd or even. The primary end point for TST is switch cost (calculated as: switch stimuli response time minus stay stimuli response time (ms)). The battery assessed processing speed, working memory, selective attention and inhibitory control. All testing was performed in a quiet room free from distraction. To account for learning effects due to repeated testing, a practice session was completed during the familiarization visit. Practice session data were not included in the final analysis. Between-day coefficient of variation (CV) and intra-class correlation coefficient (ICC) for the DSST were 9.6% and 0.78, respectively. For TST, CV = 50% and ICC = 0.40. For Stroop, CV = 7.7% and ICC = 0.82.

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BDNF measurement

Both plasma and serum BDNF were measured, given that plasma represents the unbound, bioavailable pool, and serum represents unbound and platelet-bound BDNF (Walsh & Tschakovsky, 2018). For the determination of plasma BDNF, blood was drawn in EDTA tubes and centrifuged immediately at 1500 ***g*** for 15 min at 4°C. The resultant supernatant was aliquoted and centrifuged at 10,000 ***g*** for 10 min at 4°C to remove platelets. Platelet-poor plasma was subsequently aliquoted and stored at −80°C. For the determination of serum BDNF, blood was drawn in serum separator tubes and left to clot at room temperature for 1 h based on the recommendations of Amadio *et al.* (2017). Serum tubes were then centrifuged at 1500 ***g*** for 15 min at 4°C and the resultant supernatant was aliquoted and stored at −80°C. Serum and platelet-poor plasma BDNF were measured via a commercially available enzyme-linked immunoassay (BEK-2211-2P; Biosensis, Thebarton, SA, Australia). The intra- and inter-assay coefficients of variation are 1.0% and 5.0%, respectively, as reported by an independent third party (Polacchini *et al.* 2015). Serum BDNF was diluted 100-fold in accordance with the manufacturer's recommendation and confirmed by in-house assay optimization. All other steps were followed in accordance with the manufacturer's instructions, with the exception of five-fold sample dilution for plasma to ensure that samples were within the standard curve detection range (7.8–500 pg mL^{-1}), as determined by in-house assay optimization trials.

Sample size

This investigation represents secondary analyses of the trial, for which sample size estimated *a priori* for the primary outcome of postprandial glucose control (Walsh *et al.* 2021). As such, sample size was not estimated for the outcomes reported in the present study.

Statistical analysis

Data were analysed blinded to condition allocation using R, version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented as the mean ± SD or mean difference (95% confidence interval) unless otherwise noted. *Q*–*Q* and standardized-residual plots were used to assess model assumptions within each outcome. If one or more criteria were not met, data were transformed.

A linear mixed-model analysis with fixed effects of condition (ketone *vs.* placebo) × type (an indicator variable indicating whether it is an outcome or baseline measurement) interaction and a period (indicating the order of conditions received) × type interaction, and a subject random effect was used to compare all cerebrovascular and haemodynamic variables. An unstructured covariance structure was assumed and was specified to account for the correlation present within individuals during a period (i.e. the baseline and outcome values during a particular treatment period). This model allowed us to estimate the effect of condition on our outcomes, accounting for baseline values and the order in which condition was received (period effect) (Jones & Kenward, 2014). We assumed no carry-over between conditions given the washout period of at least 2 weeks between conditions and acute ingestion of exogenous ketones elevates β-OHB for 5–6 h (Soto-Mota *et al.* 2019). Accordingly, carry-over effects were not tested formally.

Results

Anthropometric and fasting metabolic responses to the intervention

A detailed reporting of the anthropometric and metabolic responses to the intervention is available elsewhere (*Walsh et al.* 2021). Fifteen participants were randomized to either the placebo or ketone condition in the first phase of the trial; however, one participant in the ketone condition failed to follow study procedures, including consuming the supplements and meals provided, and was removed from the trial (Fig. 1). Fourteen participants (10 females) completed the trial and were included in the final analysis (Fig. 1 and Table 1). Thirteen (9 female) participants met the inclusion criteria for obesity (body mass index range = 31.1–49.9 kg m^{-2}; waist circumference range = 89.0–141.0 cm) and one participant (female) met the inclusion criteria for high fasting glucose (5.93 mM) without being overweight or obese.

There was no effect of either placebo or ketone supplementation on fasting plasma glucose, insulin, C-peptide and non-esterified fatty acids (Table 2). There was a small, systematic reduction in body weight over time, although there was no difference between conditions (Table 2). Otherwise, there were no changes in body composition including measures of fat mass, lean body mass or waist circumference.

Supplementation adherence, tolerability and efficacy

Adherence to the supplementation regimen was very high, and there was no difference in compliance between the placebo and ketone conditions (99 ± 2% *vs.* 98 ± 4%, respectively; $P = 0.25$). Both supplements were well tolerated by participants and did not impact feelings of hunger or fullness and there were no reported adverse events or unintended effects (Walsh *et al.* 2021). During the familiarization visit prior to randomization,

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participants consumed a dose of the β-OHB supplement, providing 12 g of β-OHB within a berry-flavoured ketone monoester drink (HVMN Inc.). This dose resulted in an elevation of blood β-OHB to 1.8 $\pm$ 1.3 mM (range 0.6–4.7 mM) within 15 min, confirming that the supplementation protocol was efficacious with respect to raising blood ketones (Walsh *et al.* 2021).

Cognitive function

Table 3 displays performance outcomes for the three cognitive function tests. After adjusting for baseline values and a potential period effect, DSST performance (number of correct responses in 90 s; CR) significantly improved from pre- to post-supplementation within the ketone condition (2.7 CR; range 1.3–4.1; $P = 0.0003$). There

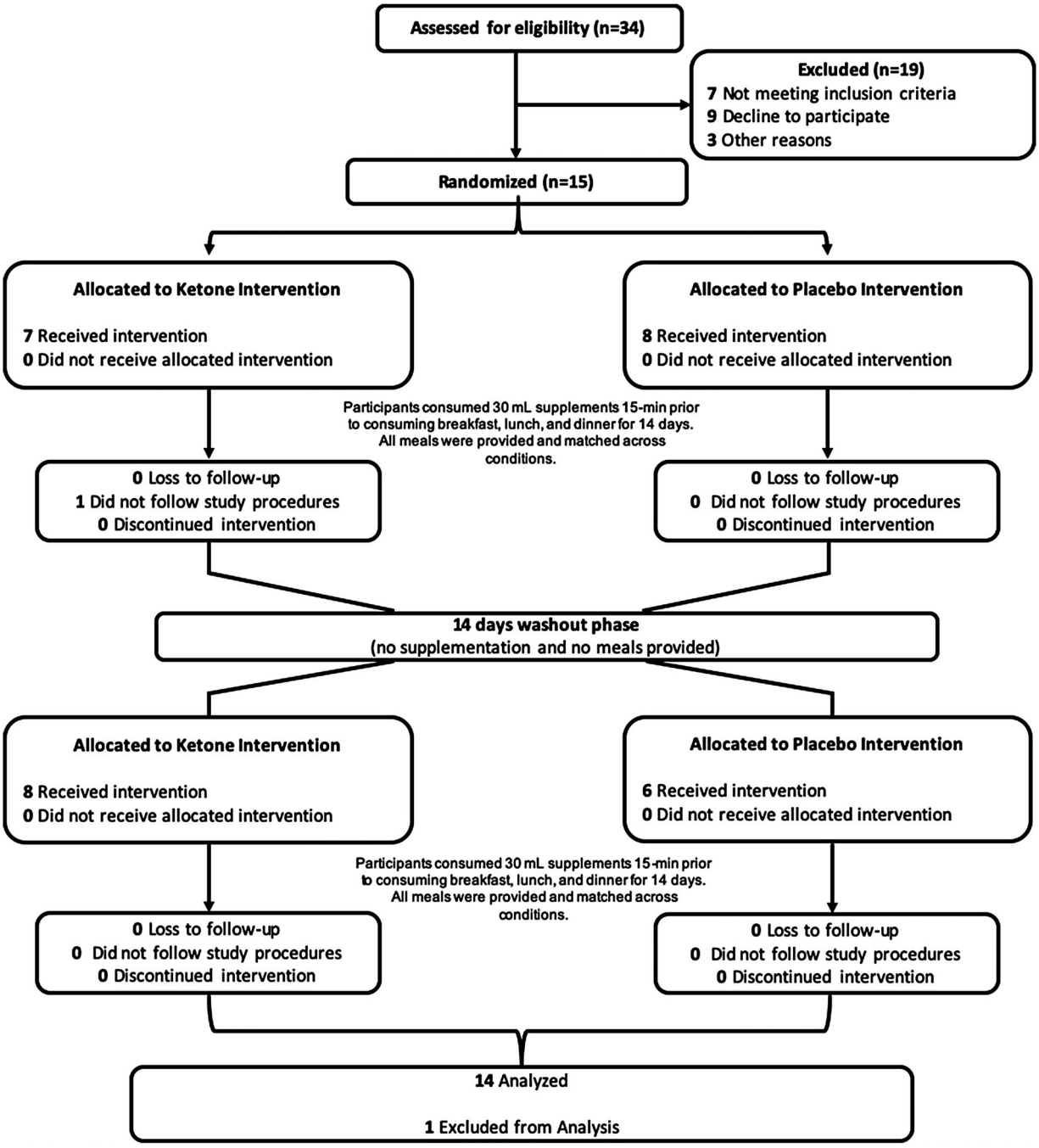

Figure 1. Trial flowchart
Trial flowchart in the form of a consort flow diagram.

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Table 1. Baseline characteristics

	Group (n = 14)	Female (n = 10)	Male (n = 4)
Age (years)	55.8 (12.4)	59.0 (8.2)	47.8 (18.4)
Body weight (kg)	95.1 (22.2)	91.5 (25.2)	104.0 (8.8)
Body mass index (kg m^{-2})	33.8 (6.9)	34.1 (8.2)	33.1 (1.5)
Waist circumference (cm)	109.2 (17.1)	108.4 (20.4)	111.3 (1.7)
Systolic blood pressure (mmHg)	132 (18)	134 (19)	128 (17)
Diastolic blood pressure (mmHg)	81 (11)	84 (10)	74 (13)
Glucose (mmol L^{-1})	4.74 (0.49)	4.61 (0.32)	4.96 (0.35)
Insulin (uIU mL^{-1})	21.58 (13.41)	20.11 (14.51)	25.27 (10.78)
C-peptide (pmol L^{-1})	2324.2 (1112.5)	2404.6 (1219.2)	2123.4 (910.2)
NEFA (mmol L^{-1})	0.63 (0.15)	0.66 (0.15)	0.54 (0.14)

NEFA, non-esterified fatty acids. Data are the mean (SD). All measures were taken in the fasting, non-supplemented state.

Table 2. Changes in anthropometric and fasting metabolic outcomes following intervention

	Placebo (n = 14) Δ_{Mean} (95% CI)	Ketone (n = 14) Δ_{Mean} (95% CI)	P value Time
Body weight (kg)	−1.08 (−1.77 to −0.39)*	−0.97 (−1.66 to −0.28)*	0.0007
Body mass index (kg m^{-2})	−0.46 (−0.72 to −0.21)*	−0.39 (−0.65 to −0.13)*	0.0001
Waist circumference (cm)	−0.01 (−1.74 to 1.71)	−1.2 (−2.9 to 0.51)	0.16
Glucose (mmol L^{-1})	0.10 (−0.18 to 0.38)	0.21 (−0.14 to 0.55)	0.09
Insulin (uIU mL^{-1})	−2.38 (−23.16 to 18.40)	−16.78 (−52.48 to 18.93)	0.23
C-peptide (pmol L^{-1})	4.77 (−647.7 to 657.3)	−364.5 (−962.3 to 233.4)	0.20
NEFA (mmol L^{-1})	−0.15 (−0.32 to 0.02)*	−0.11 (−0.25 to 0.02)*	0.004

NEFA, non-esterified fatty acids. Data are the mean differences (Δ_{Mean}) between post- minus pre-intervention measures with 95% confidence intervals (CI). The complete dataset is reported elsewhere (Walsh *et al*. 2021)
*Significant main effect of time within condition ($P < 0.05$).

Table 3. Cognitive function outcomes

	Placebo (n = 14)		Ketone (n = 14)		P value
	Pre	Post	Pre	Post	Condition
Digit-symbol substitution (number correct)	37.1 (8.7)	38.8 (7.1)	38.6 (7.4)	40.9 (7.8)*	0.0003
Task-switching (ms)	208.3 (162.7)	175.1 (106.9)	216.4 (142.2)	185.0 (150.4)	0.67
Stroop task (ms)	195.6 (104.3)	185.7 (83.5)	220.1 (174.8)	199.5 (103.2)	0.78

Digit-symbol substitution scored as number of correct items processed in 90 s. Task-switching scored as switch cost (ms): the difference in reaction time between stay conditions and switching conditions. Stroop scored as interference cost (ms): the difference in reaction time between correct congruent and incongruent stimuli. Data are the mean (SD).
*main effect of ketone supplementation after adjusting for trial period and baseline values, $P < 0.05$

was no difference in Stroop Task performance (Stroop cost, ms) (5.7 ms; range –35.2 to 46.6; $P = 0.78$) or TST performance (switch cost, ms) (13.8 ms; range –51.7 to 79.3; $P = 0.67$) following ketone supplementation compared to placebo.

Haemodynamic and cerebrovascular responses

There was no change in resting haemodynamic variables within or between interventions including: MAP, heart rate, P_{ETCO_2} and respiratory rate (Table 4). To account for intra-individual changes within and between conditions,

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Table 4. Resting haemodynamic parameters

	Placebo (n = 14)		Ketone (n = 14)		P value
	Pre	Post	Pre	Post	Condition
MAP (mmHg)	98 (13)	94 (14)	99 (13)	98 (15)	0.75
Heart rate (min^{-1})	64 (11)	62 (9)	64 (9)	62 (10)	0.83
P_{ETCO_2} (mmHg)	33 (3)	32 (2)	32 (2)	32 (3)	0.61
Respiratory rate (min^{-1})	12 (4)	13 (4)	13 (4)	12 (4)	0.06

MAP, mean arterial pressure; P_{ETCO_2}, partial pressure of end tidal carbon dioxide. Data are the mean (SD). All measures were taken in the fasting, non-supplemented state.

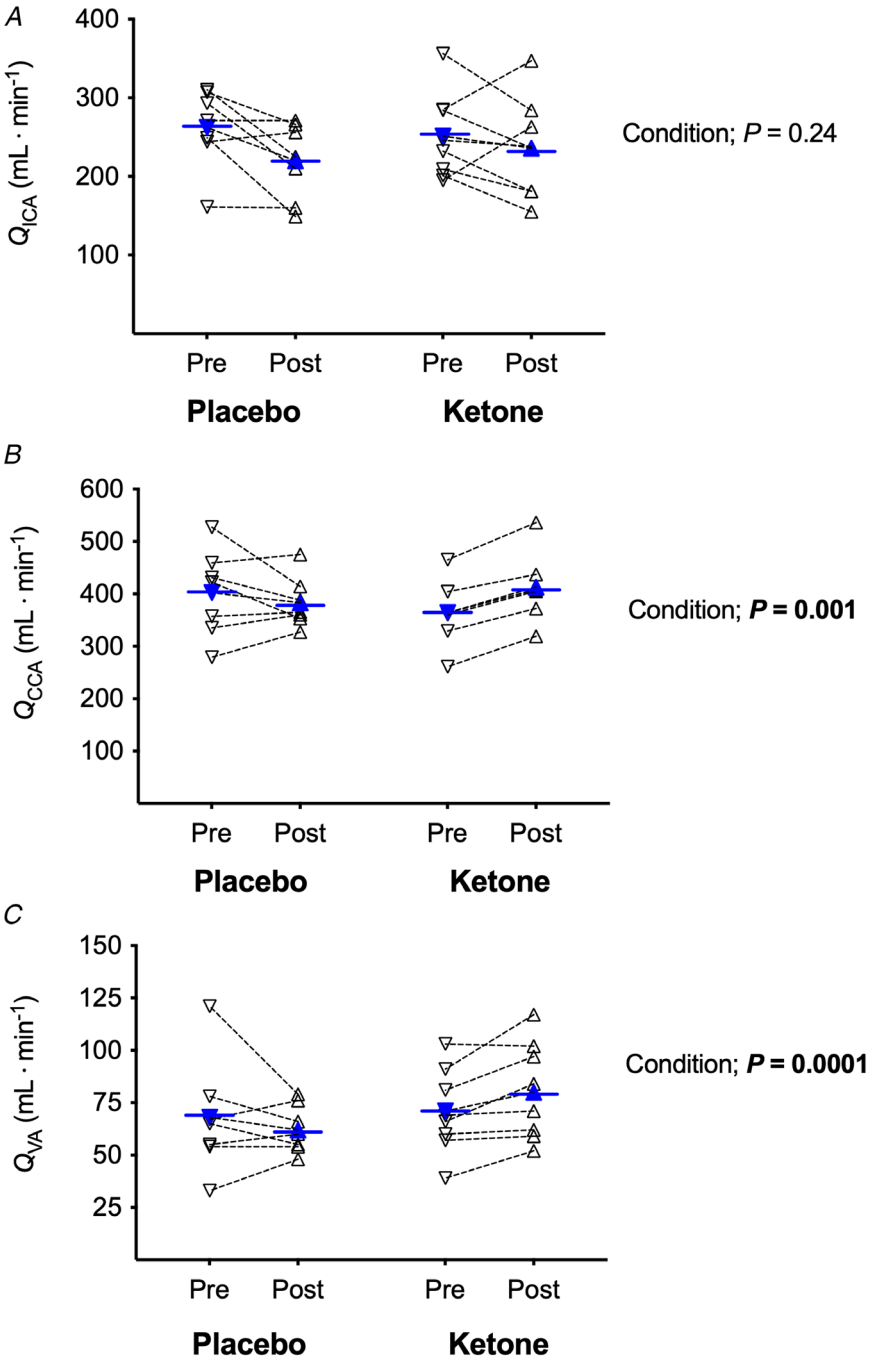

Figure 2. Cerebral blood flow responses to 14 days of either placebo or ketone supplementation
Blood flow (Q) responses to placebo or ketone supplementation in the internal carotid artery (ICA; n = 8) (*A*); common carotid artery (CCA; n = 7) (*B*); and vertebral artery (VA; n = 8) (*C*). Closed blue symbols are group means. Open symbols and hatched lines are individual responses within each condition. P values are main effect of condition after adjusting for trial period and baseline values.

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sensitivity analyses were performed separately that included MAP and P_{ETCO_2} as covariates for Q_{ICA}, Q_{VA} and Q_{CCA} responses, respectively; however, there was no significant influence of either MAP or P_{ETCO_2} on CBF. Accordingly, the models presented here are not adjusted for MAP or P_{ETCO_2}.

After adjusting for baseline values and a potential period effect, there was a significant increase in Q_{VA} (18 mL min^{-1}; range 10–26 mL min^{-1}; $P = 0.0001$), VA_{CVC} (0.08 mL min^{-1} mmHg^{-1}; range 0.01–0.15 mL min^{-1}; $P = 0.027$) and VA shear rate (34 s^{-1}; range 10–59 s^{-1}; $P = 0.008$) following ketone compared to placebo supplementation (Figs 2–4). Exploratory analysis revealed a significant, positive relationship between DSST performance and Q_{VA} (0.034 mL min^{-1} CR^{-1}; range 0.002–0.066 mL min^{-1} CR^{-1}; $P = 0.039$) and VA_{CVC} (3.32 mL min^{-1} mmHg^{-1} CR^{-1}; range 0.20–6.43 mL min^{-1} mmHg^{-1} CR^{-1}; $P = 0.04$). There was a significant increase in Q_{CCA} (25 mL min^{-1}; range 12–38 mL min^{-1}; $P = 0.001$) but no change in CCA_{CVC} or CCA shear rate following ketone supplementation compared to placebo. Exploratory analysis revealed a significant, positive relationship between DSST performance and Q_{CCA} (0.001 mL min^{-1} CR^{-1}; range 0.001–0.02; mL min^{-1} CR^{-1}; $P = 0.033$) and CCA_{CVC} (1.24 mL min^{-1} mmHg^{-1} CR^{-1}; range

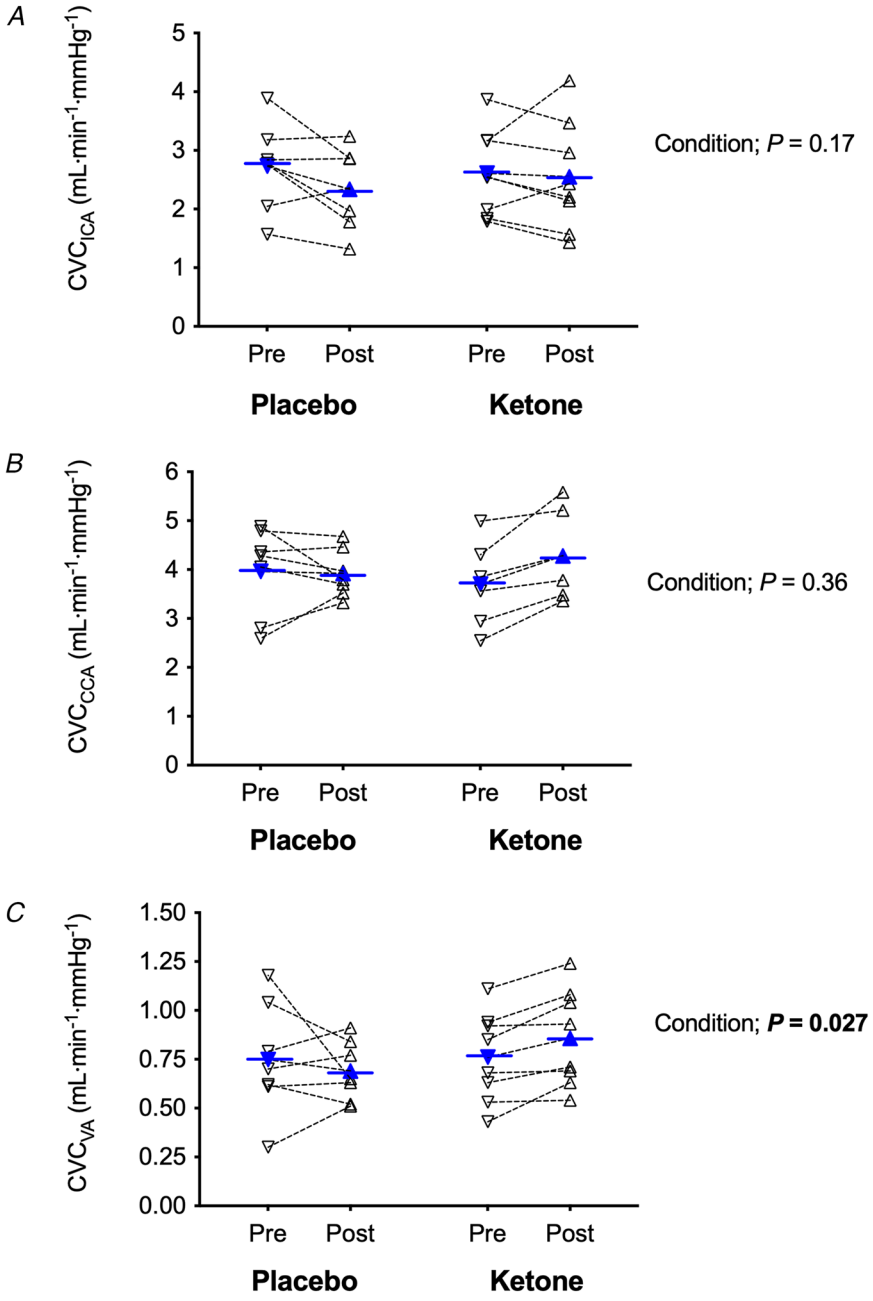

Figure 3. Cerebrovascular conductance responses to 14 days of either placebo or ketone supplementation
Cerebrovascular conductance (CVC) responses to placebo or ketone supplementation in the internal carotid artery (ICA; $n = 8$) (*A*); common carotid artery (CCA; $n = 7$) (*B*); and vertebral artery (VA; $n = 8$) (*C*). Closed blue symbols are group means. Open symbols and hatched lines are individual responses within each condition. *P* values are main effect of condition after adjusting for trial period and baseline values.

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0.24–2.24 mL min^{-1} mmHg^{-1} CR^{-1}; $P = 0.024$). ICA shear rate was significantly elevated following ketone supplementation compared to placebo (42 s^{-1}; range 15–69 s^{-1}; $P = 0.004$); however, there were no differences in Q_{ICA} and ICA_{CVC}.

BDNF responses

After adjusting for baseline values and a potential period effect, there was no difference in serum BDNF (Δ 301.87 pg mL^{-1}; range –1033.43 to 1637.18 pg mL^{-1}; $P = 0.65$) or plasma BDNF (Δ –380.4 pg mL^{-1}; range –1023.9 to 263.2 pg mL^{-1}; $P = 0.24$) following ketone supplementation compared to placebo (Fig. 5).

Discussion

The purpose of this secondary analysis was to investigate whether 14 days of pre-meal ketone supplementation would elevate CBF and BDNF, as well as to determine whether these changes would be reflected in improvements in cognitive function in adults with obesity. The main findings are that with ketone supplementation: (i) cognitive performance significantly improved on the

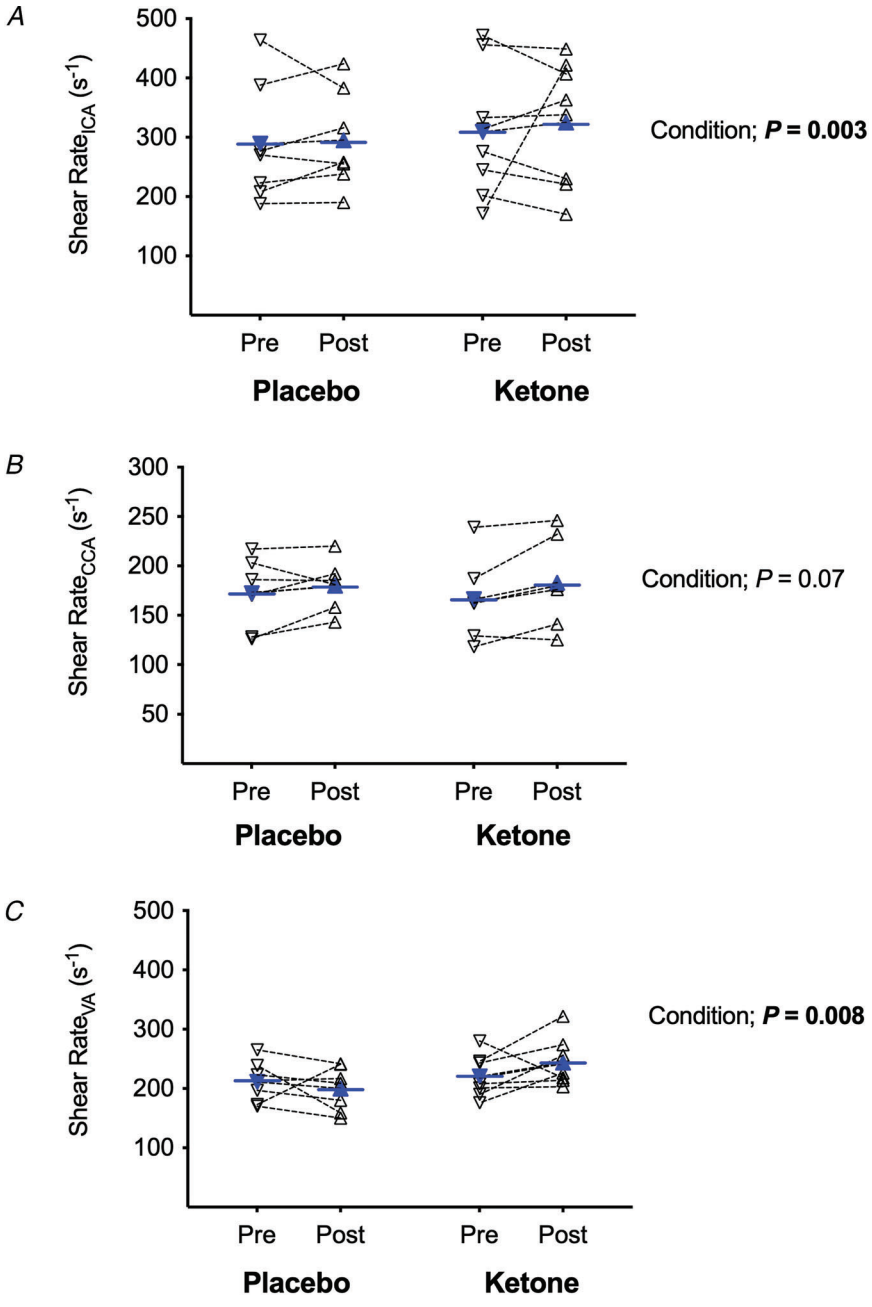

Figure 4. Shear rate responses to 14 days of either placebo or ketone supplementation
Shear rate responses to placebo or ketone supplementation in the internal carotid artery (ICA; $n = 8$) (*A*); common carotid artery (CCA; $n = 7$) (*B*); and vertebral artery (VA; $n = 8$) (*C*). Closed blue symbols are group means. Open symbols and hatched lines are individual responses within each condition. *P* values are main effect of condition after adjusting for trial period and baseline values.

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DSST; (ii) there was a significant increase in Q_{VA}, VA_{CVC} and Q_{CCA}; (iii) there was no difference in serum or plasma BDNF compared to placebo; and (iv) exploratory analyses revealed a significant positive association between DSST performance and Q_{CCA}, Q_{VA}, CCA_{CVC} and VA_{CVC}. These findings suggest that short-term ketone supplementation increases CBF, which may be beneficial for aspects of cognitive function.

In line with our hypothesis, 14 days of ketone supplementation improved cognitive function as indicated by DSST performance. The DSST is a multidomain test that involves complex visual scanning and manual dexterity, requiring processing speed and working memory (Jaeger, 2018). The psychometric properties of the DSST variant used in the present study load strongly on the working memory domain, suggesting a potential sensitivity of working memory to ketone supplementation. This aligns with the results of the study by Jensen *et al.* (2020) who found that β-OHB infusion (2.4 mM peak) improved working memory in people with T2D following 2 h of exposure. Acute β-OHB infusion would ostensibly shift cerebral metabolism, possibly altering brain network stability and facilitating an improvement in cognitive function (Hasselbalch *et al.* 1996; Mikkelsen *et al.* 2015; Mujica-Parodi *et al.* 2020). Interestingly, we assessed cognitive function in the fasting state (i.e. in the absence of β-OHB), suggesting a possible persistence of a β-OHB effect and/or facilitation of cognitive function by increased CBF. The lack of improvement in Stroop and TST performance in the present study supports the contention that β-OHB has domain-specific effects because Jensen *et al.* (2020) found no change in sustained attention, psychomotor speed and verbal memory performance with β-OHB infusion (Jensen *et al.* 2020). Furthermore, the impacts of a dietary supplement on cognitive function may depend on the degree of cognitive challenge imposed by a given test, especially in cognitively normal adults (Gratton *et al.* 2020). This raises the potential for ceiling effects on Stroop and TST performance, given the lower cognitive challenge imposed by these tests compared to the DSST. By contrast, acute *vs.* chronic β-OHB ingestion may differentially impact cognitive functions because 6 months of supplementation with a ketogenic drink (medium-chain triglyceride oil) improves measures of executive functions, processing speed, episodic memory and language abilities in people with mild cognitive impairment Fortier *et al.* 2019). However, a common pathological feature T2D and mild cognitive impairment is cerebral glucose hypometabolism as a result of insulin

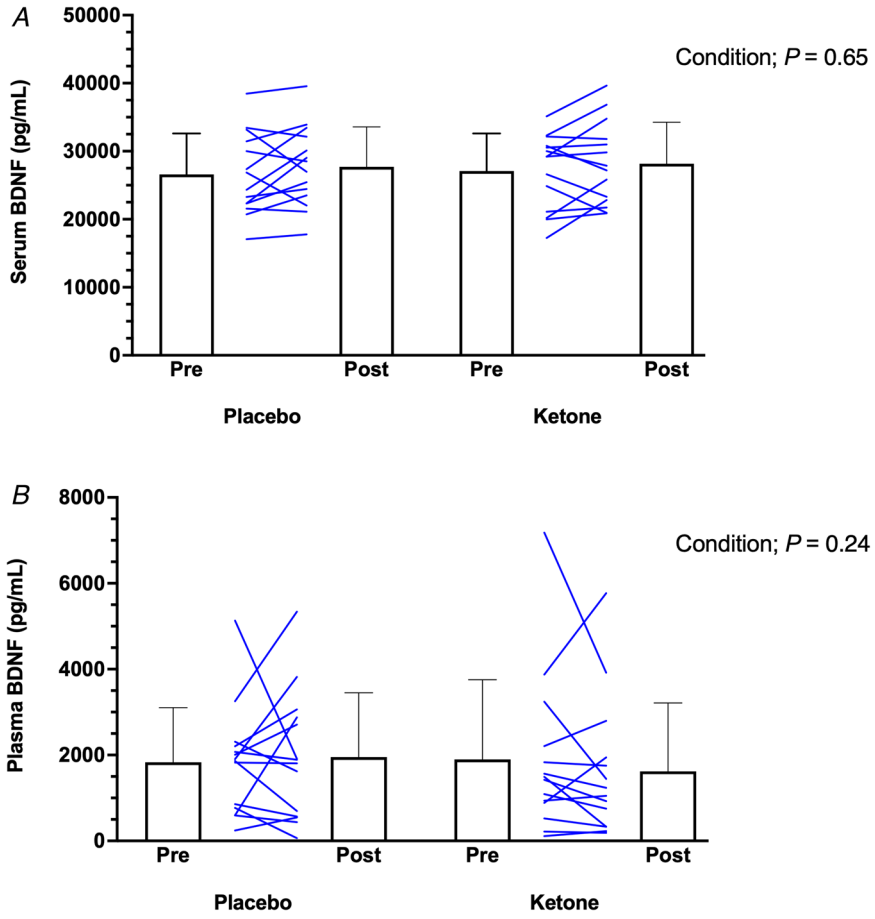

Figure 5. BDNF responses to 14 days of either placebo or ketone supplementation
Serum (n = 14) (*A*) and plasma BDNF (n = 14) (*B*) responses to 14 days of pre-meal placebo or ketone supplementation. Bars are group mean with SD, and lines are pre-post responses for individual participants. Note: Plasma BDNF is presented as raw, non-transformed data. *P* values are main effect of condition after adjusting for trial period and baseline values.

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resistance; therefore, the provision of β-OHB in these patients may compensate for a cerebral energy crisis and restore, rather than augment, the domain-specific aspects of cognitive function (Croteau *et al.* 2017; Fortier *et al.* 2019; Mujica-Parodi *et al.* 2020). Despite the presence of obesity, participants in the present study had normal fasting glucose, did not display high levels of insulin and were cognitively normal.

Adults with obesity are at increased risk of cerebrovascular disease and neurological impairment. Acute and chronic hyperglycaemia impairs systemic vascular function (Piconi *et al.* 2004; Ceriello *et al.* 2008; Shemesh *et al.* 2019) and contributes to development of vascular disease (Ceriello *et al.* 2008; Habas & Shang, 2018). We hypothesized that ketone supplementation would confer benefit on the cerebrovasculature via the direct effects of β-OHB on cerebral metabolism and CBF (Hasselbalch *et al.* 1996; Mikkelsen *et al.* 2015; Svart *et al.* 2018), as well as indirectly through its glucose-lowering effects (Neptune, 1956; Myette-Côté *et al.* 2018, 2019; Walsh *et al.* 2021). We observed a significant increase in Q_{VA}, VA_{CVC}, VA shear rate and Q_{CCA} following ketone supplementation. Interestingly, Q_{CCA}, Q_{VA}, CCA_{CVC} and VA_{CVC} were positively associated with DSST performance, suggesting a potential facilitatory effect of increased perfusion on cognitive function with ketone supplementation (Hendrikse *et al.* 2004; Kim *et al.* 2006). Increased Q_{VA} would support cognitive processes facilitated by the posterior cortex and cerebellum, including visual search efficiency, visual processing and aspects of psychomotor speed required for DSST performance (Usui *et al.* 2009; Jaeger, 2018). Furthermore, elevated Q_{CCA} could enhance perfusion to inferior frontal regions, including the dorsal lateral prefrontal cortex, which is critical for higher-ordered cognitive functions such as working memory, cognitive flexibility and inhibition (*Hendrikse et al.* 2004; Usui *et al.* 2009). The positive effects of ketone supplementation on CBF and cognition could also be related to reduced postprandial glucose and/or improved endothelial function because we recently reported the primary outcomes of this trial (Walsh *et al.* 2021). Nonetheless, β-OHB infusion consistently elevates regional and global CBF in humans (Hasselbalch *et al.* 1996; Mikkelsen *et al.* 2015; Svart *et al.* 2018). This effect may be partly a result of reductions in oxidative stress (Shimazu *et al.* 2013) and/or changes in the cytosolic NAD^+/NADH ratio in the brain (Vlassenko *et al.* 2006; Xin *et al.* 2018), which uncouples O_2 delivery-to-demand matching. Indeed, 30–40% increases in CBF across all brain regions during ketone infusion occur without concomitant changes in cerebral O_2 consumption (Hasselbalch *et al.* 1996; Svart *et al.* 2018). β-OHB has direct effects on the peripheral vasculature in rodent models (Han *et al.* 2018; Wu *et al.* 2020) and we found that 14 days of ketone supplementation increased brachial artery endothelial function in adults with obesity (Walsh *et al.* 2021). However, peripheral vascular function does not necessarily reflect cerebrovascular function (Carr *et al.* 2020).

We assessed plasma and serum BDNF as another potential mechanistic link between ketone supplementation and improvements in cognitive function. By contrast to our hypothesis, there was no effect on either plasma or serum BDNF. BDNF is lower in adults with obesity compared to normal weight adults (Krabbe *et al.* 2007; Walsh *et al.* 2020), which may have implications for cognitive dysfunction (Alosco & Gunstad, 2014; Prickett *et al.* 2018; Sun *et al.* 2018). Recently, we reported that acute β-OHB ingestion prior to an OGTT increases plasma BDNF 2 h following the consumption of a glucose load of 75 g in adults with obesity (Walsh *et al.* 2020); however, the combination of β-OHB with OGTT precludes any inferences regarding the direct effect of β-OHB on BDNF. In the present study, unchanged BDNF levels may be a result of the lack of β-OHB in the circulation in the fasting samples, and the 14 day supplementation period was probably too brief to meaningfully alter basal levels.

Future directions and limitations

The encouraging findings from this brief intervention warrant additional follow-up studies aiming to establish the effect of acute ketone monoester ingestion on brain-health outcomes, as well as whether these effects extend to a longer-duration and/or high-dose supplementation period. The results of the present study provide support for the recently reported safety and tolerability of β-OHB supplementation (Clarke *et al.* 2012*b*; Soto-Mota *et al.* 2019) with respect to cerebrovascular health. β-OHB may also be neuroprotective by positively modulating cerebral monocytes via the hydroxy-carboxylic acid receptor 2 (Rahman *et al.* 2014). Indeed, 14 days of β-OHB supplementation lowers lipopolysaccharide-stimulated monocyte activation in humans (Walsh *et al.* 2021); however, we are unable to assess cerebral immune cell function *in vivo* in humans.

A limitation of the present study is that the sample of participants were apparently healthy, aside from the presence of obesity. Furthermore, the age range of participants was large and we did not have an equal balance of males and females. β-OHB supplementation may have a more pronounced effect in middle-aged to older adults with overt health conditions such as T2D or cognitive impairment (Jensen *et al.* 2020). In addition, CBF was measured in the resting state, which precludes conclusions about the effect of β-OHB on cerebrovascular function *per se*. The present study reports secondary

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outcomes from a larger trial and may not have been powered to detect changes in BDNF. It is also possible that the BDNF response to β-OHB ingestion is transient (Walsh *et al.* 2020) and went uncaptured in the fasting, non-supplemented state. Follow-up studies are warranted aiming to systematically investigate the impact of β-OHB supplementation on circulating BDNF. There were small, yet systematic, reductions in measures of body mass and circulating non-esterified fatty acids independent of condition (main effect of time), which we attribute to the provision of a tightly controlled diet consisting primarily of unprocessed, whole foods with balanced macronutrient composition. Importantly, diet was tightly matched between conditions and there was no difference in weight loss between conditions. Despite these limitations, the present study employed a rigorous placebo-controlled, double-blind, counterbalanced, cross-over design, in accordance with CONSORT guidelines. Participants were provided with all meals, and meals were matched between conditions. This level of control and rigor provides confidence in the effects observed in the present study.

Conclusions

Fourteen days of thrice-daily supplementation with a ketone monoester drink improved cognitive function and elevated regional CBF in adults with obesity. To the best of our knowledge, the present study is the first to characterize the effect of short-term ketone supplementation on multiple aspects of brain health in adults with obesity. Given that the supplementation regimen was effective at lowering postprandial glucose and well-tolerated by participants (Walsh *et al.* 2021), our trial provides a foundation for the potential non-pharmacological therapeutic application of ketone supplementation in patient groups at risk of hyperglycaemic cerebrovascular disease and cognitive dysfunction.

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Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests. JL is the Chief Scientific Officer for the not-for-profit Institute for Personalized Therapeutic Nutrition. JL holds shares in Metabolic Insights Inc., a for-profit company developing non-invasive metabolic monitoring devices.

Author contributions

JL and JW designed research with input from HC, PA and HN. JW, HN and HC conducted research. JW, HC and HN analysed data and performed statistical analysis with input from JL and PA. EM was consulted for all statistical analysis. JW and HC wrote the paper. JW had primary responsibility for the final content. All authors approved the final version of the manuscript submitted for publication.

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Keywords

β-hydroxybutyrate, BDNF, cerebrovascular, double-blind, executive functions, intervention, placebo-controlled

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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