Cognitive, Sleep, and Autonomic Responses to Induction of a Ketogenic Diet in Military Personnel: A Pilot Study

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BACKGROUND: This pilot study examined the effect of a 2-wk ketogenic diet (KD) compared with a carbohydrate (CHO) diet in military personnel on cognitive performance, mood, sleep, and heart rate variability (HRV).

METHODS: A randomized-controlled, cross-over trial was conducted with eight male military personnel (age, 36 ± 7 yr; body mass, 83.7 ± 9.2 kg; BMI, 26.0 ± 2.3 kg · m^{-2}). Subjects ingested their habitual diet for 7 d (baseline), then an iso-energetic KD (~25 g CHO/d) or CHO diet (~285 g CHO/d) for 14 d (adaptation), separated by a 12-d washout. HRV, fasting capillary blood D-βHB, and glucose concentration, mood, and sleep were measured daily. Cognitive performance was measured on the 7th day of baseline and the 7th and 14th days of adaptation. Data were analyzed using a series of linear mixed models.

RESULTS: Mean weekly D-βHB was higher (95% CI, +0.34 to +2.38 mmol · L^{-1}) and glucose was lower (−0.45 to −0.21 mmol · L^{-1}) in the KD compared with the CHO diet. Cognitive performance (Psychomotor Vigilance Task, 2-choice reaction time, and running memory continuous performance test) and mean weekly fatigue, vigor, and sleep (sleep duration, sleep efficiency, and sleep onset latency) were similar between diets. A diet × week interaction for HRV approached significance, with exploratory analyses suggesting HRV was lower compared with baseline during week-2 adapt (−27 to +4 ms) in the KD.

DISCUSSION: A 2-wk induction to a KD in male military personnel does not appear to affect cognitive performance, mood, or sleep, but may lower HRV, indicating increased physiological stress.

KEYWORDS: ketosis, adaptation, performance, stress, actigraphy, heart rate variability.

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Induction to a very-low carbohydrate (CHO), ketogenic diet (KD) abruptly alters brain substrate metabolism.[8] The reduction in CHO availability, which is typically the primary substrate for the brain (glucose provides >95% of energy),[9] stimulates the production of ketone bodies (KB) largely from hepatic conversion of nonesterified fatty acids.[35] Aceto-acetate and D-β-hydroxybutyrate (D-βHB) are both KBs that provide an alternative energetic substrate for the brain during carbohydrate insufficiency and exert a multitude of effects unrelated to substrate provision.[1,33] Considering cognitive function, mood, and sleep are regulated by the brain, it is likely that these are affected when transitioning onto a KD. Previous research has demonstrated adherence to a KD can alter these parameters in individuals with neurodegenerative diseases;[30,42] however, underlying metabolic abnormalities in these populations limit the application to otherwise healthy individuals. The utility of the KD, therefore, depends on whether cognitive performance, mood, and sleep are altered during induction, which is important for individuals operating within stressful and safety critical environments, such as the military.

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Research investigating the effect of a KD on cognitive performance and mood in healthy populations is limited as focus has been on physical performance.[6,39] In healthy men, a 5-d low-CHO, high-fat diet (circulating KBs not measured) impaired power of attention, speed of memory, and rapid visual information processing.[16] Similarly, a 7-d KD impaired simple reaction time and power of attention, as well as reducing calmness and alertness.[12] More recently, adherence to the KD for ~29 d in healthy men and women elicited inconsistent effects on cognitive function and no effect on mood.[18] It is possible that the duration of the KD in earlier studies (5–7 d) provided an inadequate dietary adaptation period, preventing ketogenesis, ketone transport, and ketolysis to be sufficiently upregulated to overcome deficiencies in CHO availability. Previous research also did not appear to standardize cognitive testing procedures, for example, accounting for time of day and previous dietary intake, which could have masked the effects of a KD.

Similarly, the effect of a KD on sleep in healthy populations is uncertain. In the aforementioned study,[18] self-reported sleep quality following a ~29 d KD appeared unaltered; however, this may be due to subjective sleep measures lacking sensitivity to detect dietary effects. In healthy men, acute induction (48 h) to a KD increased the proportion of stage-4 (slow-wave) sleep and reduced the proportion of rapid eye movement (dream) sleep as measured by polysomnography.[2] Whether these effects persist for prolonged KD interventions is unknown, for which polysomnography becomes increasingly difficult to employ.

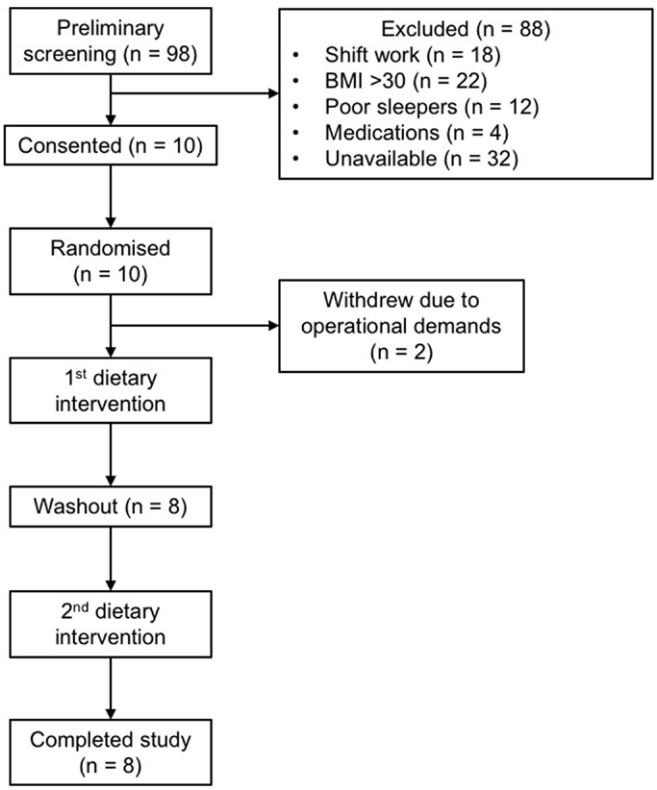

Fig. 1. Participant flow according to the CONSORT diagram.

Table I. Daily Dietary Intake During the Carbohydrate and Ketogenic Diet Interventions.

	ENERGY (MJ)	PROTEIN (g)	CHO (g)	FAT (g)
Carbohydrate diet				
Baseline	11.7 ± 3.1	136 ± 55	271 ± 93	119 ± 45
Week-1 adapt	11.9 ± 3.9	137 ± 58	277 ± 87	122 ± 51
Week-2 adapt	11.9 ± 3.2	124 ± 35	292 ± 100	114 ± 22
Ketogenic diet				
Baseline	12.1 ± 3.4	149 ± 50	235 ± 120	130 ± 52
Week-1 adapt	11.2 ± 2.8	166 ± 55	24 ± 9 [a]	207 ± 66 [a]
Week-2 adapt	11.3 ± 2.7	143 ± 53	26 ± 16 [a]	220 ± 54 [a]
P-values				
Diet × week interaction	0.67	0.69	<0.001	<0.001
Main effect of diet	0.10	0.36		

Values are presented as raw mean ± SD. Abbreviations: MJ, megajoules; *g*, grams; CHO, carbohydrate. Diet × week interaction was determined from raw values and main effect of diet was determined from change from baseline values. Diet × week interaction: different from baseline ([a]$P < 0.001$).

Actigraphy provides an alternative and feasible strategy for objectively measuring sleep duration, sleep efficiency, and sleep onset latency (SOL) in field studies and has been validated against gold-standard polysomnography.[10] Although these actigraphic measures of sleep do not appear to be affected by 1–4 d of a nonketogenic high-fat diet[27] or KD,[2] differences between low- and high-CHO diets may be observed after 4 d, as demonstrated by reduced SOL following a high-CHO diet in a sample of 44 healthy young adults.[27] It is, therefore, possible that actigraphy may elucidate the effect of extended (>4 d) adaptation to a KD on sleep.

The sudden KD-induced transition in substrate availability and metabolism also appears to invoke a physiological stress response. In healthy men, a 3-d KD increased resting concentrations of plasma cortisol, adrenaline, and noradrenaline,[25] and, in mice and rats, a 2- to 3-wk KD increased hypothalamic-pituitary-adrenal axis activation.[37] This stress response would be expected to occur concomitantly with an increase in sympathetic and decrease in parasympathetic autonomic nervous system activity, which would manifest as a reduction in heart rate variability (HRV).[23] A recent study demonstrated a 4-wk KD reduced resting HRV and increased day-to-day variability in HRV in a group of trained endurance male athletes.[29] In the same study, changes to KB and glucose availability were suggested to influence HRV during induction to the KD and reductions in HRV were suggested to predict changes in exercise capacity.[29] Considering lower HRV has been associated with impaired cognitive performance,[13] it is plausible that KD-induced changes in HRV could predict responses in cognitive performance.

The primary aim of this pilot study was to examine the effects of a rigorously controlled 2-wk KD compared with a CHO diet in military service personnel on cognitive function, mood, sleep, and HRV. The secondary aim was to evaluate whether diet-induced changes in HRV were related with cognitive performance and blood D-βHB and glucose concentrations. We hypothesized that cognitive performance, mood, and

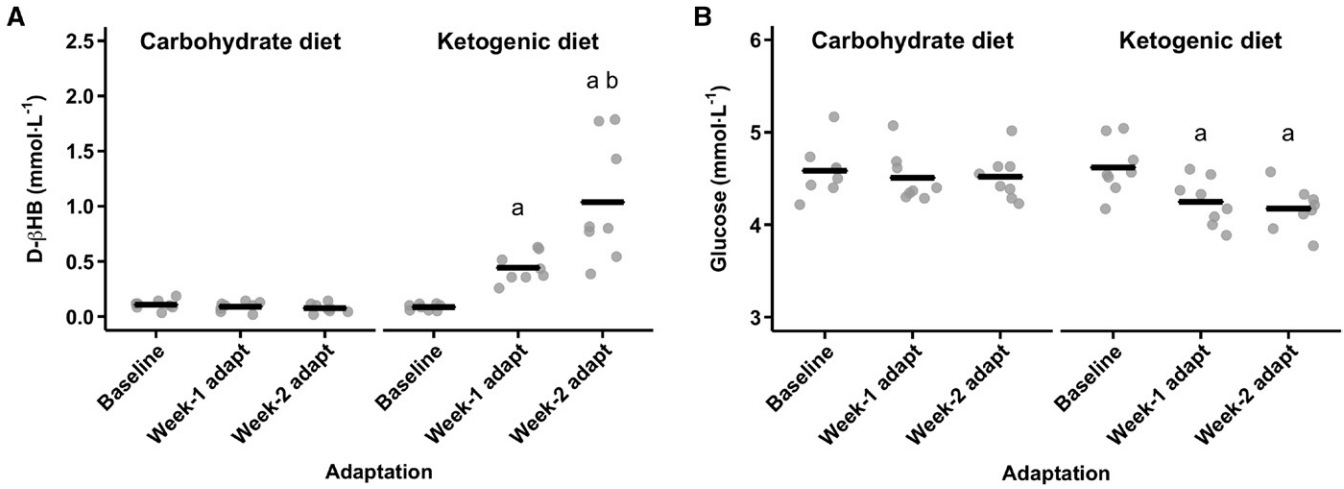

Fig. 2. A) Mean weekly capillary blood D-βHB concentration (mM) and B) mean weekly glucose concentration (mM) during the CHO diet and KD interventions. Abbreviations: D-βHB: D-β-hydroxybutyrate; mM: millimoles per liter. Data are presented as raw means with individual responses. Diet × week interaction: different from baseline ($^{a}P < 0.001$); different from week-1 adapt ($^{b}P = 0.001$).

HRV would be suppressed during induction to the KD, whereas, sleep, as measured by actigraphy, would not be affected. Furthermore, KD-induced changes in HRV would be positively correlated with changes in vigor and blood D-βHB concentration and negatively correlated with changes in fatigue, reaction time, and accuracy in cognitive performance tasks and blood glucose concentration.

METHODS

Subjects

Ethical approval was provided by the New Zealand Defence Force Ethics Committee and Massey University Human Ethics Committee (application SOA 20/47). The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12621000105842). Each participant provided written informed consent before participating. Participation was voluntary and confidentiality was strictly maintained. Subjects were recruited during January and February 2021 via a base-wide email [Royal New Zealand Air Force (RNZAF) Base Auckland]. All interested personnel volunteered for the study. Subjects were required to be: 1) male; 2) healthy; 3) ages 18–50 yr; 4) body mass index <30 kg · m^2; 5) consuming a mixed diet; and 6) habitually going to bed between 21:00–00:00 and waking between 06:00–09:00. Subjects were excluded if they: 1) habitually consumed a KD or exogenous ketone supplements in the 2 yr prior to the study; 2) smoked; 3) consumed >3 cups caffeinated beverages per day; 4) reported to habitually sleep <7 h or >9 h per night; 5) regularly consumed medications or were consuming medications acting on the central nervous system; 6) had a history of drug or alcohol abuse; 7) had food allergies or restrictive dietary patterns; 8) engaged in shift work or transmeridian travel within the 28 d prior to the study. Subjects were also required to have: Epworth Sleepiness Scale score <10;[19] global Pittsburg Sleep Quality Index score ≤ 5;[7] normal scores on the 21-item Depression Anxiety Stress Scale;[28] and scoring as either moderately evening or intermediate chronotype on the Horne-Östberg Morningness/Eveningness Questionnaire.[17] Subjects were fully informed of the rationale of the study and possible risks of the experimental procedures before providing their written consent. They were not informed of the potential effects of a KD and were requested to refrain from personal investigation to prevent biasing their results.

Equipment and Materials

To monitor dietary intake, diet records were ascertained on prespecified days using image-assisted (alongside a fiducial marker), weighed diet records reported in real-time to a registered dietitian (RD) via a mobile phone application (WhatsApp, Facebook, San Francisco, CA, USA). Subjects were trained in how to provide accurate dietary reports. Each report was coded (FoodWorks Professional Edition, Version 10, Xyris Software, Queensland, Australia) using images for validation by a student dietitian, which were checked for accuracy by an RD. To verify compliance to the KD, nutritional ketosis was classified as waking D-βHB concentration ≥ 0.4 mmol · L^{-1}. To help maintain energy balance throughout the study, subjects were requested to prevent a $>2\%$ fluctuation in body mass. Subjects had daily access to an RD for support to promote compliance. If under-reporting or noncompliance was suspected, the RD immediately intervened and the diet record was repeated the subsequent day.

To monitor daily autonomic responses, resting HRV was measured immediately after waking using photoplethysmography (PPG) via a commercially available smartphone application known as HRV4Training.[3] PPG measurements of HRV have been demonstrated to almost perfectly agree with electrocardiography (typical error, 3.8%).[32] HRV is reported as the root mean square of the sum of successive differences in R-R intervals (rMSSD), while the day-to-day variability in HRV was quantified as the standard deviation expressed as a percentage

Table II. Psychomotor Vigilance Task and Choice Reaction Time Performance During the Carbohydrate and Ketogenic Diet Interventions.

	PSYCHOMOTOR VIGILANCE TASK			2-CHOICE REACTION TIME	
	MEAN RRT(s^{-1})	10% FRT(ms)	10% SRT(ms)	MEAN RT (ms)	COGNITIVE THROUGHPUT
Carbohydrate diet					
Baseline	4.24 ± 0.22	198 ± 13	322 ± 27	424 ± 27	140 ± 9
Week-1 adapt	4.24 ± 0.30	198 ± 13	323 ± 48	427 ± 30	135 ± 18
Week-2 adapt	4.17 ± 0.25	203 ± 11	327 ± 35	423 ± 30	141 ± 13
Ketogenic diet					
Baseline	4.17 ± 0.30	201 ± 12	323 ± 41	421 ± 25	141 ± 7
Week-1 adapt	4.17 ± 0.27	202 ± 11	319 ± 36	437 ± 32	134 ± 9
Week-2 adapt	4.21 ± 0.32	197 ± 11	317 ± 32	425 ± 35	141 ± 11
P-values					
Diet × week interaction	0.72	0.22	0.87	0.50	0.99
Main effect of diet	0.61	0.26	0.55	0.15	0.91

Values are presented as raw mean ± SD. Abbreviations: RRT, Reciprocal response time (responses per second); 10% FRT, mean fastest 10% of responses; 10% SRT, mean slowest 10% of responses; RT, response time; s, seconds; ms, milliseconds. Cognitive throughput refers to the number of correct responses per minute. Diet × week interaction was determined from raw values and main effect of diet was determined from change from baseline values.

of the mean for each subject. Subjects were provided with a demonstration of how to use the PPG smartphone application and completed the application's induction following installation. Subjects were requested to perform measurements while breathing normally and remaining lying down.

To monitor daily fasted, morning (i.e., within 15 min after waking) capillary whole-blood glucose and D-βHB concentrations, fingertip blood samples were analyzed using a point-of-care, handheld device (Freestyle Optium Neo; Abbott Diabetes Care, Doncaster, Australia) and standardized techniques. Subjects were trained on how to reliably perform measurements and reported the results immediately to an RD via a mobile phone application (WhatsApp, Facebook, San Francisco, CA, USA).

To monitor weekly changes in cognitive performance, a ~11-min validated test battery was administered between 08:00–10:00, which employed the purpose-built, handheld Psychomotor Vigilance Task (PVT-192; Ambulatory Monitoring Inc., Ardsley, NY, USA), and the computerized Automated Neuropsychological Assessment Metrics test battery (ANAM®; Vista Life Sciences, Parker, CO, USA). All measurements were taken within 30 min of the first baseline measure; for example, if the baseline test was at 08:30, all subsequent tests could be between 08:00–09:00. Subjects completed the test battery five times prior to commencing the study to minimize learning effects.

Vigilant attention was measured using a 5-min version of the PVT. Subjects responded to the presentation of a visual stimulus as quickly as possible by pressing a button with the thumb or finger of their dominant hand.[11] After a random time interval (between 2–10 s), the stimulus reappeared. The PVT was included in this study because of its sensitivity to the effects of fatigue and short administration time. The PVT is also very reliable with little evidence of practice effects.[5] Variables for analysis included mean reciprocal response time (RRT), mean fastest 10% response time, and mean slowest 10% response time.

Attention and reaction time were also measured using a 3-min 2-choice reaction time (RT) test from the ANAM test battery. The task required subjects to react to two stimuli, a 0 or *, by pressing the left or right mouse button, respectively. This task measured the ability to react quickly and switch mental components of cognitive function.[21] Interstimulus interval ranged from 650 to 1100 ms. Variables for analysis included mean RT for correct responses and cognitive throughput (i.e., a measure of speed and accuracy defined as the number of correct responses per minute).[34]

Working memory and vigilant attention were measured using a 3-min running memory continuous performance test (RMCPT) from the ANAM test battery, which is a continuous RT test using a standard "one-back" paradigm. Subjects were required to recall the previous numerical digit (0–9) that appeared on the screen and respond as to whether the digit was the same or different to the digit preceding it, by a left or right mouse click, respectively. Interstimulus interval ranged from 4500 to 4600 ms and each digit was displayed for 200 ms. Variables for analysis included mean RT for correct responses and cognitive throughput.[34]

To monitor daily mood, ratings of fatigue and vigor were measured between 10:00–17:00 using the ANAM mood scale, which has been validated against the Profile of Mood States.[20] These two dimensions each consist of six adjectives rated on a 7-point Likert scale of mood intensity anchored with 0 (not at all) and 6 (very much). The fatigue adjectives are: lazy, inactive, tired, weary, sluggish, and drowsy. The vigor adjectives are: energetic, lively, alert, spirited, active, and vigorous. Each dimension is expressed as the mean score for the six adjectives, with higher mean scores for the fatigue dimension indicating more fatigue and higher mean scores for the vigor dimension indicating more vigor.

To monitor daily sleep, subjects wore a wrist actigraph (Micro Motionlogger Watch; Ambulatory Monitoring Inc., Ardsley, New York, USA) on their nondominant wrist at all times during the study, except when showering and swimming. Actigraphy provides an objective assessment of sleep-wake patterns and has been validated against gold-standard polysomnography.[10] Subjects were instructed to press an event marker on the actigraph to indicate when they started and stopped trying to sleep and completed a standardized sleep-log daily. Data were

Table III. Running Memory Continuous Performance and Mood During the Carbohydrate and Ketogenic Diet Interventions.

	RUNNING MEMORY CONTINUOUS PERFORMANCE		MOOD	
	MEAN RT (ms)	COGNITIVE THROUGHPUT	FATIGUE	VIGOR
Carbohydrate diet				
Baseline	515 ± 69	116 ± 13	2.1 ± 0.6	3.3 ± 0.7
Week-1 adapt	522 ± 95	117 ± 18	1.9 ± 0.6	3.4 ± 0.8
Week-2 adapt	501 ± 67	120 ± 14	1.9 ± 0.4	3.2 ± 0.6
Ketogenic diet				
Baseline	527 ± 93	115 ± 19	1.9 ± 0.6	3.6 ± 0.6
Week-1 adapt	515 ± 86	117 ± 16	1.8 ± 0.6	3.5 ± 0.8
Week-2 adapt	496 ± 82	122 ± 18	2.1 ± 0.7	3.2 ± 0.9
P-values				
Diet × week interaction	0.53	0.83	0.27	0.57
Main effect of diet	0.09	0.52	0.31	0.20

Values are presented as raw mean ± SD. Abbreviations: RT, Response time; s, seconds; ms, milli-seconds. Cognitive throughput refers to the number of correct responses per minute. Diet × week interaction was determined from raw values and main effect of diet was determined from change from baseline values.

recorded in 1-min epochs and subsequently downloaded to a computer and analyzed by an experienced sleep researcher using the manufacturer's software (ActionW2.7, with the Sadeh sleep-scoring algorithm applied) in conjunction with bed time information from the sleep log. For each nighttime sleep period, SOL (the first 10 consecutive minutes scored as sleep by the software algorithm), total sleep time (i.e., number of hours of sleep from sleep-onset to sleep-offset), and sleep efficiency (i.e., total sleep as a percentage of time in bed) were calculated. Although our screening criteria required subjects to habitually sleep at least 7 h on average per night for study inclusion, we did not exclude data for subjects not meeting this threshold from the analysis due to the already limited sample size and shorter sleep durations being reflective of the wider working population.

Procedures

A randomized-controlled, counterbalanced, cross-over trial was conducted with male military service personnel from the RNZAF. Neither participants nor researchers were blinded to the treatment. Subjects completed 7 d of baseline testing, then were randomized (www.randomizer.org) to either a 14-d CHO diet or KD (i.e., dietary adaptation). Following a 12-d washout period, participants completed another 7 d of baseline testing and the alternative dietary treatment. For the duration of the study, subjects were requested to abstain from over-the-counter medications, alcohol, napping, and to maintain their normal sleep and physical activity routines, and remain hydrated. Caffeine intake was limited to <100 mg $\cdot$ d^{-1} ingested prior to 12:00; subjects were provided with a handout detailing caffeine content in common dietary items. All other lifestyle choices were allowed to vary naturally during baseline testing and dietary adaptation; however, consistent patterns were requested to be maintained.

For baseline testing, subjects ingested their habitual diet (HD) for 7 d. Dietary records were reported on 3 nonconsecutive days. Sleep, HRV, capillary whole-blood glucose, and D-βHB concentration and mood (fatigue and vigor) were measured daily. Cognitive performance variables and body mass were measured on the 7th baseline day.

For dietary adaptation, subjects began ingesting their 14-d dietary allocation immediately following baseline testing. Sleep, HRV, capillary whole-blood glucose, D-βHB concentration, and mood were measured daily. Cognitive performance and body mass were measured on the 7th and 14th dietary adaptation days. An RD provided education and meal plans specific to the subjects' dietary allocation within 500 kJ of their HD intake. Subjects on the KD were also provided a comprehensive educational handout. The prescribed KD diet comprised: $<$ 5% energy intake (EI) (<40 g $\cdot$ d^{-1}) from CHO, 15–20% EI from protein; and $>$75% EI from fat, whereas the CHO diet comprised $>$45% EI from CHO, 15–20% EI from protein, and $<$40% EI from fat. During the KD, subjects were provided with electrolyte capsules (Pure Electrolyte Replacement Capsule; Pure Sports Nutrition, New Zealand), which contained 440 mg sodium chloride, 150 mg potassium citrate, 100 mg magnesium citrate, and 50 mg calcium citrate and were requested to ingest one capsule twice daily to mitigate potential reductions in blood electrolyte concentration. Diet records were reported on 3 nonconsecutive days between the 1st to 7th and 8th to 14th dietary adaptation days.

Statistical Analysis

Data were analyzed using linear mixed models with restricted maximum likelihood in the R package "lme4". Variables measured daily (D-βHB, glucose, rMSSD, fatigue, and vigor) were averaged for each week prior to entry into the models. For initial models, fixed effects factors included diet (two levels; CHO or KD) and adaptation (three levels; baseline, week-1 adapt, and week-2 adapt) and a random intercept for subject was included to adjust for interindividual homogeneity. Diet order (two levels) was also included as a fixed effect given the crossover design. Normality of distribution and homoscedasticity of the model's residuals were determined by visual inspection of Q-Q plots; if violated, data were either log, square-root, or inverse transformed and assessed for best fit prior to extracting *P*-values. Raw values were used to assess a diet × week interaction, but not a main effect of diet as these included prediet (i.e., baseline) values.

Table IV. Sleep Responses During the Carbohydrate and Ketogenic Diet Interventions.

	SLEEP DURATION (h)	SLEEP EFFICIENCY (%)	SLEEP ONSET LATENCY (min)
Carbohydrate diet			
Baseline	6.46 ± 1.18	83.6 ± 9.3	7.6 ± 4.5
Week-1 adapt	6.53 ± 1.06	84.2 ± 9.1	6.4 ± 5.1
Week-2 adapt	6.60 ± 1.09	83.5 ± 10.5	7.8 ± 5.4
Ketogenic diet			
Baseline	6.47 ± 1.08	82.0 ± 11.0	7.2 ± 4.9
Week-1 adapt	6.65 ± 1.04	82.0 ± 9.8	7.6 ± 5.8
Week-2 adapt	6.72 ± 0.97	82.1 ± 9.4	8.8 ± 11.5
P-values			
Diet × week interaction	0.69	0.85	0.85
Main effect of diet	0.29	0.54	0.21

Values are presented as raw mean ± SD. Abbreviation: h, hours; min, minutes. Diet × week interaction was determined from raw values and main effect of diet was determined from change from baseline values.

In the absence of a diet × week interaction, change from baseline values were used to assess for a diet × week interaction and main effect of diet. A main effect of week was not considered practically significant. *P*-values for fixed-effects factors were obtained using Type II Wald *F* tests with Kenward-Roger degrees of freedom in the R package "car". *P*-values for pairwise comparisons were obtained using Holm adjustment for multiplicity in the R package "emmeans", with differences given as 95% confidence intervals (CI) of estimated marginal means. The 95% CI for effect sizes (ES) based on Cohen's *d* were calculated from the linear mixed model estimates, while accounting for the study design by using the square root of the sum of all the variance components (specified random effects and residual error) in the denominator and residual degrees of freedom of the model. The standardized linear relationship between change in (Δ) daily rMSSD and A) Δ daily D-βHB and B) Δ daily glucose, while adjusting for the alternate substrate, and the standardized linear relationship between Δ mean weekly rMSSD and A) Δ cognitive performance variables and B) Δ mean weekly mood variables, while adjusting for sleep duration, for each diet were determined using the 95% CI of linear mixed model coefficients after scaling and centering the data. Data are presented as raw mean ± SD, unless otherwise stated. Significance was inferred when $P \leq 0.05$.

RESULTS

Two subjects withdrew from the study due to operational demands. A total sample size of $N = 8$ (age, 36 ± 7 yr; body mass, 83.7 ± 9.2 kg; height, 1.79 ± 0.03 m; body mass index, 26.0 ± 2.3) were used in the final analyses, unless otherwise stated. Subject flow is summarized in the CONSORT diagram (**Fig. 1**).

Table I summarizes weekly dietary intake during the CHO diet and KD interventions. There were no diet × week interactions or, when using change from baseline values, no diet × week interactions or main effects of diet for energy and protein intake (all, $P > 0.05$). There was a diet × week interaction for fat and CHO intake (all, $P < 0.001$). Compared with baseline, the KD was +29 to +126 g · d^{-1} higher in fat and −280 to −142 g · d^{-1} lower in CHO at week-1 adapt and +42 to +139 g · d^{-1} higher in fat and −278 to −140 g · d^{-1} lower in CHO at week-2 adapt (all, $P < 0.001$); there were no differences in the CHO diet (all, $P > 0.05$). For pre- and post-diet body mass, there was no diet × week interaction ($P = 0.51$); however, when using a Studentised *t*-test to compare change scores from baseline, body mass in the KD was lower by −0.9 to −3.6 kg compared to the CHO diet ($P = 0.005$).

There was a diet × week interaction for mean weekly D-βHB ($P < 0.001$), with post hoc tests indicating higher concentrations compared with baseline at week-1 adapt (+0.16 to +0.54 mmol · L^{-1}; $P < 0.001$; ES = 0.53 to 1.27) and week-2 adapt (+0.61 to +1.17 mmol · L^{-1}; $P < 0.001$; ES = 1.21 to 2.20), and higher concentrations compared with week-1 adapt at week-2 adapt (+0.23 to + 0.86 mmol · L^{-1}; $P < 0.001$; ES = +0.44 to + 1.16) in the KD; there were no differences in the CHO diet (all, $P > 0.05$) (**Fig. 2A**). Days to D-βHB concentration ≥ 0.4 mmol · L^{-1} in the KD was 4.6 ± 0.7. There was a diet × week interaction for mean weekly glucose ($P < 0.001$), with post hoc tests indicating lower concentrations compared with baseline at week-1 adapt (−0.62 to −0.13 mmol · L^{-1}; $P < 0.001$; ES = −0.91 to −0.31) and week-2 adapt (−0.69 to −0.20 mmol · L^{-1}; $P < 0.001$; ES = −1.05 to −0.42) in the KD; there were no differences in the CHO diet (all, $P > 0.05$) (**Fig. 2B**).

Table II and **Table III** summarizes weekly cognitive performance and mood variables during the CHO diet and KD interventions. There were no diet × week interactions or, when using change from baseline values, no diet × week interactions or main effects of diet for all cognitive performance variables (all, $P > 0.05$). Two subjects reported mood less than three times per week and were excluded from the analyses for mood (i.e., $N = 6$). There were no diet × week interactions or, when using change from baseline values, no diet × week interactions or main effects of diet for fatigue and vigor (all, $P > 0.05$).

Table IV summarizes sleep variables during the CHO diet and KD interventions. The were no diet × week interactions or, when using change from baseline values, no diet × week interactions or main effects of diet for sleep duration, sleep efficiency, and SOL (all, $P > 0.05$).

A diet × week interaction for mean weekly rMSSD approached significance ($P = 0.064$), with exploratory post hoc comparisons in the KD indicating lower values compared with baseline at week-2 adapt (−27 to +4 ms; ES = −0.59 to −0.10), but not week-1 adapt (−16 to +15 ms; ES = −0.24 to 0.21) and lower values compared with week-1 adapt at week-2 adapt (−28 to +3 ms; ES = −0.58 to −0.09) (**Fig. 3A**). Change from baseline values for mean weekly rMSSD also exhibited a diet × week interaction approaching significance ($P = 0.09$), but no

main effect of diet ($P = 0.25$). There was no diet × week interaction for weekly day-to-day variability in rMSSD ($P = 0.71$) (**Fig. 3B**). Change from baseline values for weekly day-to-day variability in rMSSD also exhibited no diet × week interaction ($P = 0.88$) or main effect of diet ($P = 0.34$).

The Δ daily rMSSD was inversely related with Δ daily D-βHB in the KD (95% CI linear mixed model standardized coefficients, −0.78 to −0.11) and had a positive, albeit weak, relationship with Δ daily D-βHB in the CHO diet (0.00 to 0.07). There were no clear relationships between Δ daily rMSSD and Δ daily glucose in either diet (i.e., all CIs included 0). The Δ mean weekly rMSSD was inversely related with Δ mean weekly fatigue (−1.95 to −0.31) and positively related with Δ mean weekly vigor (0.57 to 1.24) in the CHO diet; whereas no clear relationships were observed in the KD. There were no clear relationships between Δ mean weekly rMSSD or weekly cognitive performance variables in both diets.

DISCUSSION

We investigated the effect of a 2-wk induction to a KD compared with a CHO diet on cognitive performance, mood, sleep, and HRV in male military personnel. The primary observations partly supported our hypotheses and included: 1) cognitive performance (Psychomotor Vigilance Task, 2-choice reaction time, and running memory continuous performance test), mood (fatigue and vigor), and sleep (duration, efficiency, and onset latency) were not affected after 1 and 2 wk of induction to a KD compared with the CHO diet; 2) HRV appeared suppressed after 2 wk of induction to a KD; and 3) Δ HRV was inversely related with Δ D-βHB concentration in the KD diet and a positive, albeit weak, relationship in the CHO diet; 4) Δ HRV was inversely related with Δ fatigue and positively related with Δ vigor in the CHO diet, but not in the KD; and 5) no relationships between HRV and cognitive performance variables were observed in either diet. Our findings suggest that induction to a KD can be employed without compromising cognitive performance, mood, and sleep; however, it remains uncertain whether these findings persist in stressful environments, such as military operations. Furthermore, possible reductions in resting HRV following induction to a KD indicates increased physiological stress, which may be related to D-βHB concentration, but the functional and physiological implications of this remain uncertain.

We refer to 2-wk adaptation as induction since longer periods may exert additional effects. Optimal periods for adapting to a KD currently remains uncertain, which may also vary between individuals and likely depends on the targeted functional or physiological outcomes as different tissues vary in their metabolic endowment for KB metabolism and signaling. For example, during KB infusion, KB oxidation increases in the brain, but not in skeletal muscle.[26] Although we did not measure brain KB oxidation, a previous 4-d KD intervention that increased total KB concentration (i.e., AcAc + D-βHB) to 4.8 mmol · L^{-1} estimated ketone-derived energy increased from less than 5% (during the CHO diet) to ~33%.[9] By comparison, due to our study's less profound hyperketonemia (i.e., D-βHB < 2 mmol · L^{-1}) and the linear relation between brain KB oxidation rates and their blood concentration,[9] KB oxidation rates were likely lower in our study; however, this may have been offset by our extended adaptation period.[36] Hyperketonemia could also have exerted effects unrelated to substrate provision, such as increased brain blood flow[40] and increased brain γ-amino butyric acid/glutamate ratio.[36] Overall, we are confident that our strict KD intervention substantially altered brain physiology.

In the present study, cognitive performance and mood appeared to be maintained after 1 and 2 wk of ingesting a KD. Similar findings were reported following a ~29-d KD,[18] but not with shorter dietary interventions (<7 d).[12,16] It seems dietary macronutrient manipulations do not elicit marked effects on cognition[41] and, given the current evidence on nonketogenic and ketogenic low-CHO diets, the brain appears metabolically

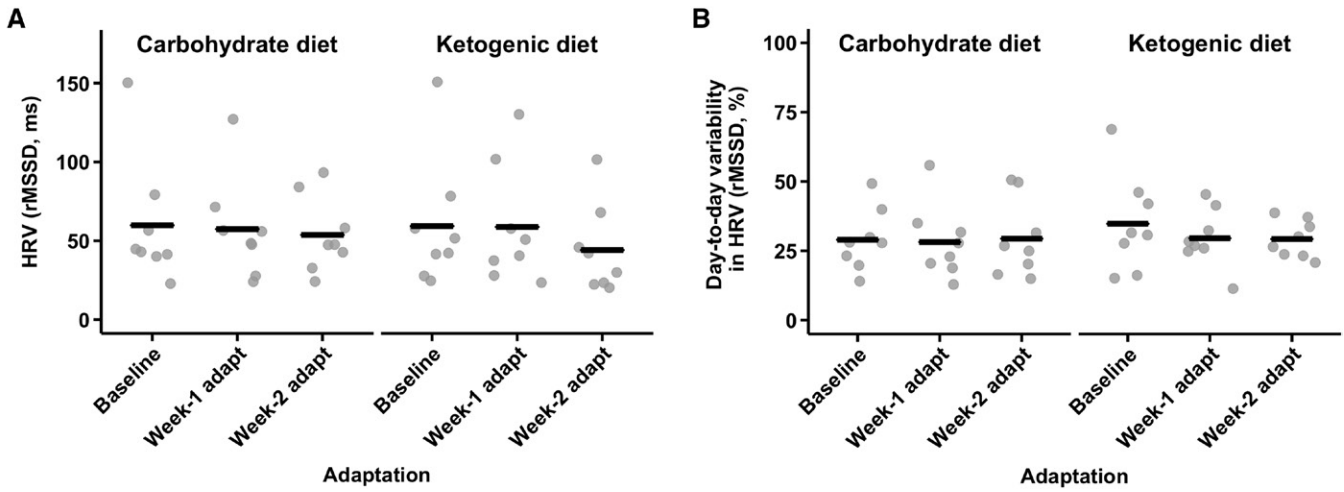

Fig. 3. A) Mean weekly HRV (rMSSD, ms) and B) day-to-day variability in weekly HRV (rMSSD, percentage) during the CHO diet and KD interventions. Data are presented as raw means with individual responses. Abbreviations: rMSSD: root mean square of the sum of successive differences in R–R intervals; ms: milliseconds.

flexible to alterations in substrate availability if given a sufficient adaptation period in order to maintain performance of brief simple cognitive tasks, as well as perceptions of fatigue and vigor. However, it is possible that additional stressors (e.g., sleep deprivation, hypoxia, or exercise) may require higher carbohydrate availability; for example, a 1-wk low-CHO diet combined with training impaired mood in trained female cyclists.[22] It is also possible that the cognitive testing protocol in our study was not sensitive to diet-induced changes in cognitive performance, despite being sensitive in other contexts. Nonetheless, the present findings suggest that induction to a KD does not impair cognitive performance and mood in the absence of additional psychological and physiological stressors.

Sleep was also unaltered in the present study, with subjects' weekly averages of sleep duration (4.4 to 7.5 h/d), sleep efficiency (64–96%), and SOL (2 to 17 min) maintained throughout both dietary interventions. It is possible, however, that the underlying sleep architecture was altered. For example, in a previous study, a very low-CHO meal and 48 h induction to a KD increased slow-wave sleep and reduced rapid eye movement sleep.[2] The authors speculated that the increase in slow-wave sleep was a compensatory mechanism to the initial fatigue often experienced during induction to the KD. Similarly, other studies also employing polysomnography report a higher amount of slow-wave sleep during the first sleep cycle following a low-CHO, high-fat meal compared with a high-CHO, low-fat meal[44] and increased slow-wave sleep and reduced REM sleep across the total sleep period following a 2-d nonketogenic low-CHO high-fat diet compared with a high-CHO, low-fat diet.[31] As changes to sleep architecture can occur after one meal (i.e., without ketosis), differences between diets may be predominantly due to shifts in substrate availability and oxidation, rather than hyperketonemia. Since actigraphy is unable to measure sleep stages, it is uncertain whether these effects were present in our study.

Resting HRV also appeared reduced during the second week of ingesting the KD in the present study, which suggests that induction to the KD increased physiological stress. This mirrored a previous study in endurance athletes that also demonstrated increased day-to-day variability in resting HRV.[29] HRV is a biomarker of interest within various populations to monitor stress-related impairments, such as the military,[15] as lower HRV could have implications for cognitive performance.[13] Nevertheless, we did not observe clear relationships between Δ weekly mean HRV and any of the cognitive performance variables within each diet. This is unsurprising given there was no difference between diets in cognitive performance variables, but this may change when under additional psychological and physiological stressors. Moreover, Δ average weekly HRV was inversely related to Δ average weekly fatigue and positively related with Δ average weekly vigor in the CHO diet, but not the KD. Since these relationships were expected in both diets, it is possible that physiological adaptations to the KD abrogated these effects.

Moreover, in the present study, Δ daily resting HRV had an inverse relationship with Δ daily fasting blood D-βHB in the KD and a positive, albeit weak, relationship with D-βHB in the CHO diet. In contrast, no clear relationship with Δ daily fasting blood glucose concentration was observed for either diet. For the KD, a positive relationship between rMSSD and D-βHB was expected as prior research has demonstrated that hypoglycemia-induced (i.e., blood glucose < 4 mmol $\cdot$ L^{-1}) increases in adrenaline, noradrenaline, and cortisol are mitigated by KB infusion.[4] Nevertheless, fasting hypoglycemia was rarely observed in the present study, which demonstrates marked metabolic flexibility of the human body to reduced dietary CHO intake to maintain blood glucose concentrations.[14] It is also possible that elevated blood βHB concentration may increase sympathetic activity by acting as an agonist for the G-protein-coupled receptor,[43] although not all studies are in agreement.[24] Moreover, additional stressors, such as endurance exercise, may exacerbate this response during induction to a KD by using available CHO, thus increasing ketogenesis to further suppress HRV[29] and increase circulating stress hormones, such as cortisol.[38]

In conclusion, a 2-wk induction to a KD in military personnel does not appear to impair cognitive performance, mood, or sleep. Despite the KD appearing to suppress resting HRV, which is indicative of increased physiological stress, there were no clear relationships between HRV and cognitive performance variables. The lack of clear findings from this pilot study were probably prevented by the low sample size; however, trials of this nature are difficult to conduct, particularly in hard to access populations. Further, considering all testing procedures were performed in a rested state, free from distractions, the findings should not be directly applied to adverse situations or environments. It is possible that additional stressors, such as sleep deprivation and exercise, experienced by military personnel during certain operations, may interact with a KD to elicit different effects on cognition and mood. Therefore, further research is required before the KD is deemed suitable within the military—or other populations—operating in stressful and safety critical environments.

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