The effects of short-term low energy availability, achieved through diet or exercise, on cognitive function in oral contraceptive users and eumenorrheic women

Martin D[1,2], Papageorgiou M[2,3], Colgan H[2], Bandelow S[4], Greeves, JP[5], Tang, JCY[6], Fraser WD[6], Cooper SB[2], Sale C[2], Elliott-Sale KJ[2]

Corresponding author: Dr Kirsty Elliott-Sale, Erasmus Darwin Building, Clifton Campus, Nottingham Trent University, NG11 8NS, UK, +44 (0)1158486338, Kirsty.elliottsale@ntu.ac.uk

[1] University of Lincoln, Lincoln, United Kingdom, damartin@lincoln.ac.uk

[2] Nottingham Trent University, Nottingham, United Kingdom, simon.cooper@ntu.ac.uk, craig.sale@ntu.ac.uk,

[3] Med Uni, Geneva, Switzerland, maria.papageorgiou@unige.ch

[4] Loughborough University, Loughborough, United Kingdom, s.bandelow@lboro.ac.uk

[5] HQ Army, Andover, United Kingdom, jpgreeves@live.co.uk

[6] University of East Anglia, Norwich, United Kingdom, jonathan.tang@uea.ac.uk, w.fraser@uea.ac.uk

Abstract

To date, no research has explored the effects of low energy availability (EA) on cognitive performance using dietary and exercise regimens relevant to athletes. Twenty female participants (10 eumenorrheic, 10 oral contraceptive [OC] users) completed three, 3-day conditions: 1) controlled-balanced EA without exercise (BAL; 45 $kcal \cdot kg \cdot LBM^{-1} \cdot day^{-1}$), 2) diet-induced low EA without exercise (DIET; 15 $kcal \cdot kg \cdot LBM^{-1} \cdot day^{-1}$) and 3) exercise-induced low EA (EX; 15 $kcal \cdot kg \cdot LBM^{-1} \cdot day^{-1}$, including 30 $kcal \cdot kg \cdot LBM^{-1} \cdot day^{-1}$ treadmill running at 70% $\dot{V}O_{2max}$). A cognitive test battery was completed before and after each 3-day condition. Mental rotation test accuracy improved in the BAL condition, but there was a decline in accuracy in the EX condition (BAL, +2.5%; EX, -1.4%; $P = 0.042$, $d = 0.85$). DIET (+1.3%) was not different to BAL or EX ($P > 0.05$). All other measures of cognitive performance were not affected by condition ($P > 0.05$) and OC use did not affect cognitive responses ($P > 0.05$). Accuracy in the mental rotation test was impaired when low EA was induced through increased exercise energy expenditure. All other aspects of cognition were unaffected by three days of low EA through diet or exercise. OC use did not mediate the effect of low EA on cognition.

Keywords: Cognition, energy availability, diet, exercise, females, oral contraceptives

Novelty

- **Cognitive function was not affected by 3 days diet-induced low energy availability (EA).**
- **Only spatial awareness was impaired during 3 days exercise-induced low EA.**
- **Reproductive hormones affected spatial awareness independent of EA.**

Introduction

Energy availability is the amount of dietary energy available to maintain physiological function after exercise training (Loucks et al. 2011). Athletes, military recruits and dieters often restrict energy intake, expend large amounts of energy in exercise, or undergo a combination of these practices, which can result in low energy availability (Loucks et al. 2011; De Souza et al. 2014). Low energy availability (<30 kcal·kgLBM^{-1}) is common in female athletes and recreationally active individuals and is associated with menstrual dysfunction and impaired bone health (Nattiv et al. 2007; De Souza et al. 2014; Slater et al. 2016) and it has been suggested that many other components of health and performance may also be affected (Mountjoy et al. 2014, 2018). The Relative Energy Deficiency in Sport (RED-S) model has proposed that low energy availability may negatively affect several factors relating to exercise performance, including aspects of cognition (Mountjoy et al. 2014), although there is currently little evidence available to support this (De Souza et al. 2014b; Williams et al. 2019) and the effects of low energy availability on cognition remain unclear.

To date, only two studies have examined the effects of short term (2-3 days) low energy availability on cognitive performance. In the first study, two days of near total calorie deprivation (~183 kcal·day^{-1} energy intake), was compared to energy-balanced conditions, consisting of either carbohydrate or carbohydrate and fat diets (~2820 kcal·day^{-1} energy intake), with all conditions including two hours of low intensity exercise (40-45% heart rate reserve) per day (Lieberman et al. 2008). Consuming the calorie-restricted diet did not impact participants' self-reported mood and had no effect on vigilance, response time, memory, and reasoning skills. In the second study, participants completed approximately four hours of exercise per day (40-65% $\dot{V}O_{2peak}$) and consumed either an energy-balanced (3935 ± 769 kcal·day^{-1} energy intake), or calorie-restricted (266 ± 61 kcal·day^{-1} energy intake) diet (Lieberman et al. 2017). Mood was significantly affected, with reduced vigour and increased tension, fatigue and total mood disturbance occurring in the calorie-restricted condition, which was associated with a significant reduction in interstitial glucose concentrations. Performance on the majority of cognitive tests was unaffected, although aspects of grammatical reasoning and choice reaction time were improved in the calorie-restricted condition compared to the energy-replete condition, potentially resulting from an evolutionary response of heightened cognitive function in the presence of food scarcity (Bronwen et al. 2007).

In these studies (Lieberman et al. 2008, 2017), changes in cognitive performance were only apparent when a greater exercise duration and intensity was used (Lieberman et al. 2017), which resulted in a greater deviation from energy balance (-3681 $kcal \cdot d^{-1}$ compared to -2138 $kcal \cdot d^{-1}$ in the earlier study), despite similar dietary energy intakes. The severity of the dietary restriction and exercise energy expenditure used in these studies would have resulted in a negative energy availability, which is only representative of extreme situations such as military training and does not represent the practices of athletes or exercising populations. Energy availability values in athletes are often between ~8-35 $kcal \cdot kgLBM^{-1}day^{-1}$ (Loucks et al. 2011; Van Heest et al. 2014; Vogt et al. 2005), which are achieved through more moderate levels of dietary energy restriction, or a failure to increase energy intake to compensate for exercise training (De Souza et al. 2014). Therefore, further research using more ecologically valid levels of energy availability and methods of achieving this (*i.e.*, dietary restriction and/or exercise) is required. Furthermore, these studies (Lieberman et al. 2008; Lieberman et al. 2017) have not used a non-exercise control, or dietary restriction only group, making it difficult to differentiate the effects of low energy availability from the effects of exercise (Chang et al. 2012; Tomporowski 2003).

Lieberman et al. (2017) identified an effect of sex on the response to calorie-restriction, whereby females ($n = 6$) performed better in tests of working memory, grammatical reasoning and vigilance in the calorie-restricted condition compared to when energy replete, while this effect was not apparent in males ($n = 17$). This sexually dimorphic response may be mediated by differences in reproductive hormone concentrations; therefore, the reproductive hormone milieu should be considered in future research. Research in exercising females should assess both hormonal contraceptive users and non-users in order to provide a representative sample (Martin et al. 2018), whilst also reflecting the different endogenous and exogenous reproductive hormone concentration between these groups. Combined oral contraceptive (OC) users consume synthetic oestrogens and progestins and have down-regulated endogenous reproductive hormone concentrations (Elliott-Sale et al. 2013), whilst oestrogen and progesterone concentrations are greater in eumenorrheic women and fluctuate in a cyclical manner across the menstrual cycle (Stricker et al. 2006). A further reason why female participants should be explored is that reproductive function is sacrificed during periods of low energy availability resulting in a down-regulation of reproductive hormones (Loucks et al. 1998). This alteration to the reproductive hormone profile could exacerbate or ameliorate the effects of low energy availability on cognition as performance in some domains of cognitive function (e.g., verbal memory) are positively correlated to oestrogen (Rosenberg and Park 2002), while performance in other domains (e.g., spatial awareness) are negatively associated with oestrogen (Hausmann et al. 2000)

Given the lack of previous research that has explored the effects of short-term low energy availability in exercising women, the aims of this study were to: 1. Compare the cognitive effects of short term balanced energy availability (45 kcal·kgLBM·day^{-1}) to a level of low energy availability representative of athletes (15 kcal·kgLBM·day^{-1}) achieved through dietary restriction or exercise and, 2. Examine whether the cognitive responses were different in eumenorrheic women and oral contraceptive users.

Materials and methods

Participants

Twenty recreationally active participants (10 eumenorrheic, 10 OC users), who exercised for at least 4 h·week^{-1} as measured by the International Physical Activity Questionnaire (IPAQ; Craig et al. 2003), volunteered to take part in the study (participant characteristics shown in Table 1). Eumenorrheic participants had a regular menstrual cycle in the 6 months prior to taking part, with a cycle length of between 24-35 days and used no form of hormonal contraception. Oral contraceptive participants used combined, monophasic low-dose formulations (Microgynon® n=5; Yasmin® n = 2; Rigevidon® n = 1; Gederal® n = 1; Milinette® n = 1) throughout the duration of the study and for at least 6 months prior to taking part. All participants were not at risk of an eating disorder, as characterised by their score (≤ 2) on the SCOFF eating disorder questionnaire (Morgan et al. 1999). Exclusion criteria for participation were: aged < 18 or > 40 years, current smokers, musculoskeletal injury, use of medication that may affect outcome measures, bone fracture in previous 12 months, history of metabolic, heart, liver or kidney disease, diabetes, thyroid disorders, breastfeeding women, women trying to become pregnant or women with amenorrhea, short, long, or irregular cycles. Participants provided their informed consent and the study was approved by the Nottingham Trent University Research (Human) Ethics Committee and the East Midlands NHS Research Ethics Committee (14/EM/1156), in accordance with the Declaration of Helsinki.

Experimental Design

The study design has previously been described elsewhere (Papageorgiou et al. 2018) as the current study forms part of a larger project. Prior to taking part in the experimental conditions, a preliminary assessment was undertaken to determine body composition and maximal oxygen uptake ($\dot{V}O_{2max}$) and to familiarise participants with the cognitive function test battery. All participants then completed three, 3-day experimental conditions in a crossover design; 1) controlled energy balance (BAL), 2) diet-induced low energy availability (DIET) and 3) exercise-induced low energy availability (EX) (Figure 1). Participants were randomly allocated to complete the experimental conditions in a counter-balanced order, using a Latin-square design, but due to participant availability, 6 out of 20 participants were unable to complete the sessions in the specified order. Dietary energy intake (DEI), exercise energy expenditure (EEE) and the resultant energy availability for each condition are displayed in Figure 1. For each condition, a blood sample and cognitive function assessment were conducted at baseline (PRE) and following (POST) the 3-day condition. PRE-testing was conducted in the morning either the day before, or the day of, the start of the experimental condition dependent upon participant availability, whilst POST-testing was always completed in the morning following completion of the 3-day experimental condition. Within-participant testing was conducted at the same time for each session (07:15-8:15) to account for diurnal variation (Sedliak et al. 2008). Participants were asked to consume 500 ml of water upon awakening and refrain from exercise in the 24 h before PRE-testing. Experimental conditions were completed in the early follicular phase of the menstrual cycle for eumenorrheic participants with POST-testing occurring within 7 days of the onset of menstrual bleeding to limit changes in reproductive hormone concentrations (Stricker et al., 2006). Testing was completed during the first week of pill consumption for OC users to ensure consistency between participants. Oral contraceptive users were asked to consume their pill 1 h prior to arrival to the laboratory and asked to consume it at this time for the duration of the study.

Experimental Protocol

Preliminary assessment

Lean body mass was (LBM) measured using a whole-body duel-energy x-ray absorptiometry (DXA) scan (Lunar iDXA, GE Healthcare, Illinois, USA) in order to prescribe individualised diet and exercise regimens. All scans were performed by a qualified DXA practitioner and calibration was conducted prior to all scans with a phantom as per manufacturer's guidelines. Participants were scanned in minimal clothing with all metal objects removed and were aligned centrally on the scanning bed. Participants were asked to arrive at the laboratory in a rested state, at least 3 h post-prandial and euhydrated due to the known effects of exercise, food intake and hydration on DXA scan results (Nana et al. 2015). Urine osmolality was checked prior to scanning and had to be < 800 $mOsm \cdot kg^{-1}$, otherwise participants consumed water until the appropriate osmolality was achieved.

$\dot{V}O_{2max}$ was determined using a two-stage method consisting of a speed lactate test to determine running speed at lactate threshold, and a ramp test to exhaustion (Jones 1998). Lactate threshold was determined using an incremental treadmill (h/p cosmos, Nuβdorf, Germany) test with 3 min stages beginning at 7-9 $km \cdot h^{-1}$ depending upon participant training history and familiarity with treadmill running. Between each stage there was a 1 min rest period in which a capillary blood sample was taken to determine blood lactate concentration (YSI 2300 Stat Plus, Ohio, USA). Treadmill speed was increased by 1 $km \cdot h^{-1}$ per stage until blood lactate concentration increased by 1 $mmol \cdot L^{-1}$ during one stage or was > 4 $mmol \cdot L^{-1}$. After a 10 min rest, participants began an exhaustive treadmill exercise test at the speed corresponding to the stage immediately before a significant increase in blood lactate concentration was observed. Initial treadmill incline was 0 % and was increased 1 % every minute until volitional exhaustion. Breath-by-breath analysis of expired air was conducted (ZAN600 CPET, nspire, Oberthulba, Germany) to determine $\dot{V}O_{2max.}$

PRE and POST condition testing

Upon arrival to the laboratory, height (Seca 217, Birmingham UK) and body mass (Seca 875, Birmingham, UK) were measured and 30 ml of blood was drawn using venepuncture for analysis of 17-β-oestradiol concentrations and other hormones and metabolic markers which are published elsewhere (Papageorgiou et al. 2018). Following this, participants completed the Brunel Mood Scale (BRUMS; Terry et al. 1999) to assess mood state (Tension, Depression, Anger, Fatigue, Confusion, Vigour) and the Pittsburgh Sleep Quality Index (PSQI; Buysse et al. 1989) to measure self-reported sleep quality. Mood and sleep quality were measured are they known to impact cognitive function (Benitez & Gunstad, 2012; Chepenik et al., 2007) and therefore can provide context to results A cognitive test battery was then completed in a quiet area, with participants seated at a desk facing a blank wall to minimise distractions. During verbal tests, the experimenter was seated directly behind the participant approximately 1 m away and participants were instructed to face away from the experimenter throughout the tests. For computer-based tasks (mental rotation test, Stroop test, rapid visual information processing [RVIP], visual search), a laptop (Elitebook, hp, California, USA) was loaded with cognitive software (Sensitive Cognitive Assessment Inventory, Loughborough, UK), lights were dimmed for optimal screen visibility and sound-cancelling headphones were worn to prevent distractions. Written instructions appeared on the screen before each cognitive task, which were reinforced with verbal instructions, and participants' understanding of the test was confirmed by checking that correct responses were provided during pre-test practice stimuli. For the computer-based tasks, participants were asked to get as many correct as possible, but to respond as quickly as they could in order to assess both accuracy and response time. All cognitive tests were performed during each PRE or POST trial and the order the tests were completed was standardised as presented below. Cognitive tests were selected to incorporate domains of cognitive function that have previously been related to reproductive hormone concentrations (verbal memory and spatial awareness; Poromaa & Gingnell, 2014) and energy restriction (memory, psychomotor function, attention and executive function; (Green et al., 1994, 2005; Kemps et al., 2005).

Rey auditory verbal learning test (RAVLT)

The RAVLT is an oral memory test that measures immediate memory span, new learning and susceptibility to interference (Rey 1941). A list of 15 words (List A) was read aloud (with a 1 s interval between words), for five consecutive trials (Trials 1 to 5), each followed by a free-recall test in which participants were asked to recall as many words as possible from the list in any order. The order of the presentation of the words remained fixed across trials. On completion of Trial 5, an interference list of 15 words (List B) was presented, followed by a free recall of that list. Immediately after this, participants were asked to recall List A without further presentation of these words (Trial 6). After a delay period in which the remainder of the cognitive tests were conducted (~20 min) the participant was then required to recall words from List A without hearing them again (Trial 7). Time limits were not imposed and participants were asked to inform the experimenter when they could not remember any more words. Acquisition (sum of words recalled across Trials 1-7 and List B), learning rate (difference between Trial 1 and Trial 5), proactive interference (difference between Trial 1 and List B), retroactive interference (difference between Trial 5 and Trial 6) and forgetting (difference between Trial 7 and Trial 5) were calculated (Strauss et al. 2006). No feedback was given regarding the number of correct responses until completion of the study. Six alternate words lists were used in a counterbalanced order for the RAVLT during the main experimental trials, which were matched for word frequency, length and serial position and have good levels of equivalency (Lezak 1983; Geffen et al. 1994; Majdan et al. 1996; Crawford et al. 1989; Shapiro and Harrison 1990).

Mental rotation test

An adapted version of the mental rotation test (Vandenberg and Kuse 1978) was used to measure spatial awareness. Participants were required to select, using the left and right arrow keys, which of the two three-dimensional shapes at the bottom of the screen could be rotated to match the shape in the centre of the screen. Each trial consisted of 6 practice stimuli in which feedback was provided for correct and incorrect responses, followed immediately by a main trial with 50 stimuli, equally distributed between 0, 20, 40, 60 and 80 degrees of rotation, relative to the central shape, in a randomised order. Task difficulty was increased with greater degrees of rotation (Cooperau and Shepard 1973) so the effects of increasing task complexity could be assessed. The variables of interest were accuracy and the response time of correct responses. For response time analysis, a minimum response time of 200 ms and a maximum response time of 20000 ms was set to remove any anticipatory or unreasonably slow response times. Tests of mental rotation have a good internal consistency (Kuder-Richardson 20 = 0.88 and Cronbachs α = 0.91; Vandenberg and Kuse 1978; Cassie et al. 2009) and test-retest reliability (0.83; Vandenberg and Kuse 1978).

Visual Search test

The Visual Search test is a computer-based test assessing response time and simple visuo-motor speed consisting of two difficulty levels. In the simple level, 20 stimuli were presented and participants were required to press the space bar as quickly as possible when a green triangle was presented on a black background. In the complex level, green dots were randomly distributed across the screen, which were redrawn every 250 ms to induce the effects of a flickering background and act as a distractor. The outline of a triangle was progressively drawn on the background in green dots, with the density of the dots increasing over time. The participants were required to press the space bar as soon as they identified the triangle for a total of 50 stimuli. In both test levels, the location of the triangle stimulus was random and the variables of interest were accuracy and the response time of correct responses. A minimum response time of 300 ms and a maximum response time of 1500 ms (simple level) and 10000 ms (complex level) was set to remove any anticipatory or unreasonably slow response times. The test has previously been shown to be reliable for both difficulty levels ($r > 0.80$; Bandelow et al. 2011).

Stroop-Colour test

The Stroop-Colour test (Stroop 1935), is a computer-based test of inhibitory control that measures the ability to suppress automated responses (Strauss et al. 2006). In the simple level, one of three words (RED, BLUE or GREEN) was written in white font in the centre of the screen, with a matching word and non-matching word either side, also in white font. Using the arrow keys, participants selected the word which matched the middle word as quickly as possible for a total of 20 stimuli. In the complex level, participants were provided with a word in the centre of the screen (e.g. GREEN), written in a different colour font (e.g. blue font). This time, the participant was asked to choose the word corresponding to the font colour of the central word, rather than the written text, as quickly as possible for a total of 50 stimuli. The variables of interest were response time of correct responses and accuracy. A minimum response time of 250 ms and a maximum response time of 2500 ms (simple level) and 4000 ms (complex level) was set to remove any anticipatory or unreasonably slow response times. The test has previously been shown to be reliable for both the simple and complex ($r > 0.85$) levels (Bandelow et al. 2011).

Rapid Visual Information Processing task

The Rapid Visual Information Processing (RVIP) task is a computer-based measure of sustained attention, working memory and selective attention, adapted from the continuous performance task (Wesnes and Warburton 1984). Digits between 2 and 9 appeared in a pseudo-random order in the centre of the screen at a rate of 100 numbers·min^{-1} for 5 min. Participants were instructed to press the space bar when either 3 consecutive even, or 3 consecutive odd numbers appeared. Correct responses were accepted for 1500 ms after the final digit of each sequence. There were a total of 40 correct sequences to identify. The proportion of correct responses out of all responses (true positive rate), the proportion of missed targets (miss rate) and the response time of correct responses was recorded. A minimum response time of 200 ms and a maximum response time of 1500 ms was set to remove any anticipatory or unreasonably slow response times. Prior to each test, participants were provided with a practice attempt to identify a total of 4 sequences, where feedback was provided to show correct or missed responses. The test has previously been shown to be reliable for both the true positive rate ($r = 0.85$) and miss rate ($r = 0.79$), however response time had moderate reliability ($r = 0.53$; Bandelow et al. 2011).

Diet and Exercise protocol

During the 3-day experimental periods, participants were provided with diets according to the experimental condition (Figure 1). Diets were individually weighed and separated into containers. The diets consisted of cereals, milk, vegetable soups, pitta bread, salad and chicken/fish and were palatable and easy for participants to prepare to enhance compliance. The macronutrient composition of the diets was 50% of energy intake from carbohydrate, 30% from fat and 20% from protein; diets were designed by a registered dietician. During each 3-day experimental period, participants were asked to consume all the food provided, have their meals at similar times both within and between conditions and not to consume any other food or caloric beverages. During the DIET condition, a multivitamin and mineral supplement (Boots A-Z, Nottingham, UK) was provided for daily consumption to provide adequate micronutrient intake and isolate the effects of low energy availability/macronutrient restriction. Participants were asked not to participate in any exercise during the experimental conditions (e.g. cycling to work, running, gym, training), unless as part of the experimental protocol, which was confirmed verbally with participants.

For the EX condition, participants performed treadmill running equivalent to a total energy expenditure of 30 kcal·kgLBM·day^{-1} (Figure 1), which was separated into two exercise sessions; one in the morning and one in the afternoon. This consisted of repeated 15 min running bouts at 70% $\dot{V}O_{max}$, with 5 min rest between bouts, until energy expenditure reached the required amount as determined through breath-by-breath analysis (ZAN, nSpire, HDpft 3000). Breath-by-breath analysis was used for the initial morning exercise session and the values were used to calculate running speed and duration for subsequent exercise sessions.

Biochemical analysis

Blood was drawn from an antecubital forearm vein and separated into serum tubes, which were left to clot at room temperature for 30 minutes. Serum was centrifuged at 3000 rev·min^{-1} at 4°C for 10 minutes, transferred into Eppendorf tubes and frozen at -80°C. 17-β-oestradiol was analysed using an electro-chemiluminescence immunoassay (ECLIA; Roche, Basel, Switzerland) with an inter-assay coefficient of variation (CV) of < 4.3% between 214.3-2156.7 pmol·L^{-1} and a detection limit of 18.4 pmol·L^{-1}.

Statistical Analysis

Eumenorrheic and OC participants' demographic information was compared using an independent samples t-test. A one-way repeated-measures ANOVA was used to assess for differences between conditions at baseline. Change from pre- to post-condition for RAVLT, RVIP, BRUMS and PSQI data were calculated and analysed using a two-way (group x condition) mixed-model ANOVAs using Statistica (Dell, Texas, USA). Three-way (group x condition x level) mixed-model ANOVAs were used to assess mental rotation test, Stroop test and visual search test performance as these tests are comprised of different difficulty levels, reflecting different aspects of cognition. 17-β-oestradiol concentration was analysed using a three-way (group x condition x time) mixed-model ANOVA. Data were checked for normality using Shapiro-Wilk tests and significant effects were explored with Bonferroni-adjusted t-tests, with effect sizes calculated using Cohens' D (0.2 = small, 0.5 = medium, 0.8 = large; Cohen and Jacob 1992). All data are presented as mean ± 1SD and statistical significance was set at $P \leq 0.05$.

Results

There were no differences in any demographic variable between eumenorrheic women and OC users (all $P < 0.05$).

Cognitive function

The mean data for all cognitive function tests can be found in Table 2. There were no differences between conditions at baseline for any of the cognitive function measures (all P > 0.05), therefore data were analysed and are presented as change from pre- to post-condition.

The changes from pre- to post-condition for the RAVLT, visual search test, Stroop test and RVIP task were not affected by condition, group or test level (visual search test, Stroop test), and there were no significant interactions between these factors (all P > 0.05). The change from pre-to post-condition was different between conditions for accuracy in the mental rotation test (main effect of condition, P = 0.045; Table 2). As change data were analysed, this is indicative of a divergence in performance over time, between conditions. *Post-hoc* tests showed that whilst accuracy improved in the BAL condition, there was a decline in accuracy in the EX condition (BAL, +2.5%; EX, -1.4%; P = 0.042, D = 0.85). DIET (+1.3%) was not different to BAL or EX (P > 0.05; Figure 2). There was a significant difference between groups for response times in the mental rotation test (main effect of group, P = 0.017), whereby response time was improved to a greater extent from pre-to post-condition in OC users (-13.7%) compared to eumenorrheic participants (-4.0%, P = 0.017; D = 0.67). Response time was improved to a greater extent from pre-to post-condition at 80° compared to 0° rotation (P = 0.005, D = 0.29). Accuracy was improved to a greater extent from pre-to post-condition at 80° compared to 0° and 20° rotation (both P < 0.005) and at 40° compared to 20° (P = 0.031).

Brunel Mood Scale

There was no effect of group on any component of the BRUMS score (main effect of group, all P > 0.05) and group did not influence the response to each condition (group x condition interaction, all P > 0.05). The condition influenced the change from pre- to post-condition for Anger, Confusion and Fatigue (main effect of condition, all P < 0.05). Post-hoc tests showed that whilst Anger increased in the DIET condition, it was reduced in the EX condition (BAL, +0.85; EX, -0.6; P = 0.010, D = 0.854; Figure 3) and was not different in BAL (-0.1) compared to DIET or EX conditions (P > 0.05). Confusion was reduced in the BAL condition and increased in the DIET condition (BAL, -0.75; DIET, +0.45; P = 0.005, D = 0.461), with EX (-0.3) not different to BAL or DIET conditions (P > 0.05). Fatigue was reduced in the BAL condition and increased in the EX condition (BAL, -0.9; EX, +1.45; P = 0.027, D = 0.802), while DIET (+0.65) was not different to BAL or EX (P > 0.05)

Pittsburgh Sleep Quality Index

There was no effect of condition on sleep quality (main effect of condition, P = 0.702) and group did not influence the response between conditions (group x condition interaction effect, P = 0.572). There was a significant difference between eumenorrheic (-0.133) and OC (+1.167) participants for PSQI score change from pre- to post-condition (main effect of group, P = 0.017, D = 0.59; Table 3).

17-β-oestradiol concentration

Mean plasma 17-β-oestradiol concentration was significantly higher in eumenorrheic (143.0 ± 62.6 pmol·L^{-1}) than OC (51.2 ± 53.5 pmol·L^{-1}) participants (main effect group; $P < 0.001$, D = 1.58). There was a significant time x group interaction ($P < 0.001$); 17-β-oestradiol concentrations were reduced from pre (73.8 ± 68.1 pmol·L^{-1}) to post (28.5 ± 11.2 pmol·L^{-1}; $P = 0.03$, D = 1.141) condition in OC participants and were not different from pre (125.4 ± 59.9 pmol·L^{-1}) to post (160.7 ± 61.1 pmol·L^{-1}) condition in eumenorrheic participants ($P = 0.129$, D = 0.58; Table 4). There was no effect of condition on 17-β-oestradiol concentrations and condition did not interact with group or time ($P > 0.05$).

Discussion

The main finding of the present study was that with the exception of mental rotation, a short-term reduction in energy availability induced by diet or exercise had no effect on cognitive function in either OC users or eumenorrheic women. Accuracy in the mental rotation test, an indicator of spatial awareness, was impaired in the exercise-induced low energy availability condition only. Response time in the mental rotation test was improved to a greater extent from pre-to post-condition in OC users compared to non-users across all conditions, suggesting that reproductive hormone status may influence spatial awareness independently of energy availability.

In line with previous research (Lieberman et al. 2008; 2017), the findings of the present study suggest that for the majority of components of cognition measured, performance was not significantly affected by low energy availability, regardless of the method by which it was achieved. Previously, only grammatical reasoning and choice reaction time were shown to be adversely affected by low energy availability when achieved through severe calorie restriction and exercise (Lieberman et al. 2017). Whilst these aspects of cognitive function were not directly measured in the current study, we showed no effect of low energy availability (achieved via diet or exercise) on visual search test or Stroop test performance, which employ similar cognitive domains to choice reaction time. These differences in findings may be due to more moderate restrictions in energy availability in the current study and differences in exercise intensity and duration. A higher exercise intensity was used for a shorter duration in the current study compared to previous research (Lieberman et al. 2008; 2017), whilst the dietary energy intakes were higher in both the exercise-induced and diet-induced low energy availability conditions. The diet and exercise regimens, and subsequent energy availability in the present study, are more representative of those employed by athletes and are therefore more ecologically valid for an active population. In the present study, the addition of non-exercise dietary restriction and controlled-energy balance conditions, provided the ability to differentiate the effects of exercise from low energy availability, which is important as exercise can have profound effects on cognitive performance (Chang et al. 2012; Tomporowski 2003) which may confound the effects of low energy availability in previous studies (e.g. Lieberman et al. 2008; 2017).

This is the first study to assess the effects of low energy availability on spatial awareness (as assessed by the mental rotation test) and the results showed that accuracy was significantly impaired after exercise-induced low energy availability compared to the balanced energy availability condition, with no significant effects of diet-induced low energy availability on mental rotation performance (Figure 2). Mental rotation performance has consistently been shown to be greater in athletes compared to non-athletes and therefore may be important for athletic performance (Jansen and Lehmann, 2013; Jansen et al. 2012; Schmidt et al. 2016). Previous research has shown that the parietal cortex is primarily involved in mental rotation tasks (Gogos et al. 2010; Milivojevic et al. 2009), although functional magnetic resonance imaging studies have shown no effects of energy restriction on parietal cortex activation (Jakobsdottir et al. 2016). There is little evidence available on the effects of exercise on parietal lobe activity, with one study showing reduced parietal operculum activation following a 20 min, moderate-intensity, cycling bout (MacIntosh et al. 2014). Given that the exercise condition influenced spatial awareness in the current study, further research is needed to provide mechanistic insights and assess whether such changes in spatial awareness are mediated by alterations in brain activation as well as the time course of such alterations. Whilst it is unclear why this cognitive domain was selectively impaired by exercise-induced low energy availability, it may be that only performance on the mental rotation task was affected as this was a complex task, requiring the longest processing time (mean > 2 s) of the test battery and therefore may be more susceptible to interference.

The current study has improved upon previous research by not employing dietary placebos. In previous research (Lieberman et al. 2009, 2017), the low energy availability conditions consisted primarily of very low-calorie gels or non-nutritive foods to blind participants to the condition they were undertaking. Placebo-controlled designs are typically the gold-standard for randomised-controlled trials, however a series of studies have shown that dietary restraint, or the conscious effort to restrict energy intake, can impair cognitive performance (Green et al. 1994; Kemps et al. 2005; Rogers and Green 1993), even in the absence of changes in weight (Green and Rogers 1995). Therefore, the use of placebos is not an ecologically valid model, as it may negate some of the psychological consequences of consciously restricting energy intake, which would be present in real-world scenarios. The present study showed that using an ecologically valid model of low energy availability has minimal effects on cognitive function.

Between-group differences were shown for response time in the mental rotation test; OC users' performance improved to a greater extent from pre-to post-condition compared to eumenorrheic participants. These effects occurred independently of changes in energy availability as they were apparent across all conditions, so it is likely that this was a result of differing reproductive hormone profiles. Endogenous 17-β-oestradiol concentrations were significantly reduced from pre-to post-condition in the OC users and increased (D = 0.584, moderate), albeit non-significantly, from pre- to-post condition in the eumenorrheic participants (group x time interaction effect, $P < 0.05$). Mental rotation performance has been shown to be inversely related to 17-β-oestradiol concentrations across the menstrual cycle, which may explain these findings (Hausmann et al. 2000; Silverman and Phillips 1993). This study provides further evidence of the importance of reproductive hormone concentration in spatial awareness, however as this study compared change in performance over 3 days, this may provide novel evidence that oestrogen influences the acquisition and/or retention of spatial awareness. These results may also be explained by the eumenorrheic participants sleep quality being impaired over the 3 days compared to OC users, with sleep having been shown to consolidate performance gains in mental rotation performance (Debarnot et al. 2013).

Conclusions

The current study has shown that the majority of cognitive functions are unaffected by 3 days low energy availability, irrespective of whether this is achieved through diet or exercise, in eumenorrheic women and OC users. These findings may have important implications for athletes who frequently exercise to induce a low energy availability, although further research should be conducted in elite athlete populations. This is also the first study to assess the effects of low energy availability on aspects of spatial awareness via a mental rotation task. Global accuracy on this test was negatively affected by exercise-induced low energy availability, but this effect was evident at all rotation angles, and hence may not be specifically related to the mental operation of rotating visually presented objects. The change in mental rotation performance over the 3 days of each condition was also different between OC users and non-users, providing further evidence of the importance of oestrogen in spatial awareness performance. This evidence supports the concept that low energy availability may potentially have detrimental effects on aspects of physiological function other than reproductive function and bone metabolism, yet highlights the importance of considering reproductive status in this area. This study has improved current understanding of the effects of energy availability on cognitive performance by using ecologically valid methods of reducing energy availability, and a wide range of cognitive function tests.

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Conflicts of interest

The authors have no conflicts of interest to report.

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Tables

Table 1. Participant characteristics for eumenorrheic (EU) and oral contraceptive (OC) participants.

	EU (n=10)	**OC (n=10)**
Age (y)	24 ± 3	26 ± 4
Height (m)	1.66 ± 0.05	1.65 ± 0.04
Body mass (kg)	61.1 ± 7.0	58.1 ± 4.7
SCOFF eating disorder score	0.50 ± 0.71	0.30 ± 0.71
Lean body mass (kg)	41.3 ± 4.1	41.1 ± 3.3
$\dot{V}O_{2max}$ (ml·kg·min^{-1})	48.1 ± 3.3	49.6 ± 6.8

$\dot{V}O_{2max}$, maximal oxygen uptake; SCOFF, Sick, Control, One, Fat, Food

Table 2. Cognitive function absolute values (mean ± 1SD) and percentage change from pre- to post-condition for controlled energy balance (BAL), diet-induced low energy availability (DIET) and exercise-induced low energy availability (EX) for pooled group data (n = 20)

	BAL-PRE	BAL-POST	Change (%)	DIET-PRE	DIET-POST	Change (%)	EX-PRE	EX-POST	Change (%)
Stroop test									
Simple RT	689 ± 109	652 ± 87	-5.4	669 ± 98	653 ±100	-2.4	668 ± 99	658 ± 101	-1.5
Simple Accuracy	0.97 ± 0.05	0.98 ± 0.04	+0.7	0.99 ± 0.03	0.99 ± 0.03	0.0	0.99 ± 0.02	0.99 ± 0.03	-0.3
Complex RT	944 ± 182	880 ± 162	-6.8	913 ± 147	885 ±138	-3.1	884 ± 149	864 ± 147	-2.3
Complex Accuracy	0.97 ± 0.04	0.97 ± 0.03	+0.1	0.97 ± 0.04	0.96 ± 0.04	-0.5	0.98 ± 0.02	0.97 ± 0.03	-1.3
Visual Search									
Simple RT	517 ± 31	522 ± 36	+0.9	514 ± 32	512 ± 20	-0.4	518 ± 36	524 ± 43	+1.1
Simple Accuracy	0.97 ± 0.03	0.99 ± 0.03	+1.5	0.99 ± 0.03	0.99 ± 0.03	+0.1	0.98 ± 0.02	0.98 ± 0.03	-0.5
Complex RT	1512 ± 221	1499 ± 234	-0.9	1569 ± 182	1576 ± 178	+0.5	1564 ± 205	1562 ± 299	-0.1
Complex Accuracy	0.99 ± 0.02	0.99 ± 0.02	+0.1	0.99 ± 0.02	0.99 ± 0.02	+0.3	0.99 ± 0.01	0.98 ± 0.03	-0.6
RVIP									
RT	523 ± 63	508 ± 58	-2.8	524 ± 62	522 ± 66	-0.4	515 ± 54	519 ± 58	+0.8
True positive rate	0.75 ± 0.22	0.82 ± 0.20	+9.7	0.83 ± 0.18	0.84 ± 0.17	+2.1	0.84 ± 0.19	0.88 ± 0.15	+4.1
Miss rate	0.44 ± 0.12	0.40 ± 0.12	-9.4	0.43 ± 0.13	0.39 ± 0.13	-9.6	0.41 ± 0.14	0.39 ± 0.14	-6.0
Mental rotation test									
0° RT	1580 ± 469	1407 ± 349	-11.0	1507 ± 447	1386 ± 348	-8.0	1446 ± 428	1464 ± 360	+1.3
0° Accuracy	0.96 ± 0.05	0.98 ± 0.07	+2.1	0.98 ± 0.08	0.96 ± 0.07	-2.1	0.98 ± 0.05	0.93 ± 0.07	-4.6
20° RT	1792 ± 704	1597 ± 418	-10.9	1732 ± 592	1514 ± 339	-12.5	1597 ± 398	1536 ± 387	-3.8
20° Accuracy	0.97 ± 0.06	0.96 ± 0.06	-0.9	0.95 ± 0.07	0.93 ± 0.09	-1.8	0.97 ± 0.06	0.90 ± 0.14	-7.4
40° RT	2270 ± 668	1981 ± 566	-12.7	2184 ± 768	1955 ± 546	-10.5	2084 ± 744	1980 ± 456	-5.0
40° Accuracy	0.94 ± 0.11	0.96 ± 0.06	+1.8	0.89 ± 0.14	0.93 ± 0.07	+4.0	0.91 ± 0.09	0.91 ± 0.09	+0.6
60° RT	2708 ± 870	2372 ± 518	-12.4	2681 ± 1111	2368 ± 649	-11.7	2525 ± 760	2369 ± 803	-6.2
60° Accuracy	0.89 ± 0.15	0.89 ± 0.09	+0.1	0.91 ± 0.12	0.92 ± 0.12	+0.8	0.87 ± 0.13	0.89 ± 0.10	+2.9
80° RT	3314 ± 1018	2803 ± 732	-15.4	3003 ± 1087	2790 ± 954	-7.1	3031 ± 933	2738 ± 636	-9.7
80° Accuracy	0.75 ± 0.18	0.83 ± 0.17	+11.4	0.80 ± 0.16	0.84 ± 0.13	+4.5	0.83 ± 0.15	0.85 ± 0.17	+3.0
Overall RT	2333 ± 685	2032 ± 464	-12.9	2221 ± 743	2003 ± 483	-9.8	2137 ± 596	2017 ± 460	-5.6
Overall Accuracy	0.90 ± 0.07	0.92 ± 0.06	+2.5	0.90 ± 0.08	0.91 ± 0.07	+0.9	0.91 ± 0.07	0.90 ± 0.07	-1.4 *
RAVLT									
Acquisition	82.3 ± 18.8	81.1 ± 19.7	-1.5	82.0 ± 19.4	79.0 ± 20.1	-3.6	82.0 ± 20.4	81.5 ± 18.7	-0.5
Learning rate	5.2 ± 1.8	5.3 ± 1.8	+1.9	4.6 ± 2.1	6.0 ± 1.7	+29.3	5.3 ± 1.9	5.8 ± 1.9	+8.5
Proactive interference	1.8 ± 2.1	0.9 ± 1.7	-50.0	2.1 ± 2.4	0.9 ± 2.8	-58.1	1.2 ± .9	1.0 ± 1.7	-20.8
Retroactive interference	1.4 ± 1.6	0.9 ± 1.6	-33.3	1.1 ± 1.4	1.5 ± 1.3	+38.1	1.1 ± 1.3	0.8 ± 1.9	-23.8
Forgetting	1.7 ± 1.8	1.5 ± 1.8	-11.7	1.6 ± 2.0	2.0 ± 2.0	+25.8	1.5 ± 1.4	1.8 ± 2.0	+20.7

***** Indicates a main effect of condition, with EX different to BAL ($P < 0.05$). Response time (RT) data are presented in ms, accuracy data are presented as proportion of correct responses and change data are presented as percentage.

Table 3. Mean ± 1SD score on the Pittsburgh Sleep Quality Index questionnaire for eumenorrheic (EU) and oral contraceptive (OC) participants pre-and post-condiction for controlled energy balance (BAL), diet-induced low energy availability (DIET) and exercise-induced low energy availability (EX).

Condition	EU (n=10)			OC (n=10)		
	PRE	**POST**	**Change (%)**	**PRE**	**POST**	**Change (%)**
BAL	8.2 ± 3.1	8.2 ± 2.3	+0.0	6.3 ± 1.8	6.8 ± 2.0	+7.9
DIET	7.6 ± 3.6	7.0 ± 3.3	-7.9	5.5 ± 1.3	7.0 ± 2.8	+27.3
EX	7.4 ± 2.0	7.6 ± 1.7	+2.7	5.9 ± 2.3	7.4 ± 2.4	+25.4

Table 4. Mean ± 1SD plasma 17-β-oestradiol concentrations (pmol·L^{-1}) for eumenorrheic (EU) and oral contraceptive (OC) participants pre-and post-condition for controlled energy balance (BAL), diet-induced low energy availability (DIET) and exercise-induced low energy availability (EX).

Condition	EU (n=10)			OC (n=10)		
	PRE	POST	Change (%)	PRE	POST	Change (%)
BAL	108.9 ± 33.6	157.3 ± 53.1	+48.3	68.8 ± 63.3	27.5 ± 9.9	-57.9
DIET	118.9 ± 29.7	157.9 ± 62.9	+32.2	71.9 ± 52.8	29.1 ± 11.4	-59.0
EX	148.3 ± 92.9	167.0 ± 72.1	+24.0	80.7 ± 89.8	29.0 ± 13.3	-72.0

Figure captions

Figure 1. Overview of the study design adapted from Papageorgiou et al. (2018). Preliminary assessments (P) were followed by controlled energy balance (BAL), diet-induced low energy availability (DIET) and exercise-induced low energy availability (EX) conditions in a crossover manner. Identification (ID) of first day of menstrual cycle or first day of pill consumption was followed by baseline testing (PRE), 3 days following a prescribed diet and/or exercise regimen (D1-3) and follow-up testing (POST) the morning after day 3 of the condition. Dietary energy intake (DEI), exercise energy expenditure (EEE) and energy availability (EA) are described for each condition proportionate to lean body mass (LBM). All groups (eumenorrheic, OC users) completed both time points (PRE, POST) for all conditions (BAL, DIET, EX) in separate menstrual or oral contraceptive cycles.

Figure 2. Mean ± 1SD percentage change in overall accuracy in the mental rotation test for controlled energy balance (BAL), diet-induced low energy availability (DIET) and exercise-induced low energy availability (EX). * indicates a significant difference between BAL and EX ($P < 0.05$).

Figure 3. Mean ± 1SD change in score on the Brunel Mood Scale (BRUMS) from pre- to post-condition for controlled energy balance (BAL), diet-induced low energy availability (DIET) and exercise-induced low energy availability (EX). * indicates a significant difference between conditions ($P < 0.05$).