

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

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31 **Abstract**

32 To date, no research has explored the effects of low energy availability (EA) on cognitive performance using
33 dietary and exercise regimens relevant to athletes. Twenty female participants (10 eumenorrheic, 10 oral
34 contraceptive [OC] users) completed three, 3-day conditions: 1) controlled-balanced EA without exercise (BAL;
35 45 kcal·kg·LBM⁻¹·day⁻¹), 2) diet-induced low EA without exercise (DIET; 15 kcal·kg·LBM⁻¹·day⁻¹) and 3)
36 exercise-induced low EA (EX; 15 kcal·kg·LBM⁻¹·day⁻¹, including 30 kcal·kg·LBM⁻¹·day⁻¹ treadmill running at
37 70% $\dot{V}O_{2max}$). A cognitive test battery was completed before and after each 3-day condition. Mental rotation test
38 accuracy improved in the BAL condition, but there was a decline in accuracy in the EX condition (BAL, +2.5%;
39 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
40 of cognitive performance were not affected by condition ($P > 0.05$) and OC use did not affect cognitive responses
41 ($P > 0.05$). Accuracy in the mental rotation test was impaired when low EA was induced through increased
42 exercise energy expenditure. All other aspects of cognition were unaffected by three days of low EA through diet
43 or exercise. OC use did not mediate the effect of low EA on cognition.

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

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46 **Novelty**

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

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58 **Introduction**

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

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71 To date, only two studies have examined the effects of short term (2-3 days) low energy availability on cognitive
72 performance. In the first study, two days of near total calorie deprivation ($\sim 183 \text{ kcal}\cdot\text{day}^{-1}$ energy intake), was
73 compared to energy-balanced conditions, consisting of either carbohydrate or carbohydrate and fat diets (~ 2820
74 $\text{kcal}\cdot\text{day}^{-1}$ energy intake), with all conditions including two hours of low intensity exercise (40-45% heart rate
75 reserve) per day (Lieberman et al. 2008). Consuming the calorie-restricted diet did not impact participants' self-
76 reported mood and had no effect on vigilance, response time, memory, and reasoning skills. In the second study,
77 participants completed approximately four hours of exercise per day (40-65% $\dot{V}O_{2\text{peak}}$) and consumed either an
78 energy-balanced ($3935 \pm 769 \text{ kcal}\cdot\text{day}^{-1}$ energy intake), or calorie-restricted ($266 \pm 61 \text{ kcal}\cdot\text{day}^{-1}$ energy intake)
79 diet (Lieberman et al. 2017). Mood was significantly affected, with reduced vigour and increased tension, fatigue
80 and total mood disturbance occurring in the calorie-restricted condition, which was associated with a significant
81 reduction in interstitial glucose concentrations. Performance on the majority of cognitive tests was unaffected,
82 although aspects of grammatical reasoning and choice reaction time were improved in the calorie-restricted
83 condition compared to the energy-replete condition, potentially resulting from an evolutionary response of
84 heightened cognitive function in the presence of food scarcity (Bronwen et al. 2007).

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86 In these studies (Lieberman et al. 2008, 2017), changes in cognitive performance were only apparent when a
87 greater exercise duration and intensity was used (Lieberman et al. 2017), which resulted in a greater deviation

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

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100 Lieberman et al. (2017) identified an effect of sex on the response to calorie-restriction, whereby females ($n = 6$)
101 performed better in tests of working memory, grammatical reasoning and vigilance in the calorie-restricted
102 condition compared to when energy replete, while this effect was not apparent in males ($n = 17$). This sexually
103 dimorphic response may be mediated by differences in reproductive hormone concentrations; therefore, the
104 reproductive hormone milieu should be considered in future research. Research in exercising females should
105 assess both hormonal contraceptive users and non-users in order to provide a representative sample (Martin et al.
106 2018), whilst also reflecting the different endogenous and exogenous reproductive hormone concentration
107 between these groups. Combined oral contraceptive (OC) users consume synthetic oestrogens and progestins and
108 have down-regulated endogenous reproductive hormone concentrations (Elliott-Sale et al. 2013), whilst oestrogen
109 and progesterone concentrations are greater in eumenorrhic women and fluctuate in a cyclical manner across the
110 menstrual cycle (Stricker et al. 2006). A further reason why female participants should be explored is that
111 reproductive function is sacrificed during periods of low energy availability resulting in a down-regulation of
112 reproductive hormones (Loucks et al. 1998). This alteration to the reproductive hormone profile could exacerbate
113 or ameliorate the effects of low energy availability on cognition as performance in some domains of cognitive
114 function (e.g., verbal memory) are positively correlated to oestrogen (Rosenberg and Park 2002), while
115 performance in other domains (e.g., spatial awareness) are negatively associated with oestrogen (Hausmann et al.
116 2000)

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118 Given the lack of previous research that has explored the effects of short-term low energy availability in exercising
119 women, the aims of this study were to: 1. Compare the cognitive effects of short term balanced energy availability
120 ($45 \text{ kcal}\cdot\text{kgLBM}\cdot\text{day}^{-1}$) to a level of low energy availability representative of athletes ($15 \text{ kcal}\cdot\text{kgLBM}\cdot\text{day}^{-1}$)
121 achieved through dietary restriction or exercise and, 2. Examine whether the cognitive responses were different
122 in eumenorrheic women and oral contraceptive users.

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124 **Materials and methods**

125 **Participants**

126 Twenty recreationally active participants (10 eumenorrheic, 10 OC users), who exercised for at least $4 \text{ h}\cdot\text{week}^{-1}$
127 as measured by the International Physical Activity Questionnaire (IPAQ; Craig et al. 2003), volunteered to take
128 part in the study (participant characteristics shown in Table 1). Eumenorrheic participants had a regular menstrual
129 cycle in the 6 months prior to taking part, with a cycle length of between 24-35 days and used no form of hormonal
130 contraception. Oral contraceptive participants used combined, monophasic low-dose formulations (Microgynon®
131 $n=5$; Yasmin® $n = 2$; Rigevidon® $n = 1$; Gederal® $n = 1$; Milinette® $n = 1$) throughout the duration of the study
132 and for at least 6 months prior to taking part. All participants were not at risk of an eating disorder, as characterised
133 by their score (≤ 2) on the SCOFF eating disorder questionnaire (Morgan et al. 1999). Exclusion criteria for
134 participation were: aged < 18 or > 40 years, current smokers, musculoskeletal injury, use of medication that may
135 affect outcome measures, bone fracture in previous 12 months, history of metabolic, heart, liver or kidney disease,
136 diabetes, thyroid disorders, breastfeeding women, women trying to become pregnant or women with amenorrhea,
137 short, long, or irregular cycles. Participants provided their informed consent and the study was approved by the
138 Nottingham Trent University Research (Human) Ethics Committee and the East Midlands NHS Research Ethics
139 Committee (14/EM/1156), in accordance with the Declaration of Helsinki.

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141 **Experimental Design**

142 The study design has previously been described elsewhere (Papageorgiou et al. 2018) as the current study forms
143 part of a larger project. Prior to taking part in the experimental conditions, a preliminary assessment was
144 undertaken to determine body composition and maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) and to familiarise participants
145 with the cognitive function test battery. All participants then completed three, 3-day experimental conditions in a
146 crossover design; 1) controlled energy balance (BAL), 2) diet-induced low energy availability (DIET) and 3)
147 exercise-induced low energy availability (EX) (Figure 1). Participants were randomly allocated to complete the

148 experimental conditions in a counter-balanced order, using a Latin-square design, but due to participant
149 availability, 6 out of 20 participants were unable to complete the sessions in the specified order. Dietary energy
150 intake (DEI), exercise energy expenditure (EEE) and the resultant energy availability for each condition are
151 displayed in Figure 1. For each condition, a blood sample and cognitive function assessment were conducted at
152 baseline (PRE) and following (POST) the 3-day condition. PRE-testing was conducted in the morning either the
153 day before, or the day of, the start of the experimental condition dependent upon participant availability, whilst
154 POST-testing was always completed in the morning following completion of the 3-day experimental condition.
155 Within-participant testing was conducted at the same time for each session (07:15-8:15) to account for diurnal
156 variation (Sedliak et al. 2008). Participants were asked to consume 500 ml of water upon awakening and refrain
157 from exercise in the 24 h before PRE-testing. Experimental conditions were completed in the early follicular phase
158 of the menstrual cycle for eumenorrheic participants with POST-testing occurring within 7 days of the onset of
159 menstrual bleeding to limit changes in reproductive hormone concentrations (Stricker et al., 2006). Testing was
160 completed during the first week of pill consumption for OC users to ensure consistency between participants. Oral
161 contraceptive users were asked to consume their pill 1 h prior to arrival to the laboratory and asked to consume it
162 at this time for the duration of the study.

163

164 **Experimental Protocol**

165 *Preliminary assessment*

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

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175 $\dot{V}O_{2\text{max}}$ was determined using a two-stage method consisting of a speed lactate test to determine running speed at
176 lactate threshold, and a ramp test to exhaustion (Jones 1998). Lactate threshold was determined using an
177 incremental treadmill (h/p cosmos, Nußdorf, Germany) test with 3 min stages beginning at $7\text{-}9 \text{ km} \cdot \text{h}^{-1}$ depending

178 upon participant training history and familiarity with treadmill running. Between each stage there was a 1 min rest
179 period in which a capillary blood sample was taken to determine blood lactate concentration (YSI 2300 Stat Plus,
180 Ohio, USA). Treadmill speed was increased by 1 km·h⁻¹ per stage until blood lactate concentration increased by
181 1 mmol·L⁻¹ during one stage or was > 4 mmol·L⁻¹. After a 10 min rest, participants began an exhaustive treadmill
182 exercise test at the speed corresponding to the stage immediately before a significant increase in blood lactate
183 concentration was observed. Initial treadmill incline was 0 % and was increased 1 % every minute until volitional
184 exhaustion. Breath-by-breath analysis of expired air was conducted (ZAN600 CPET, nspire, Oberthulba,
185 Germany) to determine $\dot{V}O_{2max}$.

186

187 *PRE and POST condition testing*

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

208 awareness; Poromaa & Gingnell, 2014) and energy restriction (memory, psychomotor function, attention and
209 executive function; (Green et al., 1994, 2005; Kemps et al., 2005).

210

211 *Rey auditory verbal learning test (RAVLT)*

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

228

229 *Mental rotation test*

230 An adapted version of the mental rotation test (Vandenberg and Kuse 1978) was used to measure spatial awareness.
231 Participants were required to select, using the left and right arrow keys, which of the two three-dimensional shapes
232 at the bottom of the screen could be rotated to match the shape in the centre of the screen. Each trial consisted of
233 6 practice stimuli in which feedback was provided for correct and incorrect responses, followed immediately by
234 a main trial with 50 stimuli, equally distributed between 0, 20, 40, 60 and 80 degrees of rotation, relative to the
235 central shape, in a randomised order. Task difficulty was increased with greater degrees of rotation (Cooperau and
236 Shepard 1973) so the effects of increasing task complexity could be assessed. The variables of interest were
237 accuracy and the response time of correct responses. For response time analysis, a minimum response time of 200

238 ms and a maximum response time of 20000 ms was set to remove any anticipatory or unreasonably slow response
239 times. Tests of mental rotation have a good internal consistency (Kuder-Richardson 20 = 0.88 and Cronbachs α =
240 0.91; Vandenberg and Kuse 1978; Cassie et al. 2009) and test-retest reliability (0.83; Vandenberg and Kuse 1978).

241

242 *Visual Search test*

243 The Visual Search test is a computer-based test assessing response time and simple visuo-motor speed consisting
244 of two difficulty levels. In the simple level, 20 stimuli were presented and participants were required to press the
245 space bar as quickly as possible when a green triangle was presented on a black background. In the complex level,
246 green dots were randomly distributed across the screen, which were redrawn every 250 ms to induce the effects
247 of a flickering background and act as a distractor. The outline of a triangle was progressively drawn on the
248 background in green dots, with the density of the dots increasing over time. The participants were required to
249 press the space bar as soon as they identified the triangle for a total of 50 stimuli. In both test levels, the location
250 of the triangle stimulus was random and the variables of interest were accuracy and the response time of correct
251 responses. A minimum response time of 300 ms and a maximum response time of 1500 ms (simple level) and
252 10000 ms (complex level) was set to remove any anticipatory or unreasonably slow response times. The test has
253 previously been shown to be reliable for both difficulty levels ($r > 0.80$; Bandelow et al. 2011).

254

255 *Stroop-Colour test*

256 The Stroop-Colour test (Stroop 1935), is a computer-based test of inhibitory control that measures the ability to
257 suppress automated responses (Strauss et al. 2006). In the simple level, one of three words (RED, BLUE or
258 GREEN) was written in white font in the centre of the screen, with a matching word and non-matching word
259 either side, also in white font. Using the arrow keys, participants selected the word which matched the middle
260 word as quickly as possible for a total of 20 stimuli. In the complex level, participants were provided with a word
261 in the centre of the screen (e.g. GREEN), written in a different colour font (e.g. blue font). This time, the
262 participant was asked to choose the word corresponding to the font colour of the central word, rather than the
263 written text, as quickly as possible for a total of 50 stimuli. The variables of interest were response time of correct
264 responses and accuracy. A minimum response time of 250 ms and a maximum response time of 2500 ms (simple
265 level) and 4000 ms (complex level) was set to remove any anticipatory or unreasonably slow response times. The
266 test has previously been shown to be reliable for both the simple and complex ($r > 0.85$) levels (Bandelow et al.
267 2011).

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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.

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

303

304 *Biochemical analysis*

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

310

311 **Statistical Analysis**

312 Eumenorrhic and OC participants' demographic information was compared using an independent samples t-test.
313 A one-way repeated-measures ANOVA was used to assess for differences between conditions at baseline. Change
314 from pre- to post-condition for RAVLT, RVIP, BRUMS and PSQI data were calculated and analysed using a two-
315 way (group x condition) mixed-model ANOVAs using Statistica (Dell, Texas, USA). Three-way (group x
316 condition x level) mixed-model ANOVAs were used to assess mental rotation test, Stroop test and visual search
317 test performance as these tests are comprised of different difficulty levels, reflecting different aspects of cognition.
318 17- β -oestradiol concentration was analysed using a three-way (group x condition x time) mixed-model ANOVA.
319 Data were checked for normality using Shapiro-Wilk tests and significant effects were explored with Bonferroni-
320 adjusted t-tests, with effect sizes calculated using Cohens' D (0.2 = small, 0.5 = medium, 0.8 = large; Cohen and
321 Jacob 1992). All data are presented as mean \pm 1SD and statistical significance was set at $P \leq 0.05$.

322

323 **Results**

324 There were no differences in any demographic variable between eumenorrhic women and OC users (all $P <$
325 0.05).

326

327 *Cognitive function*

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

331 The changes from pre- to post-condition for the RAVLT, visual search test, Stroop test and RVIP task were not
332 affected by condition, group or test level (visual search test, Stroop test), and there were no significant interactions
333 between these factors (all $P > 0.05$). The change from pre-to post-condition was different between conditions for
334 accuracy in the mental rotation test (main effect of condition, $P = 0.045$; Table 2). As change data were analysed,
335 this is indicative of a divergence in performance over time, between conditions. *Post-hoc* tests showed that whilst
336 accuracy improved in the BAL condition, there was a decline in accuracy in the EX condition (BAL, +2.5%; EX,
337 -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
338 significant difference between groups for response times in the mental rotation test (main effect of group, $P =$
339 0.017), whereby response time was improved to a greater extent from pre-to post-condition in OC users (-13.7%)
340 compared to eumenorrhic participants (-4.0%, $P = 0.017$; $D = 0.67$). Response time was improved to a greater
341 extent from pre-to post-condition at 80° compared to 0° rotation ($P = 0.005$, $D = 0.29$). Accuracy was improved
342 to a greater extent from pre-to post-condition at 80° compared to 0° and 20° rotation (both $P < 0.005$) and at 40°
343 compared to 20° ($P = 0.031$).

344

345 *Brunel Mood Scale*

346 There was no effect of group on any component of the BRUMS score (main effect of group, all $P > 0.05$) and
347 group did not influence the response to each condition (group x condition interaction, all $P > 0.05$). The condition
348 influenced the change from pre- to post-condition for Anger, Confusion and Fatigue (main effect of condition, all
349 $P < 0.05$). *Post-hoc* tests showed that whilst Anger increased in the DIET condition, it was reduced in the EX
350 condition (BAL, +0.85; EX, -0.6; $P = 0.010$, $D = 0.854$; Figure 3) and was not different in BAL (-0.1) compared
351 to DIET or EX conditions ($P > 0.05$). Confusion was reduced in the BAL condition and increased in the DIET
352 condition (BAL, -0.75; DIET, +0.45; $P = 0.005$, $D = 0.461$), with EX (-0.3) not different to BAL or DIET
353 conditions ($P > 0.05$). Fatigue was reduced in the BAL condition and increased in the EX condition (BAL, -0.9;
354 EX, +1.45; $P = 0.027$, $D = 0.802$), while DIET (+0.65) was not different to BAL or EX ($P > 0.05$)

355

356 *Pittsburgh Sleep Quality Index*

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

361

362 *17- β -oestradiol concentration*

363 Mean plasma 17- β -oestradiol concentration was significantly higher in eumenorrheic (143.0 ± 62.6 pmol·L⁻¹) than
364 OC (51.2 ± 53.5 pmol·L⁻¹) participants (main effect group; $P < 0.001$, $D = 1.58$). There was a significant time x
365 group interaction ($P < 0.001$); 17- β -oestradiol concentrations were reduced from pre (73.8 ± 68.1 pmol·L⁻¹) to
366 post (28.5 ± 11.2 pmol·L⁻¹; $P = 0.03$, $D = 1.141$) condition in OC participants and were not different from pre
367 (125.4 ± 59.9 pmol·L⁻¹) to post (160.7 ± 61.1 pmol·L⁻¹) condition in eumenorrheic participants ($P = 0.129$, $D =$
368 0.58 ; Table 4). There was no effect of condition on 17- β -oestradiol concentrations and condition did not interact
369 with group or time ($P > 0.05$).

370

371 **Discussion**

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

378

379 In line with previous research (Lieberman et al. 2008; 2017), the findings of the present study suggest that for the
380 majority of components of cognition measured, performance was not significantly affected by low energy
381 availability, regardless of the method by which it was achieved. Previously, only grammatical reasoning and
382 choice reaction time were shown to be adversely affected by low energy availability when achieved through severe
383 calorie restriction and exercise (Lieberman et al. 2017). Whilst these aspects of cognitive function were not
384 directly measured in the current study, we showed no effect of low energy availability (achieved via diet or
385 exercise) on visual search test or Stroop test performance, which employ similar cognitive domains to choice
386 reaction time. These differences in findings may be due to more moderate restrictions in energy availability in the

387 current study and differences in exercise intensity and duration. A higher exercise intensity was used for a shorter
388 duration in the current study compared to previous research (Lieberman et al. 2008; 2017), whilst the dietary
389 energy intakes were higher in both the exercise-induced and diet-induced low energy availability conditions. The
390 diet and exercise regimens, and subsequent energy availability in the present study, are more representative of
391 those employed by athletes and are therefore more ecologically valid for an active population. In the present study,
392 the addition of non-exercise dietary restriction and controlled-energy balance conditions, provided the ability to
393 differentiate the effects of exercise from low energy availability, which is important as exercise can have profound
394 effects on cognitive performance (Chang et al. 2012; Tomporowski 2003) which may confound the effects of low
395 energy availability in previous studies (e.g. Lieberman et al. 2008; 2017).

396

397 This is the first study to assess the effects of low energy availability on spatial awareness (as assessed by the
398 mental rotation test) and the results showed that accuracy was significantly impaired after exercise-induced low
399 energy availability compared to the balanced energy availability condition, with no significant effects of diet-
400 induced low energy availability on mental rotation performance (Figure 2). Mental rotation performance has
401 consistently been shown to be greater in athletes compared to non-athletes and therefore may be important for
402 athletic performance (Jansen and Lehmann, 2013; Jansen et al. 2012; Schmidt et al. 2016). Previous research has
403 shown that the parietal cortex is primarily involved in mental rotation tasks (Gogos et al. 2010; Milivojevic et al.
404 2009), although functional magnetic resonance imaging studies have shown no effects of energy restriction on
405 parietal cortex activation (Jakobsdottir et al. 2016). There is little evidence available on the effects of exercise on
406 parietal lobe activity, with one study showing reduced parietal operculum activation following a 20 min,
407 moderate-intensity, cycling bout (MacIntosh et al. 2014). Given that the exercise condition influenced spatial
408 awareness in the current study, further research is needed to provide mechanistic insights and assess whether such
409 changes in spatial awareness are mediated by alterations in brain activation as well as the time course of such
410 alterations. Whilst it is unclear why this cognitive domain was selectively impaired by exercise-induced low
411 energy availability, it may be that only performance on the mental rotation task was affected as this was a complex
412 task, requiring the longest processing time (mean > 2 s) of the test battery and therefore may be more susceptible
413 to interference.

414

415 The current study has improved upon previous research by not employing dietary placebos. In previous research
416 (Lieberman et al. 2009, 2017), the low energy availability conditions consisted primarily of very low-calorie gels

417 or non-nutritive foods to blind participants to the condition they were undertaking. Placebo-controlled designs are
418 typically the gold-standard for randomised-controlled trials, however a series of studies have shown that dietary
419 restraint, or the conscious effort to restrict energy intake, can impair cognitive performance (Green et al. 1994;
420 Kemps et al. 2005; Rogers and Green 1993), even in the absence of changes in weight (Green and Rogers 1995).
421 Therefore, the use of placebos is not an ecologically valid model, as it may negate some of the psychological
422 consequences of consciously restricting energy intake, which would be present in real-world scenarios. The
423 present study showed that using an ecologically valid model of low energy availability has minimal effects on
424 cognitive function.

425

426 Between-group differences were shown for response time in the mental rotation test; OC users' performance
427 improved to a greater extent from pre-to post-condition compared to eumenorrhic participants. These effects
428 occurred independently of changes in energy availability as they were apparent across all conditions, so it is likely
429 that this was a result of differing reproductive hormone profiles. Endogenous 17- β -oestradiol concentrations were
430 significantly reduced from pre-to post-condition in the OC users and increased ($D = 0.584$, moderate), albeit non-
431 significantly, from pre- to-post condition in the eumenorrhic participants (group x time interaction effect, $P <$
432 0.05). Mental rotation performance has been shown to be inversely related to 17- β -oestradiol concentrations across
433 the menstrual cycle, which may explain these findings (Hausmann et al. 2000; Silverman and Phillips 1993). This
434 study provides further evidence of the importance of reproductive hormone concentration in spatial awareness,
435 however as this study compared change in performance over 3 days, this may provide novel evidence that
436 oestrogen influences the acquisition and/or retention of spatial awareness. These results may also be explained by
437 the eumenorrhic participants sleep quality being impaired over the 3 days compared to OC users, with sleep
438 having been shown to consolidate performance gains in mental rotation performance (Debarnot et al. 2013).

439

440 **Conclusions**

441 The current study has shown that the majority of cognitive functions are unaffected by 3 days low energy
442 availability, irrespective of whether this is achieved through diet or exercise, in eumenorrhic women and OC
443 users. These findings may have important implications for athletes who frequently exercise to induce a low energy
444 availability, although further research should be conducted in elite athlete populations. This is also the first study
445 to assess the effects of low energy availability on aspects of spatial awareness via a mental rotation task. Global
446 accuracy on this test was negatively affected by exercise-induced low energy availability, but this effect was

447 evident at all rotation angles, and hence may not be specifically related to the mental operation of rotating visually
448 presented objects. The change in mental rotation performance over the 3 days of each condition was also different
449 between OC users and non-users, providing further evidence of the importance of oestrogen in spatial awareness
450 performance. This evidence supports the concept that low energy availability may potentially have detrimental
451 effects on aspects of physiological function other than reproductive function and bone metabolism, yet highlights
452 the importance of considering reproductive status in this area. This study has improved current understanding of
453 the effects of energy availability on cognitive performance by using ecologically valid methods of reducing energy
454 availability, and a wide range of cognitive function tests.

455

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457 None.

458

459 **Conflicts of interest**

460 The authors have no conflicts of interest to report.

461

462

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628 **Tables**629 **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

630 $\dot{V}O_{2max}$, maximal oxygen uptake; SCOFF, Sick, Control, One, Fat, Food

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648 **Table 2.** Cognitive function absolute values (mean \pm 1SD) and percentage change from pre- to post-condition for controlled energy balance (BAL), diet-induced low energy
 649 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 (%)
<i>Stroop test</i>									
Simple RT	689 \pm 109	652 \pm 87	-5.4	669 \pm 98	653 \pm 100	-2.4	668 \pm 99	658 \pm 101	-1.5
Simple Accuracy	0.97 \pm 0.05	0.98 \pm 0.04	+0.7	0.99 \pm 0.03	0.99 \pm 0.03	0.0	0.99 \pm 0.02	0.99 \pm 0.03	-0.3
Complex RT	944 \pm 182	880 \pm 162	-6.8	913 \pm 147	885 \pm 138	-3.1	884 \pm 149	864 \pm 147	-2.3
Complex Accuracy	0.97 \pm 0.04	0.97 \pm 0.03	+0.1	0.97 \pm 0.04	0.96 \pm 0.04	-0.5	0.98 \pm 0.02	0.97 \pm 0.03	-1.3
<i>Visual Search</i>									
Simple RT	517 \pm 31	522 \pm 36	+0.9	514 \pm 32	512 \pm 20	-0.4	518 \pm 36	524 \pm 43	+1.1
Simple Accuracy	0.97 \pm 0.03	0.99 \pm 0.03	+1.5	0.99 \pm 0.03	0.99 \pm 0.03	+0.1	0.98 \pm 0.02	0.98 \pm 0.03	-0.5
Complex RT	1512 \pm 221	1499 \pm 234	-0.9	1569 \pm 182	1576 \pm 178	+0.5	1564 \pm 205	1562 \pm 299	-0.1
Complex Accuracy	0.99 \pm 0.02	0.99 \pm 0.02	+0.1	0.99 \pm 0.02	0.99 \pm 0.02	+0.3	0.99 \pm 0.01	0.98 \pm 0.03	-0.6
<i>RVIP</i>									
RT	523 \pm 63	508 \pm 58	-2.8	524 \pm 62	522 \pm 66	-0.4	515 \pm 54	519 \pm 58	+0.8
True positive rate	0.75 \pm 0.22	0.82 \pm 0.20	+9.7	0.83 \pm 0.18	0.84 \pm 0.17	+2.1	0.84 \pm 0.19	0.88 \pm 0.15	+4.1
Miss rate	0.44 \pm 0.12	0.40 \pm 0.12	-9.4	0.43 \pm 0.13	0.39 \pm 0.13	-9.6	0.41 \pm 0.14	0.39 \pm 0.14	-6.0
<i>Mental rotation test</i>									
0° RT	1580 \pm 469	1407 \pm 349	-11.0	1507 \pm 447	1386 \pm 348	-8.0	1446 \pm 428	1464 \pm 360	+1.3
0° Accuracy	0.96 \pm 0.05	0.98 \pm 0.07	+2.1	0.98 \pm 0.08	0.96 \pm 0.07	-2.1	0.98 \pm 0.05	0.93 \pm 0.07	-4.6
20° RT	1792 \pm 704	1597 \pm 418	-10.9	1732 \pm 592	1514 \pm 339	-12.5	1597 \pm 398	1536 \pm 387	-3.8
20° Accuracy	0.97 \pm 0.06	0.96 \pm 0.06	-0.9	0.95 \pm 0.07	0.93 \pm 0.09	-1.8	0.97 \pm 0.06	0.90 \pm 0.14	-7.4
40° RT	2270 \pm 668	1981 \pm 566	-12.7	2184 \pm 768	1955 \pm 546	-10.5	2084 \pm 744	1980 \pm 456	-5.0
40° Accuracy	0.94 \pm 0.11	0.96 \pm 0.06	+1.8	0.89 \pm 0.14	0.93 \pm 0.07	+4.0	0.91 \pm 0.09	0.91 \pm 0.09	+0.6
60° RT	2708 \pm 870	2372 \pm 518	-12.4	2681 \pm 1111	2368 \pm 649	-11.7	2525 \pm 760	2369 \pm 803	-6.2
60° Accuracy	0.89 \pm 0.15	0.89 \pm 0.09	+0.1	0.91 \pm 0.12	0.92 \pm 0.12	+0.8	0.87 \pm 0.13	0.89 \pm 0.10	+2.9
80° RT	3314 \pm 1018	2803 \pm 732	-15.4	3003 \pm 1087	2790 \pm 954	-7.1	3031 \pm 933	2738 \pm 636	-9.7
80° Accuracy	0.75 \pm 0.18	0.83 \pm 0.17	+11.4	0.80 \pm 0.16	0.84 \pm 0.13	+4.5	0.83 \pm 0.15	0.85 \pm 0.17	+3.0
Overall RT	2333 \pm 685	2032 \pm 464	-12.9	2221 \pm 743	2003 \pm 483	-9.8	2137 \pm 596	2017 \pm 460	-5.6
Overall Accuracy	0.90 \pm 0.07	0.92 \pm 0.06	+2.5	0.90 \pm 0.08	0.91 \pm 0.07	+0.9	0.91 \pm 0.07	0.90 \pm 0.07	-1.4 *
<i>RAVLT</i>									
Acquisition	82.3 \pm 18.8	81.1 \pm 19.7	-1.5	82.0 \pm 19.4	79.0 \pm 20.1	-3.6	82.0 \pm 20.4	81.5 \pm 18.7	-0.5
Learning rate	5.2 \pm 1.8	5.3 \pm 1.8	+1.9	4.6 \pm 2.1	6.0 \pm 1.7	+29.3	5.3 \pm 1.9	5.8 \pm 1.9	+8.5
Proactive interference	1.8 \pm 2.1	0.9 \pm 1.7	-50.0	2.1 \pm 2.4	0.9 \pm 2.8	-58.1	1.2 \pm .9	1.0 \pm 1.7	-20.8
Retroactive interference	1.4 \pm 1.6	0.9 \pm 1.6	-33.3	1.1 \pm 1.4	1.5 \pm 1.3	+38.1	1.1 \pm 1.3	0.8 \pm 1.9	-23.8
Forgetting	1.7 \pm 1.8	1.5 \pm 1.8	-11.7	1.6 \pm 2.0	2.0 \pm 2.0	+25.8	1.5 \pm 1.4	1.8 \pm 2.0	+20.7

650 * 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
 651 responses and change data are presented as percentage.

652 **Table 3.** Mean \pm 1SD score on the Pittsburgh Sleep Quality Index questionnaire for eumenorrheic (EU) and oral
 653 contraceptive (OC) participants pre-and post-condition for controlled energy balance (BAL), diet-induced low
 654 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 \pm 3.1	8.2 \pm 2.3	+0.0	6.3 \pm 1.8	6.8 \pm 2.0	+7.9
DIET	7.6 \pm 3.6	7.0 \pm 3.3	-7.9	5.5 \pm 1.3	7.0 \pm 2.8	+27.3
EX	7.4 \pm 2.0	7.6 \pm 1.7	+2.7	5.9 \pm 2.3	7.4 \pm 2.4	+25.4

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673 **Table 4.** Mean \pm 1SD plasma 17- β -oestradiol concentrations (pmol·L⁻¹) for eumenorrheic (EU) and oral
 674 contraceptive (OC) participants pre-and post-condition for controlled energy balance (BAL), diet-induced low
 675 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 \pm 33.6	157.3 \pm 53.1	+48.3	68.8 \pm 63.3	27.5 \pm 9.9	-57.9
DIET	118.9 \pm 29.7	157.9 \pm 62.9	+32.2	71.9 \pm 52.8	29.1 \pm 11.4	-59.0
EX	148.3 \pm 92.9	167.0 \pm 72.1	+24.0	80.7 \pm 89.8	29.0 \pm 13.3	-72.0

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691 **Figure captions**

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

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

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