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Effect of Menstrual Cycle Phase and Hormonal Contraceptives on Resting Metabolic Rate and

Body Composition

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Running Head: Resting metabolic rate and menstrual cycle phase.

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1   Abstract (250/250)

2   The cyclical changes in sex hormones across the menstrual cycle (MC) are associated with various

3   biological changes that may alter resting metabolic rate (RMR) and body composition estimates.

4   Hormonal contraceptive (HC) use must also be considered given their impact on endogenous sex

5   hormone concentrations and synchronous exogenous profiles. The purpose of this study was to

6   determine if RMR and dual-energy X-ray absorptiometry (DXA) body composition estimates

7   change across the MC and differ compared to HC users. This was accomplished during a 5-week

8   training camp involving naturally cycling (NC) athletes (n=11) and HC users (n= 7 subdermal

9   progestin implant, n= 4 combined-monophasic oral contraceptive pill, n=1 injection) from the

10   National Rugby League Indigenous Women's Academy. MC phase was retrospectively confirmed

11   via serum estradiol and progesterone concentrations and a positive ovulation test. HC users had

12   serum estradiol and progesterone concentrations assessed at the time point of testing. Results were

13   analyzed using general linear mixed model. There was no effect of MC phase on absolute RMR

14   (p=0.877), relative RMR (p=0.957), or DXA body composition estimates (p>0.05). There was no

15   effect of HC use on absolute RMR (p=0.069), relative RMR (p=0.679), or fat mass estimates

16   (p=0.766), but HC users had a greater FFM and LBM than NC athletes (p=0.044). Our findings

17   suggest that RMR and DXA body composition estimates do not significantly differ due to changes

18   in sex hormones in a group of athletes, and measurements can be compared between MC phases

19   or with HC usage without variations in sex hormones causing additional noise.

20

21   Keywords: female athletes, sex hormones, RMR

Introduction

There is increasing awareness that sports nutrition guidelines are predominantly based on research that has been conducted in men and may not always be suitable or optimal for female athletes (Costello et al., 2014; Kuikman, McKay, et al., 2023; Kuikman, Smith, et al., 2023; Smith et al., 2022). A key consideration for female athletes is whether these guidelines need to account for changes in circulating estrogen and progesterone concentration that occur across the menstrual cycle (MC) or with hormonal contraceptive (HC) use (Elliott-Sale et al., 2021). One area of interest involves metabolic rate, which has significance in contributing to the female athlete's energy requirements as well as playing a potential role in assessing her energy status (Sterringer & Larson-Meyer, 2022).

Resting metabolic rate (RMR) represents the minimal energy cost of living (Hulbert & Else, 2004) and makes up one of the components of total daily energy requirements (Trexler et al., 2014). A 2020 meta-analysis of studies involving non-athlete populations found a significant small effect favouring an increased RMR during the luteal compared to the follicular phase of the MC, suggesting an increase in energy expenditure with elevated concentrations of progesterone and estrogen (Benton et al., 2020). However, the unique hormonal profiles within the follicular and luteal phase of the MC are rarely comprehensively assessed, with most studies simply comparing the early-mid follicular phase of the MC (with theoretically low estrogen and progesterone concentrations) to the mid-luteal phase (with theoretically elevated estrogen and progesterone concentrations) (Elliott-Sale et al., 2021). Results of this specific meta-analysis are further limited by inclusion of studies with poor methodological control of ovarian hormones (Benton et al., 2020). Finally, given the high prevalence of hormonal contraceptives (HC) use by athletes (Martin

45 et al., 2018; McNamara et al., 2022; Oxfeldt et al., 2020) and their associated effects on

46 endogenous sex hormone concentrations and synchronous exogenous profiles (Elliott et al., 2005;

47 Hirschberg, 2022), the effects of HC use on RMR should also be assessed.

48

49 Although the practical relevance of meaningful differences in RMR on energy requirements of

50 athletes needs to be considered, another scenario for the measurement of RMR in sports nutrition

51 involves its potential use as a screening tool for metabolic suppression in response to low energy

52 availability (LEA) (Sterringer & Larson-Meyer, 2022). This is done either by determining the

53 ratio of measured RMR against a value predicted by an equation (Schofield et al., 2019), or by

54 expressing measured RMR relative to fat free mass (FFM) (Loucks et al., 2011). Although the

55 RMR ratio (measured:predicted) appears to have some utility in identifying female athletes with

56 indices of metabolic suppression (Strock et al., 2020), it has not been recognized as a primary or

57 secondary indicator of Relative Energy Deficiency in Sport (REDs) in the IOC REDs Clinical

58 Assessment Tool - Version 2 (CAT2) due to current concern around its specificity and sensitivity

59 (Stellingwerff et al., 2023). Part of this concern relates to known technical and biological

60 variability in RMR measurements (Siedler et al., 2023), the latter of which could include the effects

61 of endogenous and exogenous sex hormone concentrations. Better understanding of this variability

62 might help to improve the interpretation of RMR measurements and their use as a diagnostic tool.

63 Accordingly, the aim of this study was to investigate the effects of MC phase and HC usage on

64 RMR in a cohort of female athletes using Best Practice Guidelines for the control of ovarian

65 hormones (Elliott-Sale et al., 2021). Additionally, we measured changes in body composition

66 estimates using dual-energy X-ray absorptiometry (DXA) across MC phase and with HC usage as

67 DXA scans are often performed alongside RMR measurements to interpret findings.

68

Methods

Participants

The participants of this study are part of a larger study known as the Female Athlete Research Camp (FARC) with 25 female athletes (McKay et al., 2022) being recruited for a 5-week training camp at the Australian Institute of Sport, as previously described (McKay et al., 2023). Athletes were from the National Rugby League's Indigenous Women's Academy and of Tier 3 calibre (Highly trained/National level) (McKay et al., 2022). Both naturally cycling (NC) athletes and HC users were included. Data from two HC users were excluded from analysis– one for failure to complete the training camp and one for failure to comply with the standardized protocol for body composition and RMR measurements. Information on the remaining NC athletes (n=11) and HC users (n=12) is summarized in Table 1. Of the HC users, seven used a subdermal progestin implant (Implanon), four used combined-monophasic version of the oral contraceptive pill (COC), and one used hormonal injection (depot medroxyprogesterone acetate). The hormonal compositions of the HC can be found elsewhere (McKay et al., 2023). The study was approved by the Human Ethics Research Committee at Australian Catholic University (2021-285H).

-Table 1-

Experimental design

NC athletes tracked their MC for 11 weeks prior to study commencement, using an online reporting system (REDCap). This also involved confirming ovulation via urinary luteinising hormone (LH) surge testing (Elliott-Sale et al., 2021) with urinary ovulation kits (Advanced Digital Ovulation Test, Clearblue, Geneva, Switzerland). NC athletes tested for ovulation from day 8 of the MC until ovulation occurred or until day 17, if ovulation was not detected (McKay et al., 2023). This

91   information was used to prospectively plan testing dates for three physiologically-specific MC

92   phases (Elliott-Sale et al., 2021) during the training camp (see Figure 1): 1) Phase 1: begins at the

93   onset of bleeding, when estrogen and progesterone concentrations are low; 2) Phase 2: 14-26 hours

94   prior to ovulation and the LH surge, when estrogen concentrations are at their highest and

95   progesterone concentrations remain low; and 3) Phase 4: Seven days after ovulation when

96   progesterone concentrations are at their highest and estrogen concentration are also elevated. To

97   anticipate the day of Phase 2 during the training camp, NC athletes used dual hormone ovulation

98   kits (Advanced Digital Ovulation Test, Clearblue, Geneva, Switzerland) with a rise in estrogen

99   prior to a rise in LH being identified with a "flashing smile." Venous blood samples were taken

100   on the day of testing, and concentrations of estrogen and progesterone were retrospectively used

101   to confirm that testing occurred at the correct MC phase. HC users were tested on three occasions

102   spaced by 7-10 days (also referred to as "Phase 1", "Phase 2", and "Phase 4"). COC users were

103   tested on active pill-taking days to avoid the withdrawal bleed and athletes taking all other types

104   of HC (implant and injection) were tested at any time given the continuous nature of these

105   contraceptives. HC users also had serum estradiol and progesterone concentrations at the time

106   point of testing established. Further details regarding the MC tracking can be found elsewhere

107   (McKay et al., 2023).

108   -Figure 1-

109   ***Dietary control***

110   As confirmation of MC phase was dependent on menstrual reporting that occurred mid-morning,

111   a standardized diet was implemented from lunch onwards to prepare for the next day's laboratory

112   testing. Thus, participants consumed an *ad libitum* breakfast, with the controlled diet thereafter

113   providing 80% of their estimated requirements, which were set as 5 g·kg body mass (BM)$^{-1}$day^{-1}

114   of carbohydrate, 1.5 g·kg BM^{-1}day^{-1} or protein, and 1 g·kg BM^{-1}day^{-1} as fat. For athletes with a

115   body mass index (BMI) >110% of 25.0 kg/m^2 (n=3 NC athletes, n=5 HC users), an adjusted BM

116   (ABM) was used to calculate dietary needs to prevent excessive energy intake (Krenitsky, 2005).

117   ABM was calculated as: (actual BM – ideal BM) x 0.25 + ideal BM with ideal BM representing a

118   BM that would equate to a BMI of 25.0 kg/m^2. Diets were designed by a dietitian and prepared by

119   professional chef with all meals being weighed and provided in a dining hall setting. The dining

120   hall had a rotating 2-week menu, so athletes did not necessarily receive the same food during each

121   standardized diet period that preceded testing. Meals were supervised by a member of the research

122   team, but snacks were consumed throughout the day without supervision. Athletes self-reported

123   any deviations from standardized diets, and this was accounted for when analysing standardized

124   diets.

125

126   ***Measurements***

127   *Body composition*

128   On test mornings, athletes arrived at the laboratory in an overnight fasted and rested state such that

129   no food or fluid was consumed, or exercise was performed prior to testing. DXA was used to assess

130   body composition, using the Best Practice Protocols of the Australian Institute of Sport involving

131   standardized positioning (Slater et al., 2023). Athlete scans were performed in the same mode (GE

132   Lunar iDXA) and analyzed using GE encore by the same trained researcher to assess FFM, lean

133   body mass (LBM), and fat mass (FM). Data from the first and last DXA scan were used to assess

134   changes in body composition over the training camp.

135

136   *Resting metabolic rate*

137   RMR was determined either immediately before or after the DXA scan. As such, athletes were

138   overnight fasted and rested. A protocol of rest and familiarisation was used, which produces

139   comparable results to RMR measured in an inpatient setting and has good interday reliability (ICC

140   0.87; Typical error as CV 5.9%)(Bone & Burke, 2018). With this protocol, athletes first rested on

141   a bed in a dark and quiet room for 10 minutes before being given a one-way mouthpiece (bite size

142   rubber mouthpiece connected to silicone diaphragm that allows flow in one direction only through

143   a valve) for a 15-minute familiarisation period. Athletes were instructed not to fall asleep during

144   the measurement, and this was verbally confirmed following the measurement. The room was

145   temperature controlled, and athletes had access to a blanket during the measurement so that they

146   were at a comfortable temperature. Following the rest and familiarisation period, expired air was

147   collected into a gas-impermeable Douglas bag for 10 minutes for two consecutive data collection

148   periods. Ametek Oxygen (O_2) and carbon dioxide (CO_2) analyzers (VacuMed, Ventura, CA) were

149   calibrated with two known gas concentrations (14.38% O_2,2.510% CO_2; and 16.30% O_2, 4.173%

150   CO_2) before use. The expirate from each bag was sampled for one minute with the gas sampling

151   time and flow rate being recorded. The volume of the remaining expirate was then determined

152   using a Tissot spirometer via an evacuation pump. RMR results were reported as absolute over 24

153   hours (kcal·day^{-1}) for each Douglas bag and then a mean RMR was computed from the two

154   Douglas bags. RMR results were also reported relative to FFM (kcal·kg FFM^{-1}·day^{-1}). It was not

155   possible to control pre-trial exercise since scheduled team training took place each afternoon

156   during the camp, but >12 hours separated training and laboratory testing. Training sessions

157   included gym or fielding sessions with duration and rating of perceived exertion (RPE) being used

158   to calculate training load (RPE x duration).

159

160   *Indicators of low energy availability*

161   At each testing time point across the camp, a RMR ratio (measured:predicted) was calculated using

162   three RMR predictive equations as outlined in Table 2 (Cunningham, 1982, 1991; Harris &

163   Benedict, 1918). These predictive equations were selected as they have validated thresholds to

164   indicate energy deficiency with an athlete being classified as having a suppressed RMR if the ratio

165   fell below this threshold (see Table 2)(Strock et al., 2020), and/or presented with a relative RMR

166    $<30 \text{ kcal·kg FFM}^{-1}\text{·day}^{-1}$ (Loucks et al., 2011). Alongside screening for a suppressed RMR, blood

167   indicators of LEA as per the updated IOC REDs CAT2 were measured (Stellingwerff et al., 2023)

168   and athletes completed the Low Energy Availability in Females Questionnaire (LEAF-Q) prior to

169   commencing the study (Melin et al., 2014).

170   -Table 2-

171   *Blood samples*

172   Following the DXA scan and RMR measurement, an 8.5 mL venous blood sample was collected

173   from an antecubital vein into a serum separator tube by a trained phlebotomist. Blood tubes were

174   left to clot at room temperature for 30 minutes, prior to centrifugation at 2200 G for 10 minutes at

175   4 °C. Remaining serum was split into aliquots and stored at -80 °C until batch analysis could occur.

176   Estradiol and progesterone were measured via an Access 2 Immunoassay System (Beckman

177   Coulter, Brea, CA, USA) and used to retrospectively confirm MC phase and to establish hormonal

178   profiles for HC users. Intra-assay coefficient of variations were 5% for estradiol and 11% for

179   progesterone. Lipids, cortisol, insulin-like growth factor 1 (IGF-1) and triiodothyronine (T3) were

180   measured by chemiluminescent immunoassay through a commercial laboratory (Laverty

181   Pathology, Bruce, ACT, Australia).

182

183   *Statistics*

184   Statistical analyses were performed using R Studio (v3.5.2) with statistical significance accepted

185   at an α level of $p \leq 0.05$ using general linear mixed models. Fixed effects for the model included

186   MC phase and menstrual status (NC athletes or HC users). Subject ID and test order were included

187   as random effects within the models. The initial model included all possible interactions, with non-

188   significant interactions being dropped. Statistical significance of the fixed effect was determined

189   using a Type II Wald tests with Kenward-Roger degrees of freedom, and where significant fixed

190   effects were evident, a Tukey's post-hoc comparison was used to identify where differences exist.

191   For NC athletes only, a repeated measures correlation was used to assess the relationship between

192   changes in relative RMR and serum concentrations of estradiol, progesterone, and the ratio of

193   estradiol to progesterone. Non-normally distributed data (absolute RMR, FM, estradiol levels, and

194   progesterone levels) were log-transformed for statistical analyses.

195

196   **Results**

197   Assessment of actual intake of the ~18 hour standardized diet showed no difference between trials

198   or groups for energy and macronutrient intake (p>0.05) with actual intake achieving the

199   standardized diet targets (Table 3). Over the training camp, FM was reduced in both NC athletes

200   and HC users (~0.5 kg; p=0.0001). FFM increased in HC users (+0.9 kg; p<0.0001), but not in NC

201   athletes (+0.2 kg; p=0.770). There was no difference in training load the day prior to testing with

202   MC phase (p=0.331), but NC athletes had a greater training load than HC users (455±308 AU vs.

203   287±325 AU; p=0.042).

204   -Table 3

205　Comprehensive results of menstrual status confirmation from ovulation testing and retrospective

206　analysis of serum estradiol and progesterone concentrations have been previously reported

207　elsewhere (McKay et al., 2023). In summary, five NC athletes were identified as having a

208　menstrual irregularity during the training camp (n=3 oligomenorrheic, n=1 anovulatory, n=1 luteal

209　phase deficiency). Of the remaining 6 NC athletes, only one presented with the expected Phase 2

210　hormonal profile. Thus, Phase 2 metrics were not analyzed due to the measurement variability.

211　Furthermore, Phase 1 versus Phase 4 analysis excluded data due to an insufficient rise in serum

212　progesterone concentrations from Phase 1 to Phase 4 of the MC (n= 3 NC athlete), technical issues

213　with RMR measurements (n=1 NC athlete, n= 1 HC user), and an erroneous serum estradiol

214　measurement (>3SD above mean, n=1 HC user). For the remaining athletes, as expected, there

215　was an increase in serum estradiol and progesterone concentrations from Phase 1 to Phase 4 in NC

216　athletes (estradiol, $p=0.0003$; progesterone, $p<0.0001$), but not in HC users (estradiol, $p=0.967$;

217　progesterone, $p=0.323$; Table 4).

218　　　　　　　　　　　　　　　　　　　　-Table 4-

219　There was no effect of MC phase ($p=0.877$) or HC usage ($p=0.069$) on absolute RMR nor was

220　there an effect of MC phase ($p=0.957$) or HC usage ($p=0.679$) on relative RMR (Figure 2).

221　　　　　　　　　　　　　　　　　　　-Figure 2-

222　There was no effect of MC phase ($p=0.118$) or HC usage ($p=0.766$) on FM estimates. While there

223　was also no effect of MC phase on FFM estimates ($p=0.225$) and LBM estimates ($p=0.248$), HC

224　users had a greater FFM ($p=0.028$) and LBM ($p=0.028$) than NC athletes (Figure 3).

225　　　　　　　　　　　　　　　　　　-Figure 3-

226　There was no within athlete correlation between changes in relative RMR and estradiol

227　concentrations ($r=0.31$, $p=0.179$); progesterone concentrations ($r=0.06$, $p=0.805$), or the ratio of

228 concentrations of estradiol to progesterone (r=0.11, p=0.640) from Phase 1, Phase 2, and Phase 4

229 of the MC in NC athletes (Figure 4).

230 <center>-Figure 4-</center>

231 There was no effect of MC phase on the RMR ratio calculated from the Harris Benedict (HB)

232 equation using actual BM (p=0.958) or ABM (p=0.141), Cunningham$_{1980}$ equation (p=0.865), or

233 Cunningham$_{1991}$ equation (p=0.831) nor was there an effect of HC usage on the RMR ratio

234 calculated from the HB equation using actual BM (p=0.398), Cunningham$_{1980}$ equation (p=0.911),

235 or Cunningham$_{1991}$ equation (p=0.714; Table 5). However, the RMR ratio calculated from the HB

236 equation using ABM was greater in HC athletes than NC athletes (p=0.020).

237 <center>-Table 5-</center>

238 At the first testing measurement, no athlete presented with a suppressed RMR. However, at the

239 second and/or third test, six athletes presented with a relative RMR <30 kcal·kg FFM^{-1}·day^{-1} and

240 one of these athletes also presented with a RMR ratio <0.90 when using the HB equation (Figure

241 5). No athlete presented with an RMR ratio considered suppressed when using the Cunningham$_{1980}$

242 or Cunningham$_{1991}$ equation or when using ABM in the HB equation.

243 <center>-Figure 5-</center>

244 **Discussion**

245 Our results showed that assessments of RMR and DXA-derived body composition estimates did

246 not differ due to changes in ovarian hormones in a group of female athletes. Specifically, there

247 were no systematic differences in RMR and DXA-derived body composition between Phase 1 and

248 Phase 4 of the MC or with HC usage in female athletes, nor any correlation with changes in

249 absolute or ratios of concentrations of serum estradiol and progesterone. These findings have

250 implications for the dietary recommendations given to female athletes and the Best Practice

251 Protocols for the measurement of RMR and body composition. Specifically, our results suggest

252 female athletes do not need to alter their energy requirements due changes in RMR across MC

253 phase or with HC use, and that MC phase and HC use does not need to be standardized when

254 measuring RMR.

255

256 Previous studies have provided some evidence for a modulation of RMR due to alterations in

257 estrogen concentrations. For example, the suppression of serum estrogen and progesterone to

258 postmenopausal concentrations in premenopausal women was associated with a ~40 kcal/day

259 reduction (~3%) in RMR compared to the follicular phase and ~70 kcal/day (~5%) compared to

260 the luteal phase (Day et al., 2005). Furthermore, this reduction in RMR was prevented with

261 concurrent transdermal estrogen administration that maintained serum estrogen concentrations to

262 that expected in the mid to late follicular phase of the MC (Melanson et al., 2015). Such effects of

263 estrogen increasing RMR have been attributed to estrogen increasing brown adipose tissue (BAT)

264 activity both directly by acting on BAT and through its effect on the ventromedial hypothalamus

265 nucleus and sympathetic nervous system signalling (Gavin et al., 2018; Vigil et al., 2022). In the

266 current study, despite the large increase in serum estradiol concentrations during Phase 4 of the

267 MC in NC athletes (Table 4), we did not show an effect of MC phase or HC usage on RMR.

268 Furthermore, a repeated measures correlation did not show any association between RMR and

269 estradiol concentrations. These findings suggest that estrogen did not achieve a significant

270 modulation of RMR in our cohort of female athletes.

271

272 Several explanations might underpin the discrepancies between our results and other studies

273 showing an increase in RMR with elevated estrogen concentrations. Firstly, any variation in RMR

274 due to sex hormones may be overshadowed by other factors that contribute to the 3-5% day-to-

275 day variability in measured RMR (Compher et al., 2006). Although precautions were taken in this

276 study to minimize factors that may contribute to technical error and variability in RMR (e.g.,

277 standardizing dietary intake the day prior to testing), consideration should be given to the

278 magnitude of change in RMR across the MC reported in previous studies. For instance, a female

279 athlete with an absolute RMR of 1200-1800 calories/day would require a change >60-90

280 calories/day to exceed a 5% day-to-day variation in RMR. Yet, of the seventeen studies in the 2020

281 meta-analysis that undertook testing in a fasted and rested state and provided RMR results, the

282 mean change in RMR was ~45 calories/day, with only five studies reporting an increase >60

283 calories/day above the follicular phase (Benton et al., 2020). Since the publication of this meta-

284 analysis, only one study could be located that found differences in RMR between MC phases with

285 RMR being 37 calories/day lower in the follicular compared to the luteal phase of the MC (Malo-

286 Vintimilla et al., 2023). However, ovulation was not confirmed, and nine of the nineteen

287 participants had serum progesterone concentrations <5 ng/mL in the luteal phase, which suggests

288 anovulatory cycles (Malo-Vintimilla et al., 2023). Furthermore, these studies were not conducted

289 specifically in athletic cohorts whom may have a greater prevalence of menstrual disturbances (De

290 Souza et al., 2010) and a greater exercise energy expenditure and energy intake resulting in a

291 greater energy flux compared to sedentary individuals (Bullough et al., 1995; Goran et al., 1994;

292 Paris et al., 2016). Interestingly, an increased RMR seen during periods of high energy flux, and

293 with elevated serum estrogen and progesterone concentrations are both thought to occur via beta-

294 adrenergic support of RMR (Bell et al., 2004; Day et al., 2005). Modulations in RMR due to

295 energy flux and sex hormones may not be additive, resulting in changes in RMR across the MC in

296 sedentary females, but not trained female athletes. Furthermore, variations in training or

297   competition prior to RMR measurements may contribute to greater variability in the RMR

298   measurements of female athletes compared to sedentary women.

299

300   A further reason for discrepancy between our results and previous studies that have demonstrated

301   changes in RMR across MC phase might be attributed to differences in methodology used to

302   identify MC phase. Few studies assessing changes in RMR across the MC have confirmed

303   ovulation and measured serum concentrations of estradiol and progesterone, which could result in

304   measurements that are occurring unknowingly in an incorrect phase and hormonal profile. For

305   instance, a common strategy is to assume that ovulation occurred half-way through the MC without

306   confirming an LH surge has occurred despite follicular phase length being variable (Mihm et al.,

307   2011). If ovulation is assumed to occur at day 14 of the MC, but a woman has an extended follicular

308   phase, measurements that are thought to have occurred in the luteal phase could actually have

309   occurred in the late follicular phase of the MC. This is problematic as these phases have different

310   hormonal profiles (Elliott-Sale et al., 2021). This technique also does not consider anovulatory

311   cycles or luteal phase defects, which will result in an incorrect hormonal profile during the luteal

312   phase of the MC (De Souza, 2003). Evidently, when assessing changes across MC phase it is vital

313   to follow the Best Practice Protocols for control of ovarian hormones (Elliott-Sale et al., 2021). It

314   should be noted that while a strength of our study was following the Best Practice Protocols for

315   ovarian hormone control, this contributed to a small sample size, which is a notable limitation of

316   our study.

317

318   The effect of hormonal profiles seen in Phase 2 of the MC on RMR could not be assessed in this

319   study. Because this phase lasts only 12-26 hours (Elliott-Sale et al., 2021), may be absent if the

320 athlete has an anovulatory cycle, and requires resources to ascertain its presence (i.e. blood sample

321 analysis to determine estrogen and progesterone concentrations, measuring for a LH surge etc),

322 there is little utility in considering its effect on nutrition recommendations for female athletes.

323 However, since Phase 2 of the MC provides an opportunity to investigate the effects of estrogen

324 on RMR with minimal progesterone (Elliott-Sale et al., 2021), this would provide mechanistic

325 insight into the control of RMR. Furthermore, the identification of any meaningful noise in RMR

326 measurement could be taken into account when Best Practice Protocols for RMR assessment in

327 athletic cohorts are established. Therefore, future studies are needed to assess if RMR changes

328 across Phase 2 of the MC, so that the need to control for this phase of the MC can be determined.

329

330 There are multiple forms of HC with unique effects on the endogenous hormonal milieu (Elliott-

331 Sale et al., 2021). Among our study cohort, 3 different types of HC (COC, injection and implant)

332 were used, with a further differentiation in the brands of COC (4 brands). The hormonal profile of

333 HC users established in this study demonstrated variations in sex hormones across testing time

334 point with a ~20% increase in estradiol concentrations and ~70% increase in progesterone

335 concentrations from "Phase 1" to "Phase 4"(Table 4). Notably, endogenous concentrations of

336 estradiol and progesterone were measured, and exogenous sex hormones may have higher receptor

337 affinity that exceed the effects of endogenous sex hormones (Hirschberg, 2022). Additionally,

338 such changes in sex hormones for HC users were small in comparison to the large increases in

339 concentrations of estradiol (~500%) and progesterone (~2900%) seen from Phase 1 to Phase 4 in

340 NC athletes. HC usage had no effect on RMR such that RMR did not differ between NC athletes

341 and HC users or between testing time points in HC users. This is in agreement with the majority

342 of studies comparing the RMR of COC users and non-users (Duhita et al., 2017, 2019; Eck et al.,

343   1997; Jensen & Levine, 1998), although one study reported a higher RMR in COC users when

344   FFM and FM were included as covariates (Diffey et al., 1997). Like COC, most studies examining

345   the effects of depot-medroxyprogesterone acetate injection on RMR have found no effect on RMR

346   (Pelkman et al., 2001; Steward et al., 2016), and reports of changes in RMR with usage are likely

347   secondary to changes in body composition (Batista et al., 2017). Notably, seven athletes in our

348   cohort used the hormonal implant, Implanon, which is a single-rod progestin-only implant that is

349   inserted in the upper arm for up to three years (Fischer, 2008). No study could be located assessing

350   the effect of Implanon on RMR, which likely reflects the relatively new approval of this HC and

351   usage trends compared to other HC (King et al., 2021). Overall, our study suggests that HC usage

352   has no effect on RMR. However, a limitation of this study was the lack of homogeneity with the

353   type of HC used within our cohort leading to an increased variability in endogenous and exogenous

354   hormonal profiles. As such, further studies are needed to assess the effect of HC usage on RMR.

355

356   Estimates of total body composition using DXA depend on the assumption of a constant lean soft

357   tissue hydration (Pietrobelli et al., 1996), but this will vary with extracellular and intracellular fluid

358   distribution (St-Onge et al., 2004). Variations in sex hormones with MC phase or HC usage may

359   introduce a source of error in DXA body composition estimates as estrogen and progesterone cause

360   a shift in osmoregulation that may alter water distribution within the extracellular fluid space

361   (Stachenfeld, 2008). Differences in FM estimates by DXA scan have been reported in the early

362   versus mid-follicular phase (~0.30 kg, ~1.6% change)(Gould et al., 2021) and the late follicular

363   phase compared with the early follicular phase (~0.31 kg, ~1.9%) and mid-luteal phase (~0.35 kg,

364   ~2.1%)(Thompson et al., 2021). However, this is not a consistent finding with others reporting no

365   difference between the mid-luteal and mid-follicular MC phase (Jürimäe et al., 2011; Koşar et al.,

366   2022; Ong et al., 2022). Additionally, for studies that have reported differences across MC phase,

367   this difference was below the ~4.7% least significant change (LSC) in consecutive day precision

368   error for whole body FM DXA estimates (Slater et al., 2023). Differences in DXA body

369   composition estimates have also been reported across the oral contraceptive pill (OCP) cycle of

370   monophasic COC users with lower LBM estimates during the early hormone phase compared to

371   the non-active pill phase (~0.29 kg, ~0.7% change) and late pill phase (~0.34 kg, ~0.9% change)

372   (Thompson et al., 2021). We were unable to assess change across the OCP cycle of the four COC

373   users as all purposefully manipulated their cycles to avoid a withdrawal bleed during the camp

374   (McKay et al., 2023). However, like FM estimates across MC phase, differences in DXA body

375   composition previously reported across the OCP cycle were below the ~1.4% LSC in consecutive

376   day precision error for LBM DXA estimates (Slater et al., 2023). Overall, our findings suggest that

377   any underlying shifts in fluid balance due to changing sex hormones with MC phase and HC usage

378   are not sufficient to produce meaningful change in DXA body composition estimates (Figure 3).

379   Practically, this suggests researchers and practitioners can measure total body composition via a

380   DXA scan in Phase 1 or Phase 4 of the MC without sex hormones creating additional noise.

381

382   Subtle menstrual disturbances were identified in five NC athletes within this cohort (Figure 5), and

383   it is possible that this was due to LEA exposure (De Souza, 2003). While a suppressed RMR has

384   been demonstrated in female athletes with menstrual disturbances (Koehler et al., 2016; Melin et

385   al., 2015; Myerson et al., 1991; Strock et al., 2020), none of the athletes with menstrual irregularity

386   during the training camp presented with an RMR measurement that met any of the criteria

387   suggesting RMR suppression. This suggests either that the menstrual issues observed were not

388   underpinned by exposure to LEA or that RMR assessment does not provide a universal tool to

389   diagnose metabolic suppression. Of the six athletes who did present with a suppressed RMR

390   measurement across the training camp, only two also presented with an indicator of LEA.

391   Furthermore, for five of the six athletes, this suppressed RMR was based solely on a relative RMR

392   <30 kcal·kgFFM^{-1}·day^{-1}. The use of relative RMR to indicate metabolic suppression may not be

393   appropriate for the physique characteristics within this cohort of athletes, as the RMR to FFM ratio

394   changes with anthropometrics such that there is a reduced ratio with an increased BM and FFM

395   (Cameron et al., 2002; Weinsier et al., 1992). The use of RMR measurements to diagnose LEA

396   should be used with caution and alongside other markers until Best Practice Methods for the

397   measurements of RMR are developed.

398

399   ***Conclusion***

400   In conclusion, the results of this study suggest that RMR and body composition estimates do not

401   significantly differ between Phase 1 and Phase 4 of the MC or with HC use in female athletes.

402   Accordingly, measurements of RMR and body composition via DXA can be compared in Phase 1

403   or Phase 4 of the MC, or varying HC approaches in female athletes, without variations in sex

404   hormones or shifts in fluid balance causing additional noise. Furthermore, female athletes do not

405   need to purposefully alter energy intake during Phase 1 or Phase 4 of the MC or with HC usage

406   to address changes in RMR and should continue to focus on matching energy intake with

407   nutritional goals and training/competition need. Finally, as subtle menstrual irregularities are

408   difficult to identify, tracking of MC phase for clinical or research activities should not assume that

409   an athlete is within a particular phase of the MC unless confirmed using Best Practice Guidelines

410   (Elliott-Sale et al., 2021).

411

Authorship

This study was designed by M.A. Kuikman, A.K.A. McKay, R. Harris, C. Minahan, and L.M. Burke. Data was analyzed and collected by M.A. Kuikman, A.K.A. McKay, E.S. Smith, R. McCormick, N. Tee, and L.M. Burke. The data interpretation and manuscript preparation were undertaken by M.A. Kuikman, A.K.A. McKay, R. Harris. K.J. Elliott-Sale, T. Stellingwerff, E.S. Smith, R. McCormick, N. Tee, C. Minahan, J. Skinner, K.E. Ackerman, L.M. Burke.

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Table 1 Baseline athlete characteristics with menstrual status with body mass, lean body mass, fat free mass and fat mass from first dual-energy X-ray absorptiometry scan.

	NC	HC
Age (yrs)	20.8±3.2	22.4±3.5
Menarche (yrs)	13.0±2.0	12.9±1.6
BMI (kg/m^2)	27.1±3.4	28.4±5.0
Body mass (kg)	70.8±8.1	79.1±14.2
Lean body mass (kg)	45.0±2.5	49.9±4.7
Fat free mass (kg)	47.6±2.6	52.8±4.9
Fat mass (kg)	23.2±7.2	26.6±10.9

Note. Data are presented as mean±SD. NC, naturally cycling athletes; HC, hormonal contraceptive users.

Table 2: Equations used to calculate RMR$_{ratio}$ and relative RMR, and low energy availability indicators with corresponding threshold to indicate a suppressed RMR or low energy availability

RMR equation		Threshold	LEA Indicator	Threshold
HB	RMR$_M$ ÷ ((655.1 + (9.563xBM) + (1.850xHt)) – (4.676xAge))	<0.90	Total cholesterol	>5.2 mmol/L
ABM in HB	RMR$_M$ ÷ ((655.1 + (9.563xABM) + (1.850xHt)) – (4.676xAge)	<0.90	LDL	>3.4 mmol/L
Cunningham$_{1980}$	RMR$_M$ ÷ (500 + (22xLBM))	<0.90	Cortisol	>620 nmol/L
Cunningham$_{1991}$	RMR$_M$ ÷ (370 + (21.6xFFM))	<0.92	T3 and IGF-1	Within or below lowest quartile*
Relative RMR	RMR$_M$ ÷ FFM	<30kcal·kgFFM^{-1}·day^{-1}	LEAF-Q Score	>8

Note. RMR, resting metabolic rate; RMR$_M$, Measured RMR; HB, Harris, Benedict; BM, body mass; Ht, height; ABM, adjusted body mass; LBM, lean body mass; FFM, fat free mass; LEA, low energy availability; LDL, low density lipoprotein; T3, triiodothyronine; IGF-1, insulin-like growth factor 1; LEAF-Q; Low Energy Availability in Females Questionnaire. *Using lab-specific age dependent range.

Table 3: Energy and macronutrient intake during the standardised diet period the day prior to testing during Phase 1, Phase 2, and Phase 4 of the menstrual cycle for naturally cycling athletes and hormonal contraceptive users.

	Phase 1		Phase 2		Phase 4		P		
	NC	HC	NC	HC	NC	HC	Phase	MS	Interaction
Energy (kcal)	1943±162	2108±315	1968±202	1997±183	1947±157	2030±162	0.527	0.215	0.286
Carbohydrate (g)	271±24	295±34	268±36	288±18	275±24	290±23	0.402	0.055	0.565
Carbohydrate (g/kg)	4.0±0.2	4.1±0.3	4.0±0.5	4.1±0.3	4.1±0.3	4.1±0.1	0.447	0.672	0.451
Protein (g)	83±9	93±17	86±11	86±10	83±7	87±6	0.581	0.181	0.221
Protein (g/kg)	1.2±0.1	1.3±0.2	1.3±0.1	1.2±0.1	1.2±0.1	1.2±0.1	0.555	0.668	0.191
Fat (g)	57±7	61±14	60±19	55±9	56±5	57±5	0.526	0.955	0.259
Fat (g/kg)	0.9±0.1	0.9±0.2	0.9±0.3	0.8±0.1	0.8±0.1	0.8±0.1	0.522	0.131	0.249

Note. Data are presented as mean±SD. NC, naturally cycling athletes; HC, hormonal contraceptive users; MS, menstrual status.

Table 4: Serum estradiol and progesterone concentrations during Phase 1, and Phase 4 of the menstrual cycle for naturally cycling athletes and hormonal contraceptive users.

	Phase 1		Phase 4	
	NC	HC	NC	HC
Estradiol (pg/mL)	26.3±3.9	57.1±58.9	159.1±63.0*	42.9±30.4
Progesterone (nmol/L)	1.4±0.6	1.7±1.9	43.0±37.9*	2.57±2.8

Note: Data are presented as mean±SD. *Indicates significant difference from Phase 1 and compared to HC users. NC, naturally cycling athletes; HC, hormonal contraceptive users

Table 5. Resting metabolic rate ratio (measured:predicted) calculated with the Harris Benedict, Cunningham 1980 and Cunningham 1991 equation during Phase 1, and Phase 4 of the menstrual cycle for naturally cycling athletes and hormonal contraceptive users.

	Phase 1		Phase 4	
	NC	HC	NC	HC
Harris Benedict	1.05±0.04	1.08±0.11	1.05±0.06	1.07±0.08
Harris Benedict with ABM (*n=3 NC/5HC*)	1.09±0.06	1.11±0.07	1.08±0.03	1.18±0.03
Cuningham$_{1980}$	1.09±0.07	1.08±0.07	1.08±0.08	1.08±0.08
Cunningham$_{1991}$	1.16±0.07	1.15±0.08	1.15±0.09	1.14±0.09

Note: Data are presented as mean±SD. NC, naturally cycling athletes; HC, hormonal contraceptive users; ABM, adjusted body mass.

Figure 1: Overview of experimental protocol with measurements occurring during Phase 1, Phase 2, and Phase 4 of the menstrual cycle in naturally cycling athletes (A) and measurements occurring during three spaced occasions for hormonal contraceptive users. For combined-monophasic oral contraceptive users, testing occurred on active pill-taking days. For all other hormonal contraceptive users (injection and implant), testing occurred at any given time (B).

Figure 2: Absolute resting metabolic rate (A), and relative resting metabolic rate (D) with menstrual cycle phase and hormonal contraceptive usage. Data shown as mean with individual data points.

Figure 3: Fat mass estimates (A) fat free mass estimates (B), and lean body mass estimates (D) with menstrual cycle phase and hormonal contraceptive usage. Data shown as mean with individual data points. *Indicates significant difference between groups.

Figure 4: Repeated measures correlation between relative resting metabolic rate (RMR) and serum estradiol concentrations (A), serum progesterone concentrations (B) and the concentration of estradiol to progesterone ratio (C). Each coloured line represents an individual naturally cycling athlete with Phase 1, Phase 2, and Phase 4 measurements (n=11).

Figure 5: Number of athletes who presented with a suppressed RMR or menstrual irregularity across the training camp, and indicators of low energy availability within each cohort. *Note.* HC, hormonal contraceptive; NC, naturally cycling; RMR, resting metabolic rate; LEAF-Q, the Low Energy Availability in Females Questionnaire; TC, total cholesterol; LDL, low-density lipoprotein; T3, triiodothyronine