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

Megan A Kuikman[1], Alannah KA McKay[1], Clare Minahan[2], Rachel Harris[3,4], Kirsty J Elliott-Sale[5], Trent Stellingwerff[6,7], Ella S Smith[1], Rachel McCormick[1], Nicolin Tee[1], Jessica Skinner[8], Kathryn E Ackerman[9], Louise M Burke[1]

[1]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Victoria, Australia
[2]Griffith Sports Science, Griffith University, Gold Coast, QLD, Australia
[3]Female Performance and Health Initiative, Australian Institute of Sport, Canberra, ACT Australia
[4]Perth Orthopaedic and Sports Medicine Research Institute, West Perth, WA, Australia
[5]Department of Sport and Exercise Sciences, Institute of Sport, Manchester Metropolitan University, Manchester, UK.
[6]Canadian Sport Institute-Pacific, Pacific Institute for Sport Excellence, Victoria, BC, Canada
[7]Exercise Science, Physical and Health Education, University of Victoria, Victoria, BC, Canada
[8]National Rugby League, Sydney, NSW, Australia
[9]Female Athlete Program, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA

Running Head: Resting metabolic rate and menstrual cycle phase.

Address for correspondence
Megan Kuikman, Mary MacKillop Institute for Health Research,
Australian Catholic University, Melbourne, Victoria, Australia 3000
Email: Megan.Kuikman@acu.edu.au

Abstract (250/250)

The cyclical changes in sex hormones across the menstrual cycle (MC) are associated with various biological changes that may alter resting metabolic rate (RMR) and body composition estimates. Hormonal contraceptive (HC) use must also be considered given their impact on endogenous sex hormone concentrations and synchronous exogenous profiles. The purpose of this study was to determine if RMR and dual-energy X-ray absorptiometry (DXA) body composition estimates change across the MC and differ compared to HC users. This was accomplished during a 5-week training camp involving naturally cycling (NC) athletes (n=11) and HC users (n= 7 subdermal progestin implant, n= 4 combined-monophasic oral contraceptive pill, n=1 injection) from the National Rugby League Indigenous Women's Academy. MC phase was retrospectively confirmed via serum estradiol and progesterone concentrations and a positive ovulation test. HC users had serum estradiol and progesterone concentrations assessed at the time point of testing. Results were analyzed using general linear mixed model. There was no effect of MC phase on absolute RMR ($p=0.877$), relative RMR ($p=0.957$), or DXA body composition estimates ($p>0.05$). There was no effect of HC use on absolute RMR ($p=0.069$), relative RMR ($p=0.679$), or fat mass estimates ($p=0.766$), but HC users had a greater FFM and LBM than NC athletes ($p=0.044$). Our findings suggest that RMR and DXA body composition estimates do not significantly differ due to changes in sex hormones in a group of athletes, and measurements can be compared between MC phases or with HC usage without variations in sex hormones causing additional noise.

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

et al., 2018; McNamara et al., 2022; Oxfeldt et al., 2020) and their associated effects on endogenous sex hormone concentrations and synchronous exogenous profiles (Elliott et al., 2005; Hirschberg, 2022), the effects of HC use on RMR should also be assessed.

Although the practical relevance of meaningful differences in RMR on energy requirements of athletes needs to be considered, another scenario for the measurement of RMR in sports nutrition involves its potential use as a screening tool for metabolic suppression in response to low energy availability (LEA) (Sterringer & Larson-Meyer, 2022). This is done either by determining the ratio of measured RMR against a value predicted by an equation (Schofield et al., 2019), or by expressing measured RMR relative to fat free mass (FFM) (Loucks et al., 2011). Although the RMR ratio (measured:predicted) appears to have some utility in identifying female athletes with indices of metabolic suppression (Strock et al., 2020), it has not been recognized as a primary or secondary indicator of Relative Energy Deficiency in Sport (REDs) in the IOC REDs Clinical Assessment Tool - Version 2 (CAT2) due to current concern around its specificity and sensitivity (Stellingwerff et al., 2023). Part of this concern relates to known technical and biological variability in RMR measurements (Siedler et al., 2023), the latter of which could include the effects of endogenous and exogenous sex hormone concentrations. Better understanding of this variability might help to improve the interpretation of RMR measurements and their use as a diagnostic tool. Accordingly, the aim of this study was to investigate the effects of MC phase and HC usage on RMR in a cohort of female athletes using Best Practice Guidelines for the control of ovarian hormones (Elliott-Sale et al., 2021). Additionally, we measured changes in body composition estimates using dual-energy X-ray absorptiometry (DXA) across MC phase and with HC usage as DXA scans are often performed alongside RMR measurements to interpret findings.

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

information was used to prospectively plan testing dates for three physiologically-specific MC phases (Elliott-Sale et al., 2021) during the training camp (see Figure 1): 1) Phase 1: begins at the onset of bleeding, when estrogen and progesterone concentrations are low; 2) Phase 2: 14-26 hours prior to ovulation and the LH surge, when estrogen concentrations are at their highest and progesterone concentrations remain low; and 3) Phase 4: Seven days after ovulation when progesterone concentrations are at their highest and estrogen concentration are also elevated. To anticipate the day of Phase 2 during the training camp, NC athletes used dual hormone ovulation kits (Advanced Digital Ovulation Test, Clearblue, Geneva, Switzerland) with a rise in estrogen prior to a rise in LH being identified with a "flashing smile." Venous blood samples were taken on the day of testing, and concentrations of estrogen and progesterone were retrospectively used to confirm that testing occurred at the correct MC phase. HC users were tested on three occasions spaced by 7-10 days (also referred to as "Phase 1", "Phase 2", and "Phase 4"). COC users were tested on active pill-taking days to avoid the withdrawal bleed and athletes taking all other types of HC (implant and injection) were tested at any time given the continuous nature of these contraceptives. HC users also had serum estradiol and progesterone concentrations at the time point of testing established. Further details regarding the MC tracking can be found elsewhere (McKay et al., 2023).

-Figure 1-

Dietary control

As confirmation of MC phase was dependent on menstrual reporting that occurred mid-morning, a standardized diet was implemented from lunch onwards to prepare for the next day's laboratory testing. Thus, participants consumed an *ad libitum* breakfast, with the controlled diet thereafter providing 80% of their estimated requirements, which were set as 5 g·kg body mass (BM)$^{\cdot -1}$day^{-1}

of carbohydrate, 1.5 g·kg BM$^{\cdot -1}$day^{-1} or protein, and 1 g·kg BM$^{\cdot -1}$day^{-1} as fat. For athletes with a body mass index (BMI) >110% of 25.0 kg/m^2 (n=3 NC athletes, n=5 HC users), an adjusted BM (ABM) was used to calculate dietary needs to prevent excessive energy intake (Krenitsky, 2005). ABM was calculated as: (actual BM – ideal BM) x 0.25 + ideal BM with ideal BM representing a BM that would equate to a BMI of 25.0 kg/m^2. Diets were designed by a dietitian and prepared by professional chef with all meals being weighed and provided in a dining hall setting. The dining hall had a rotating 2-week menu, so athletes did not necessarily receive the same food during each standardized diet period that preceded testing. Meals were supervised by a member of the research team, but snacks were consumed throughout the day without supervision. Athletes self-reported any deviations from standardized diets, and this was accounted for when analysing standardized diets.

Measurements

Body composition

On test mornings, athletes arrived at the laboratory in an overnight fasted and rested state such that no food or fluid was consumed, or exercise was performed prior to testing. DXA was used to assess body composition, using the Best Practice Protocols of the Australian Institute of Sport involving standardized positioning (Slater et al., 2023). Athlete scans were performed in the same mode (GE Lunar iDXA) and analyzed using GE encore by the same trained researcher to assess FFM, lean body mass (LBM), and fat mass (FM). Data from the first and last DXA scan were used to assess changes in body composition over the training camp.

Resting metabolic rate

RMR was determined either immediately before or after the DXA scan. As such, athletes were overnight fasted and rested. A protocol of rest and familiarisation was used, which produces comparable results to RMR measured in an inpatient setting and has good interday reliability (ICC 0.87; Typical error as CV 5.9%)(Bone & Burke, 2018). With this protocol, athletes first rested on a bed in a dark and quiet room for 10 minutes before being given a one-way mouthpiece (bite size rubber mouthpiece connected to silicone diaphragm that allows flow in one direction only through a valve) for a 15-minute familiarisation period. Athletes were instructed not to fall asleep during the measurement, and this was verbally confirmed following the measurement. The room was temperature controlled, and athletes had access to a blanket during the measurement so that they were at a comfortable temperature. Following the rest and familiarisation period, expired air was collected into a gas-impermeable Douglas bag for 10 minutes for two consecutive data collection periods. Ametek Oxygen (O_2) and carbon dioxide (CO_2) analyzers (VacuMed, Ventura, CA) were calibrated with two known gas concentrations (14.38% O_2,2.510% CO_2; and 16.30% O_2, 4.173% CO_2) before use. The expirate from each bag was sampled for one minute with the gas sampling time and flow rate being recorded. The volume of the remaining expirate was then determined using a Tissot spirometer via an evacuation pump. RMR results were reported as absolute over 24 hours ($kcal \cdot day^{-1}$) for each Douglas bag and then a mean RMR was computed from the two Douglas bags. RMR results were also reported relative to FFM ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$). It was not possible to control pre-trial exercise since scheduled team training took place each afternoon during the camp, but >12 hours separated training and laboratory testing. Training sessions included gym or fielding sessions with duration and rating of perceived exertion (RPE) being used to calculate training load (RPE x duration).

Indicators of low energy availability

At each testing time point across the camp, a RMR ratio (measured:predicted) was calculated using three RMR predictive equations as outlined in Table 2 (Cunningham, 1982, 1991; Harris & Benedict, 1918). These predictive equations were selected as they have validated thresholds to indicate energy deficiency with an athlete being classified as having a suppressed RMR if the ratio fell below this threshold (see Table 2)(Strock et al., 2020), and/or presented with a relative RMR <30 kcal·kg FFM^{-1}·day^{-1} (Loucks et al., 2011). Alongside screening for a suppressed RMR, blood indicators of LEA as per the updated IOC REDs CAT2 were measured (Stellingwerff et al., 2023) and athletes completed the Low Energy Availability in Females Questionnaire (LEAF-Q) prior to commencing the study (Melin et al., 2014).

-Table 2-

Blood samples

Following the DXA scan and RMR measurement, an 8.5 mL venous blood sample was collected from an antecubital vein into a serum separator tube by a trained phlebotomist. Blood tubes were left to clot at room temperature for 30 minutes, prior to centrifugation at 2200 G for 10 minutes at 4 °C. Remaining serum was split into aliquots and stored at -80 °C until batch analysis could occur. Estradiol and progesterone were measured via an Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA) and used to retrospectively confirm MC phase and to establish hormonal profiles for HC users. Intra-assay coefficient of variations were 5% for estradiol and 11% for progesterone. Lipids, cortisol, insulin-like growth factor 1 (IGF-1) and triiodothyronine (T3) were measured by chemiluminescent immunoassay through a commercial laboratory (Laverty Pathology, Bruce, ACT, Australia).

Statistics

Statistical analyses were performed using R Studio (v3.5.2) with statistical significance accepted at an α level of $p \leq 0.05$ using general linear mixed models. Fixed effects for the model included MC phase and menstrual status (NC athletes or HC users). Subject ID and test order were included as random effects within the models. The initial model included all possible interactions, with non-significant interactions being dropped. Statistical significance of the fixed effect was determined using a Type II Wald tests with Kenward-Roger degrees of freedom, and where significant fixed effects were evident, a Tukey's post-hoc comparison was used to identify where differences exist. For NC athletes only, a repeated measures correlation was used to assess the relationship between changes in relative RMR and serum concentrations of estradiol, progesterone, and the ratio of estradiol to progesterone. Non-normally distributed data (absolute RMR, FM, estradiol levels, and progesterone levels) were log-transformed for statistical analyses.

Results

Assessment of actual intake of the ~18 hour standardized diet showed no difference between trials or groups for energy and macronutrient intake ($p>0.05$) with actual intake achieving the standardized diet targets (Table 3). Over the training camp, FM was reduced in both NC athletes and HC users (~0.5 kg; $p=0.0001$). FFM increased in HC users (+0.9 kg; $p<0.0001$), but not in NC athletes (+0.2 kg; $p=0.770$). There was no difference in training load the day prior to testing with MC phase ($p=0.331$), but NC athletes had a greater training load than HC users (455±308 AU vs. 287±325 AU; $p=0.042$).

-Table 3

Comprehensive results of menstrual status confirmation from ovulation testing and retrospective analysis of serum estradiol and progesterone concentrations have been previously reported elsewhere (McKay et al., 2023). In summary, five NC athletes were identified as having a menstrual irregularity during the training camp (n=3 oligomenorrheic, n=1 anovulatory, n=1 luteal phase deficiency). Of the remaining 6 NC athletes, only one presented with the expected Phase 2 hormonal profile. Thus, Phase 2 metrics were not analyzed due to the measurement variability. Furthermore, Phase 1 versus Phase 4 analysis excluded data due to an insufficient rise in serum progesterone concentrations from Phase 1 to Phase 4 of the MC (n= 3 NC athlete), technical issues with RMR measurements (n=1 NC athlete, n= 1 HC user), and an erroneous serum estradiol measurement (>3SD above mean, n=1 HC user). For the remaining athletes, as expected, there was an increase in serum estradiol and progesterone concentrations from Phase 1 to Phase 4 in NC athletes (estradiol, $p=0.0003$; progesterone, $p<0.0001$), but not in HC users (estradiol, $p=0.967$; progesterone, $p=0.323$; Table 4).

-Table 4-

There was no effect of MC phase ($p=0.877$) or HC usage ($p=0.069$) on absolute RMR nor was there an effect of MC phase ($p=0.957$) or HC usage ($p=0.679$) on relative RMR (Figure 2).

-Figure 2-

There was no effect of MC phase ($p=0.118$) or HC usage ($p=0.766$) on FM estimates. While there was also no effect of MC phase on FFM estimates ($p=0.225$) and LBM estimates ($p=0.248$), HC users had a greater FFM ($p=0.028$) and LBM ($p=0.028$) than NC athletes (Figure 3).

-Figure 3-

There was no within athlete correlation between changes in relative RMR and estradiol concentrations ($r=0.31$, $p=0.179$); progesterone concentrations ($r=0.06$, $p=0.805$), or the ratio of

concentrations of estradiol to progesterone (r=0.11, p=0.640) from Phase 1, Phase 2, and Phase 4 of the MC in NC athletes (Figure 4).

-Figure 4-

There was no effect of MC phase on the RMR ratio calculated from the Harris Benedict (HB) equation using actual BM (p=0.958) or ABM (p=0.141), $Cunningham_{1980}$ equation (p=0.865), or $Cunningham_{1991}$ equation (p=0.831) nor was there an effect of HC usage on the RMR ratio calculated from the HB equation using actual BM (p=0.398), $Cunningham_{1980}$ equation (p=0.911), or $Cunningham_{1991}$ equation (p=0.714; Table 5). However, the RMR ratio calculated from the HB equation using ABM was greater in HC athletes than NC athletes (p=0.020).

-Table 5-

At the first testing measurement, no athlete presented with a suppressed RMR. However, at the second and/or third test, six athletes presented with a relative RMR <30 kcal·kg FFM^{-1}·day^{-1} and one of these athletes also presented with a RMR ratio <0.90 when using the HB equation (Figure 5). No athlete presented with an RMR ratio considered suppressed when using the $Cunningham_{1980}$ or $Cunningham_{1991}$ equation or when using ABM in the HB equation.

-Figure 5-

Discussion

Our results showed that assessments of RMR and DXA-derived body composition estimates did not differ due to changes in ovarian hormones in a group of female athletes. Specifically, there were no systematic differences in RMR and DXA-derived body composition between Phase 1 and Phase 4 of the MC or with HC usage in female athletes, nor any correlation with changes in absolute or ratios of concentrations of serum estradiol and progesterone. These findings have implications for the dietary recommendations given to female athletes and the Best Practice

Protocols for the measurement of RMR and body composition. Specifically, our results suggest female athletes do not need to alter their energy requirements due changes in RMR across MC phase or with HC use, and that MC phase and HC use does not need to be standardized when measuring RMR.

Previous studies have provided some evidence for a modulation of RMR due to alterations in estrogen concentrations. For example, the suppression of serum estrogen and progesterone to postmenopausal concentrations in premenopausal women was associated with a ~40 kcal/day reduction (~3%) in RMR compared to the follicular phase and ~70 kcal/day (~5%) compared to the luteal phase (Day et al., 2005). Furthermore, this reduction in RMR was prevented with concurrent transdermal estrogen administration that maintained serum estrogen concentrations to that expected in the mid to late follicular phase of the MC (Melanson et al., 2015). Such effects of estrogen increasing RMR have been attributed to estrogen increasing brown adipose tissue (BAT) activity both directly by acting on BAT and through its effect on the ventromedial hypothalamus nucleus and sympathetic nervous system signalling (Gavin et al., 2018; Vigil et al., 2022). In the current study, despite the large increase in serum estradiol concentrations during Phase 4 of the MC in NC athletes (Table 4), we did not show an effect of MC phase or HC usage on RMR. Furthermore, a repeated measures correlation did not show any association between RMR and estradiol concentrations. These findings suggest that estrogen did not achieve a significant modulation of RMR in our cohort of female athletes.

Several explanations might underpin the discrepancies between our results and other studies showing an increase in RMR with elevated estrogen concentrations. Firstly, any variation in RMR

due to sex hormones may be overshadowed by other factors that contribute to the 3-5% day-to-day variability in measured RMR (Compher et al., 2006). Although precautions were taken in this study to minimize factors that may contribute to technical error and variability in RMR (e.g., standardizing dietary intake the day prior to testing), consideration should be given to the magnitude of change in RMR across the MC reported in previous studies. For instance, a female athlete with an absolute RMR of 1200-1800 calories/day would require a change >60-90 calories/day to exceed a 5% day-to-day variation in RMR. Yet, of the seventeen studies in the 2020 meta-analysis that undertook testing in a fasted and rested state and provided RMR results, the mean change in RMR was ~45 calories/day, with only five studies reporting an increase >60 calories/day above the follicular phase (Benton et al., 2020). Since the publication of this meta-analysis, only one study could be located that found differences in RMR between MC phases with RMR being 37 calories/day lower in the follicular compared to the luteal phase of the MC (Malo-Vintimilla et al., 2023). However, ovulation was not confirmed, and nine of the nineteen participants had serum progesterone concentrations <5 ng/mL in the luteal phase, which suggests anovulatory cycles (Malo-Vintimilla et al., 2023). Furthermore, these studies were not conducted specifically in athletic cohorts whom may have a greater prevalence of menstrual disturbances (De Souza et al., 2010) and a greater exercise energy expenditure and energy intake resulting in a greater energy flux compared to sedentary individuals (Bullough et al., 1995; Goran et al., 1994; Paris et al., 2016). Interestingly, an increased RMR seen during periods of high energy flux, and with elevated serum estrogen and progesterone concentrations are both thought to occur via beta-adrenergic support of RMR (Bell et al., 2004; Day et al., 2005). Modulations in RMR due to energy flux and sex hormones may not be additive, resulting in changes in RMR across the MC in sedentary females, but not trained female athletes. Furthermore, variations in training or

competition prior to RMR measurements may contribute to greater variability in the RMR measurements of female athletes compared to sedentary women.

A further reason for discrepancy between our results and previous studies that have demonstrated changes in RMR across MC phase might be attributed to differences in methodology used to identify MC phase. Few studies assessing changes in RMR across the MC have confirmed ovulation and measured serum concentrations of estradiol and progesterone, which could result in measurements that are occurring unknowingly in an incorrect phase and hormonal profile. For instance, a common strategy is to assume that ovulation occurred half-way through the MC without confirming an LH surge has occurred despite follicular phase length being variable (Mihm et al., 2011). If ovulation is assumed to occur at day 14 of the MC, but a woman has an extended follicular phase, measurements that are thought to have occurred in the luteal phase could actually have occurred in the late follicular phase of the MC. This is problematic as these phases have different hormonal profiles (Elliott-Sale et al., 2021). This technique also does not consider anovulatory cycles or luteal phase defects, which will result in an incorrect hormonal profile during the luteal phase of the MC (De Souza, 2003). Evidently, when assessing changes across MC phase it is vital to follow the Best Practice Protocols for control of ovarian hormones (Elliott-Sale et al., 2021). It should be noted that while a strength of our study was following the Best Practice Protocols for ovarian hormone control, this contributed to a small sample size, which is a notable limitation of our study.

The effect of hormonal profiles seen in Phase 2 of the MC on RMR could not be assessed in this study. Because this phase lasts only 12-26 hours (Elliott-Sale et al., 2021), may be absent if the

athlete has an anovulatory cycle, and requires resources to ascertain its presence (i.e. blood sample analysis to determine estrogen and progesterone concentrations, measuring for a LH surge etc), there is little utility in considering its effect on nutrition recommendations for female athletes. However, since Phase 2 of the MC provides an opportunity to investigate the effects of estrogen on RMR with minimal progesterone (Elliott-Sale et al., 2021), this would provide mechanistic insight into the control of RMR. Furthermore, the identification of any meaningful noise in RMR measurement could be taken into account when Best Practice Protocols for RMR assessment in athletic cohorts are established. Therefore, future studies are needed to assess if RMR changes across Phase 2 of the MC, so that the need to control for this phase of the MC can be determined.

There are multiple forms of HC with unique effects on the endogenous hormonal milieu (Elliott-Sale et al., 2021). Among our study cohort, 3 different types of HC (COC, injection and implant) were used, with a further differentiation in the brands of COC (4 brands). The hormonal profile of HC users established in this study demonstrated variations in sex hormones across testing time point with a ~20% increase in estradiol concentrations and ~70% increase in progesterone concentrations from “Phase 1” to “Phase 4”(Table 4). Notably, endogenous concentrations of estradiol and progesterone were measured, and exogenous sex hormones may have higher receptor affinity that exceed the effects of endogenous sex hormones (Hirschberg, 2022). Additionally, such changes in sex hormones for HC users were small in comparison to the large increases in concentrations of estradiol (~500%) and progesterone (~2900%) seen from Phase 1 to Phase 4 in NC athletes. HC usage had no effect on RMR such that RMR did not differ between NC athletes and HC users or between testing time points in HC users. This is in agreement with the majority of studies comparing the RMR of COC users and non-users (Duhita et al., 2017, 2019; Eck et al.,

1997; Jensen & Levine, 1998), although one study reported a higher RMR in COC users when FFM and FM were included as covariates (Diffey et al., 1997). Like COC, most studies examining the effects of depot-medroxyprogesterone acetate injection on RMR have found no effect on RMR (Pelkman et al., 2001; Steward et al., 2016), and reports of changes in RMR with usage are likely secondary to changes in body composition (Batista et al., 2017). Notably, seven athletes in our cohort used the hormonal implant, Implanon, which is a single-rod progestin-only implant that is inserted in the upper arm for up to three years (Fischer, 2008). No study could be located assessing the effect of Implanon on RMR, which likely reflects the relatively new approval of this HC and usage trends compared to other HC (King et al., 2021). Overall, our study suggests that HC usage has no effect on RMR. However, a limitation of this study was the lack of homogeneity with the type of HC used within our cohort leading to an increased variability in endogenous and exogenous hormonal profiles. As such, further studies are needed to assess the effect of HC usage on RMR.

Estimates of total body composition using DXA depend on the assumption of a constant lean soft tissue hydration (Pietrobelli et al., 1996), but this will vary with extracellular and intracellular fluid distribution (St-Onge et al., 2004). Variations in sex hormones with MC phase or HC usage may introduce a source of error in DXA body composition estimates as estrogen and progesterone cause a shift in osmoregulation that may alter water distribution within the extracellular fluid space (Stachenfeld, 2008). Differences in FM estimates by DXA scan have been reported in the early versus mid-follicular phase (~0.30 kg, ~1.6% change)(Gould et al., 2021) and the late follicular phase compared with the early follicular phase (~0.31 kg, ~1.9%) and mid-luteal phase (~0.35 kg, ~2.1%)(Thompson et al., 2021). However, this is not a consistent finding with others reporting no difference between the mid-luteal and mid-follicular MC phase (Jürimäe et al., 2011; Koşar et al.,

2022; Ong et al., 2022). Additionally, for studies that have reported differences across MC phase, this difference was below the ~4.7% least significant change (LSC) in consecutive day precision error for whole body FM DXA estimates (Slater et al., 2023). Differences in DXA body composition estimates have also been reported across the oral contraceptive pill (OCP) cycle of monophasic COC users with lower LBM estimates during the early hormone phase compared to the non-active pill phase (~0.29 kg, ~0.7% change) and late pill phase (~0.34 kg, ~0.9% change) (Thompson et al., 2021). We were unable to assess change across the OCP cycle of the four COC users as all purposefully manipulated their cycles to avoid a withdrawal bleed during the camp (McKay et al., 2023). However, like FM estimates across MC phase, differences in DXA body composition previously reported across the OCP cycle were below the ~1.4% LSC in consecutive day precision error for LBM DXA estimates (Slater et al., 2023). Overall, our findings suggest that any underlying shifts in fluid balance due to changing sex hormones with MC phase and HC usage are not sufficient to produce meaningful change in DXA body composition estimates (Figure 3). Practically, this suggests researchers and practitioners can measure total body composition via a DXA scan in Phase 1 or Phase 4 of the MC without sex hormones creating additional noise.

Subtle menstrual disturbances were identified in five NC athletes within this cohort (Figure 5), and it is possible that this was due to LEA exposure (De Souza, 2003). While a suppressed RMR has been demonstrated in female athletes with menstrual disturbances (Koehler et al., 2016; Melin et al., 2015; Myerson et al., 1991; Strock et al., 2020), none of the athletes with menstrual irregularity during the training camp presented with an RMR measurement that met any of the criteria suggesting RMR suppression. This suggests either that the menstrual issues observed were not underpinned by exposure to LEA or that RMR assessment does not provide a universal tool to

diagnose metabolic suppression. Of the six athletes who did present with a suppressed RMR measurement across the training camp, only two also presented with an indicator of LEA. Furthermore, for five of the six athletes, this suppressed RMR was based solely on a relative RMR <30 kcal·kgFFM^{-1}·day^{-1}. The use of relative RMR to indicate metabolic suppression may not be appropriate for the physique characteristics within this cohort of athletes, as the RMR to FFM ratio changes with anthropometrics such that there is a reduced ratio with an increased BM and FFM (Cameron et al., 2002; Weinsier et al., 1992). The use of RMR measurements to diagnose LEA should be used with caution and alongside other markers until Best Practice Methods for the measurements of RMR are developed.

Conclusion

In conclusion, the results of this study suggest that RMR and body composition estimates do not significantly differ between Phase 1 and Phase 4 of the MC or with HC use in female athletes. Accordingly, measurements of RMR and body composition via DXA can be compared in Phase 1 or Phase 4 of the MC, or varying HC approaches in female athletes, without variations in sex hormones or shifts in fluid balance causing additional noise. Furthermore, female athletes do not need to purposefully alter energy intake during Phase 1 or Phase 4 of the MC or with HC usage to address changes in RMR and should continue to focus on matching energy intake with nutritional goals and training/competition need. Finally, as subtle menstrual irregularities are difficult to identify, tracking of MC phase for clinical or research activities should not assume that an athlete is within a particular phase of the MC unless confirmed using Best Practice Guidelines (Elliott-Sale et al., 2021).

412 **Authorship**

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

418 **Funding:** This study was embedded within the Female Athlete Research Camp, and was co-
419 funded by Australian Catholic University, Wu Tsai Human Performance Alliance and the
420 Australian Institute of Sport Female Performance and Health Initiative.

421 **Conflict of interest:** The authors declare no conflict of interests.

References

Batista, G. A., de Souza, A. L., Marin, D. M., Sider, M., Melhado, V. C., Fernandes, A. M., & Alegre, S. M. (2017). Body composition, resting energy expenditure and inflammatory markers: Impact in users of depot medroxyprogesterone acetate after 12 months follow-up. *Archives of Endocrinology and Metabolism*, *61*(1), 70–75. https://doi.org/10.1590/2359-3997000000202

Bell, C., Day, D. S., Jones, P. P., Christou, D. D., Petitt, D. S., Osterberg, K., Melby, C. L., & Seals, D. R. (2004). High energy flux mediates the tonically augmented β-adrenergic support of resting metabolic rate in habitually exercising older adults. *Journal of Clinical Endocrinology and Metabolism*, *89*(7), 3573–3578. https://doi.org/10.1210/jc.2003-032146

Benton, M. J., Hutchins, A. M., & Dawes, J. J. (2020). Effect of menstrual cycle on resting metabolism: A systematic review and meta-analysis. *PLoS ONE*, *15*(7), e0236025. https://doi.org/10.1371/JOURNAL.PONE.0236025

Bone, J. L., & Burke, L. M. (2018). No difference in young adult athletes' resting energy expenditure when measured under inpatient or outpatient conditions. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(5), 464–467. https://doi.org/10.1123/ijsnem.2016-0315

Bullough, R. C., Gillette, C. A., Harris, M. A., & Melby, C. L. (1995). Interaction of acute changes in exercise energy expenditure and energy intake on resting metabolic rate. *American Journal of Clinical Nutrition*, *61*(3), 473–481. https://doi.org/10.1093/ajcn/61.3.473

Cameron, V. A., Autelitano, D. J., Evans, J. J., Ellmers, L. J., Espiner, E. A., Gary Nicholls, M., & Mark Richards, A. (2002). Body-size dependence of resting energy expenditure can be attributed to nonenergetic homogeneity of fat-free mass. *American Journal of Physiology - Endocrinology and Metabolism*, *282*(1), E132-138. https://doi.org/10.1152/AJPENDO.2002.282.1.E132/ASSET/IMAGES/LARGE/H10120627008.JPEG

Compher, C., Frankenfield, D., Keim, N., & Roth-Yousey, L. (2006). Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *Journal of the American Dietetic Association*, *106*(6), 881–903. https://doi.org/10.1016/J.JADA.2006.02.009

Costello, J. T., Bieuzen, F., & Bleakley, C. M. (2014). Where are all the female participants in Sports and Exercise Medicine research? *European Journal of Sport Science*, *14*(8), 847–851. https://doi.org/10.1080/17461391.2014.911354

Cunningham, J. (1982). Body composition and resting metabolic rate: The myth of feminine metabolism. *American Journal of Clinical Nutrition*, *36*(4), 721–726. https://doi.org/10.1093/ajcn/36.4.721

Cunningham, J. (1991). Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. *Am J Clin Nutr*, *54*(6), 963–969.

Day, D. S., Gozansky, W. S., Van Pelt, R. E., Schwartz, R. S., & Kohrt, W. M. (2005). Sex hormone suppression reduces resting energy expenditure and β-adrenergic support of resting energy expenditure. *Journal of Clinical Endocrinology and Metabolism*, *90*(6), 3312–3317. https://doi.org/10.1210/JC.2004-1344

De Souza, M. J. (2003). Menstrual disturbances in athletes: A focus on luteal phase defects. *Medicine and Science in Sports and Exercise*, *35*(9), 1553–1563. https://doi.org/10.1249/01.MSS.0000084530.31478.DF

De Souza, M. J., Toombs, R. J., Scheid, J. L., O'Donnell, E., West, S. L., & Williams, N. I. (2010). High prevalence of subtle and severe menstrual disturbances in exercising women: Confirmation using daily hormone measures. *Human Reproduction*, *25*(2), 491–503. https://doi.org/10.1093/humrep/dep411

Diffey, B., Piers, L. S., Soares, M. J., & O'dea, K. (1997). The effect of oral contraceptive agents on the basal metabolic rate of young women. *British Journal of Nutrition*, *77*(6), 853–862. https://doi.org/10.1079/bjn19970084

Duhita, M. R., Schutz, Y., Montani, J. P., Dulloo, A. G., & Miles-Chan, J. L. (2017). Oral contraceptive pill alters acute dietary protein-induced thermogenesis in young women. *Obesity*, *25*(9), 1482–1485. https://doi.org/10.1002/OBY.21919

Duhita, M. R., Schutz, Y., Montani, J. P., Dulloo, A. G., & Miles-Chan, J. L. (2019). Assessment of the dose-response relationship between meal protein content and postprandial thermogenesis: Effect of sex and the oral contraceptive pill. *Nutrients*, *11*(7), 1599. https://doi.org/10.3390/NU11071599

Eck, L. H., Bennett, A. G., Egan, B. M., Ray, J. W., Mitchell, C. O., Smith, M. A., & Klesges, R. C. (1997). Differences in macronutrient selections in users and nonusers of an oral contraceptive. *The American Journal of Clinical Nutrition*, *65*(2), 419–424. https://doi.org/10.1093/AJCN/65.2.419

Elliott, K. J., Cable, N. T., & Reilly, T. (2005). Does oral contraceptive use affect maximum force production in women? *British Journal of Sports Medicine*, *39*(1), 15–19. https://doi.org/10.1136/bjsm.2003.009886

Elliott-Sale, K. J., Minahan, C. L., Janse de Jonge, X. A. K., Ackerman, K. E., Sipila, S., Constantini, N. W., Lebrun, C. M., & Hackney, A. C. (2021). Methodological considerations for studies in sport and exercise science with women as participants: A working guide for standards of practice for research on women. *Sports Medicine*, *51*(5), 843–861. https://doi.org/10.1007/s40279-021-01435-8

Fischer, M. A. (2008). Implanon: A new contraceptive implant. *JOGNN - Journal of Obstetric, Gynecologic, and Neonatal Nursing*, *37*(3), 361–368. https://doi.org/10.1111/j.1552-6909.2008.00247.x

Gavin, K. M., Kohrt, W. M., Klemm, D. J., & Melanson, E. L. (2018). Modulation of energy expenditure by estrogens and exercise in women. *Exercise and Sport Sciences Reviews*, *46*(4), 232. https://doi.org/10.1249/JES.0000000000000160

Goran, M. I., Calles-Escandon, J., Poehlman, E. T., O'Connell, M., & Danforth, E. (1994). Effects of increased energy intake and/or physical activity on energy expenditure in young healthy men. *Journal of Applied Physiology*, *77*(1), 366–372. https://doi.org/10.1152/jappl.1994.77.1.366

Gould, L. M., Cabre, H. E., Brewer, G. J., Hirsch, K. R., Blue, M. N. M., & Smith-Ryan, A. E. (2021). Impact of follicular menstrual phase on body composition measures and resting metabolism. *Medicine and Science in Sports and Exercise*, *53*(11), 2396–2404. https://doi.org/10.1249/MSS.0000000000002702

Harris, J. A., & Benedict, F. G. (1918). A biometric study of human basal metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, *4*(12), 370–373. https://doi.org/10.1073/PNAS.4.12.370

Hirschberg, A. L. (2022). Challenging aspects of research on the influence of the menstrual cycle and oral contraceptives on physical performance. *Sports Medicine*, *52*(7), 1453–1456. https://doi.org/10.1007/s40279-021-01616-5

Hulbert, A. J., & Else, P. L. (2004). Basal metabolic rate: History, composition, regulation, and usefulness. *Physiological and Biochemical Zoology*, *77*(6), 869–876.

Jensen, M. D., & Levine, J. (1998). Effects of oral contraceptives on free fatty acid metabolism in women. *Metabolism: Clinical and Experimental*, *47*(3), 280–284. https://doi.org/10.1016/S0026-0495(98)90257-8

Jürimäe, J., Vaiksaar, S., Mäestu, J., Purge, P., & Jürimäe, T. (2011). Adiponectin and bone metabolism markers in female rowers: Eumenorrheic and oral contraceptive users. *Journal of Endocrinological Investigation*, *34*(11), 835–839. https://doi.org/10.3275/7415

King, L. A., Michels, K. A., Graubard, B. I., & Trabert, B. (2021). Trends in oral contraceptive and intrauterine device use among reproductive-aged women in the US from 1999 to 2017. *Cancer Causes & Control*, *32*(6), 587–595. https://doi.org/10.1007/S10552-021-01410-8

Koehler, K., Williams, N. I., Mallinson, R. J., Southmayd, E. A., Allaway, H. C. M., & De Souza, M. J. (2016). Low resting metabolic rate in exercise-associated amenorrhea is not due to a reduced proportion of highly active metabolic tissue compartments. *American Journal of Physiology - Endocrinology and Metabolism*, *311*(2), E480–E487. https://doi.org/10.1152/ajpendo.00110.2016

Koşar, Ş. N., Güzel, Y., Köse, M. G., Kin İşler, A., & Hazır, T. (2022). Whole and segmental body composition changes during mid-follicular and mid-luteal phases of the menstrual cycle in recreationally active young women. *Annals of Human Biology*, *49*(2), 124–132. https://doi.org/10.1080/03014460.2022.2088857

Krenitsky, J. (2005). Adjusted body weight, pro: Evidence to support the use of adjusted body weight in calculating calorie requirements. *Nutrition in Clinical Practice*, *20*(4), 468–473. https://doi.org/10.1177/0115426505020004468

Kuikman, M. A., McKay, A. K. A., Smith, E. S., Ackerman, K. E., Harris, R., Elliott-sale, K. J., Stellingwerff, T., & Burke, L. M. (2023). Female athlete representation and dietary control methods among studies assessing chronic carbohydrate approaches to training. *International Journal of Sport Nutrition & Exercise Metabolism*, *33*(4), 198–208. https://doi.org/10.1123/ijsnem.2022-0214

Kuikman, M. A., Smith, E. S., McKay, A. K. A., Ackerman, K. E., Harris, R., Elliott-Sale, K. J., Stellingwerff, T., & Burke, L. M. (2023). Fueling the Female Athlete: Auditing Her Representation in Studies of Acute Carbohydrate Intake for Exercise. *Medicine and Science in Sports and Exercise*, *55*(3), 569–580. https://doi.org/10.1249/MSS.0000000000003056

Loucks, A. B., Kiens, B., & Wright, H. H. (2011). Energy availability in athletes. *Journal of Sports Sciences*, *29*(SUPPL. 1), S7-15. https://doi.org/10.1080/02640414.2011.588958

Malo-Vintimilla, L., Aguirre, C., Vergara, A., Fernández-Verdejo, R., & Galgani, J. E. (2023). Resting energy metabolism and sweet taste preference during the menstrual cycle in healthy women. *British Journal of Nutrition*, 1–19. https://doi.org/10.1017/S0007114523001927

Martin, D., Sale, C., Cooper, S. B., & Elliott-Sale, K. J. (2018). Period prevalence and perceived side effects of hormonal contraceptive use and the menstrual cycle in elite athletes. *International Journal of Sports Physiology and Performance*, *13*(7), 926–932. https://doi.org/10.1123/ijspp.2017-0330

McKay, A. K. A., Minahan, C., Harris, R., McCormick, R., Skinner, J., Ackerman, K. E., & Burke, L. M. (2023). Female Athlete Research Camp: A unique model for conducting research in high performance female athletes. *Medicine and Science in Sport and Exercise*.

McKay, A. K. A., Stellingwerff, T., Smith, E. S., Martin, D. T., Mujika, I., Goosey-Tolfrey, V. L., Sheppard, J., & Burke, L. M. (2022). Defining training and performance caliber: A participant classification framework. *International Journal of Sports Physiology and Performance*, *17*(2), 317–331. https://doi.org/10.1123/IJSPP.2021-0451

McNamara, A., Harris, R., & Minahan, C. (2022). 'That time of the month' … for the biggest event of your career! Perception of menstrual cycle on performance of Australian athletes training for the 2020 Olympic and Paralympic Games. *BMJ Open Sport — Exercise Medicine*, *8*(2), e001300. https://doi.org/10.1136/BMJSEM-2021-001300

Melanson, E. L., Gavin, K. M., Shea, K. L., Wolfe, P., Wierman, M. E., Schwartz, R. S., & Kohrt, W. M. (2015). Regulation of energy expenditure by estradiol in premenopausal women. *Journal of Applied Physiology*, *119*(9), 975. https://doi.org/10.1152/JAPPLPHYSIOL.00473.2015

Melin, A., Tornberg, Å. B., Skouby, S., Faber, J., Ritz, C., Sjödin, A., & Sundgot-Borgen, J. (2014). The LEAF questionnaire: A screening tool for the identification of female athletes at risk for the female athlete triad. *British Journal of Sports Medicine*, *48*(7), 540–545. https://doi.org/10.1136/bjsports-2013-093240

Melin, A., Tornberg, B., Skouby, S., Møller, S. S., Sundgot-Borgen, J., Faber, J., Sidelmann, J. J., Aziz, M., & Sjödin, A. (2015). Energy availability and the female athlete triad in elite endurance athletes. *Scandinavian Journal of Medicine and Science in Sports*, *25*(5), 610–622. https://doi.org/10.1111/sms.12261

Mihm, M., Gangooly, S., & Muttukrishna, S. (2011). The normal menstrual cycle in women. *Animal Reproduction Science*, *124*(3–4), 229–236. https://doi.org/10.1016/j.anireprosci.2010.08.030

Myerson, M., Gutin, B., Warren, M. P., May, M. T., Contento, I., Lee, M., Pi-Sunyer, F. X., Pierson, R. N., & Brooks-Gunn, J. (1991). Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. *Medicine and Science in Sports and Exercise*, *23*(1), 15–22. https://doi.org/10.1249/00005768-199101000-00004

Ong, J. N., Ducker, K. J., Furzer, B. J., Dymock, M., & Landers, G. J. (2022). Measures of body composition via Dual-energy X-ray absorptiometry, ultrasound and skinfolds are not impacted by the menstrual cycle in active eumenorrheic females. *Journal of Science and Medicine in Sport*, *25*(2), 115–121. https://doi.org/10.1016/j.jsams.2021.09.192

Oxfeldt, M., Dalgaard, L. B., Jørgensen, A. A., & Hansen, M. (2020). Hormonal contraceptive use, menstrual dysfunctions, and self-reported side effects in elite athletes in Denmark. *International Journal of Sports Physiology and Performance*, *15*(10), 1377–1384. https://doi.org/10.1123/ijspp.2019-0636

Paris, H. L., Foright, R. M., Werth, K. A., Larson, L. C., Beals, J. W., Cox-York, K., Bell, C., & Melby, C. L. (2016). Increasing energy flux to decrease the biological drive toward weight regain after weight loss - A proof-of-concept pilot study. *Clinical Nutrition ESPEN*, *11*, e12–e20. https://doi.org/10.1016/j.clnesp.2015.11.005

Pelkman, C. L., Chow, M., Heinbach, R. A., & Rolls, B. J. (2001). Short-term effects of a progestational contraceptive drug on food intake, resting energy expenditure, and body weight in young women. *The American Journal of Clinical Nutrition*, *73*(1), 19–26. https://doi.org/10.1093/AJCN/73.1.19

Pietrobelli, A., Formica, C., Wang, Z., & Heymsfield, S. B. (1996). Dual-energy X-ray absorptiometry body composition model: review of physical concepts. *American Journal of Physiology - Endocrinology and Metabolism*, *271*(6 Pt 1), E941-51.

Schofield, K. L., Thorpe, H., & Sims, S. T. (2019). Resting metabolic rate prediction equations and the validity to assess energy deficiency in the athlete population. *Experimental Physiology*, *104*(4), 469–475. https://doi.org/10.1113/EP087512

Siedler, M. R., De Souza, M. J., Albracht-Schulte, K., Sekiguchi, Y., & Tinsley, G. M. (2023). The influence of energy balance and availability on resting metabolic rate: Implications for assessment and future research directions. *Sports Medicine*, *53*(8), 1507–1526. https://doi.org/10.1007/s40279-023-01856-7

Slater, G., Farley, A., Hogarth, L., Areta, J. L., Paulsen, G., & Garthe, I. (2023). Impact of 24-Hr diet and physical activity control on short-term precision error of dual-energy x-ray absorptiometry physique assessment. *International Journal of Sport Nutrition and Exercise Metabolism*, *33*(1), 30–38. https://doi.org/10.1123/ijsnem.2022-0125

Slater, G., Townsend, N., Morabito, A., Burke, L., Schultz, C., Brookes, D., Farley, A., Meerkin, J., & Ducker, K. (2023). *Australian high performance sport system: Practitioner Best Practice Guidelines for DXA Assessment of Body Composition*.

Smith, E. S., McKay, A. K. A., Kuikman, M., Ackerman, K. E., Harris, R., Elliott-Sale, K. J., Stellingwerff, T., & Burke, L. M. (2022). Auditing the representation of female versus male athletes in sports science and sports medicine research: Evidence-based performance supplements. *Nutrients*, *14*(5), 953. https://doi.org/10.3390/NU14050953

Stachenfeld, N. S. (2008). Sex hormone effects on body fluid regulation. *Exercise and Sport Sciences Reviews*, *36*(3), 152–159. https://doi.org/10.1097/JES.0b013e31817be928

Stellingwerff, T., Mountjoy, M., McClusky, W. T. P., & et al. (2023). A review of the scientific rationale, development, and validation of the IOC Relative Energy Deficiency in Sport Clinical Assessment Tool - Version 2 (IOC REDs CAT2): by a sub-group of the IOC consensus on REDs. *British Journal of Sports Medicine*, *In Press*, 1110–1123. https://doi.org/10.1136/bjsports-2023-106914

Sterringer, T., & Larson-Meyer, D. E. (2022). RMR ratio as a surrogate marker for low energy availability. *Current Nutrition Reports*, *1*, 1–10. https://doi.org/10.1007/S13668-021-00385-X/TABLES/2

Steward, R. G., Bateman, L. A., Slentz, C., Stanczyk, F. Z., & Price, T. M. (2016). The impact of short-term depot-medroxyprogesterone acetate treatment on resting metabolic rate. *Contraception*, *93*(4), 317–322. https://doi.org/10.1016/J.CONTRACEPTION.2016.01.001

St-Onge, M. P., Wang, Z. M., Horlick, M., Wang, J., & Heymsfield, S. B. (2004). Dual-energy X-ray absorptiometry lean soft tissue hydration: Independent contributions of intra- and extracellular water. *American Journal of Physiology - Endocrinology and Metabolism*, *287*(5), 842–847. https://doi.org/10.1152/ajpendo.00361.2003

Strock, N. C. A., Koltun, K. J., Southmayd, E. A., Williams, N. I., & Souza, M. J. De. (2020). Indices of resting metabolic rate accurately reflect energy deficiency in exercising women. *International Journal of Sport Nutrition and Exercise Metabolism*, *30*(1), 14–24. https://doi.org/10.1123/ijsnem.2019-0199

Thompson, B. M., Hillebrandt, H. L., Sculley, D. V., Barba-Moreno, L., & Janse de Jonge, X. A. K. (2021). The acute effect of the menstrual cycle and oral contraceptive cycle on measures of body composition. *European Journal of Applied Physiology*, *121*(11), 3051–3059. https://doi.org/10.1007/s00421-021-04771-9

Trexler, E. T., Smith-Ryan, A. E., & Norton, L. E. (2014). Metabolic adaptation to weight loss: Implications for the athlete. *Journal of the International Society of Sports Nutrition*, *11*(1), 1–7. https://doi.org/10.1186/1550-2783-11-7

Vigil, P., Meléndez, J., Petkovic, G., & Del Río, J. P. (2022). The importance of estradiol for body weight regulation in women. *Frontiers in Endocrinology*, *13*, 951186. https://doi.org/10.3389/fendo.2022.951186

Weinsier, R. L., Schutz, Y., & Bracco, D. (1992). Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *American Journal of Clinical Nutrition*, *55*(4), 790–794. https://doi.org/10.1093/ajcn/55.4.790

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	$RMR_M \div ((655.1 + (9.563xBM) + (1.850xHt)) – (4.676xAge))$	<0.90	Total cholesterol	>5.2 mmol/L
ABM in HB	$RMR_M \div ((655.1 + (9.563xABM) + (1.850xHt)) – (4.676xAge)$	<0.90	LDL	>3.4 mmol/L
$Cunningham_{1980}$	$RMR_M \div (500 + (22xLBM))$	<0.90	Cortisol	>620 nmol/L
$Cunningham_{1991}$	$RMR_M \div (370 + (21.6xFFM))$	<0.92	T3 and IGF-1	Within or below lowest quartile*
Relative RMR	$RMR_M \div FFM$	$<30kcal \cdot kgFFM^{-1} \cdot day^{-1}$	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